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Prevalence and Associated Factors of Hand Contamination with Gram-Negative Bacilli among Healthcare Workers in University Hospital Centers (CHUs) in Benin

Sènami Evelyne Soclo Dansi[1,2,3*], Comlan Cyriaque Degbey[1,2,3], Comlan Ulrich Djossou[4], Affi Diane Agbokou[1,3], Yves Roland Sèmèvo Bokossa[5], Leslie Carmen Houessou[1,3], Honoré Sourou Bankolé[6]

[1]Clinique Universitaire d'Hygiène Hospitalière (CUHH), Centre National Hospitalier Universitaire-Hubert Koutoukou Maga (CNHU-HKM), Cotonou, Benin
[2]Institut Régional de Santé Publique-Comlan Alfred QUENUM (IRSP), Université d'Abomey-Calavi (UAC), Ouidah, Benin
[3]Société Béninoise d'Hygiène Hospitalière, Sécurité Patients et Santé Environnement, Ouidah, Bénin
[4]Institut de Formation et de Recherche en Informatique (IFRI), Université d'Abomey-Calavi, Abomey-Calavi, Benin
[5]Faculté des Sciences de la Santé (FSS), Université d'Abomey-Calavi, Cotonou, Benin
[6]École Polytechnique d'Abomey-Calavi (EPAC), Université d'Abomey-Calavi, Abomey-Calavi, Bénin
Email: *socloevelyne2@gmail.com

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Abstract

Introduction: The dissemination of multidrug-resistant bacteria (MDRB) in hospital settings represents a major public health challenge, particularly in resource-limited countries. Healthcare workers' hands constitute a critical yet insufficiently documented vector for the transmission of multidrug-resistant Gram-negative bacilli (MDR-GNB). This study aimed to determine the prevalence and associated factors of hand contamination with Gram-negative bacilli among healthcare workers in University Hospital Centers (CHUs) in Benin. **Methods:** This was a cross-sectional analytical study conducted in six CHUs in Benin. The study population consisted of healthcare workers directly involved in patient care, including physicians, nurses, and nursing assistants. Hand hygiene compliance was assessed through direct non-participant observation. In addition, microbiological analysis of hand samples collected from healthcare workers after hand hygiene was performed to detect Gram-negative bacilli (GNB), followed by antibiotic susceptibility testing for each isolated GNB. **Results:** Among the 339 healthcare workers surveyed, 267 (78.76%) demonstrated a low level of hand hygiene compliance. Microbiological analysis revealed that 186 healthcare workers (54.87%) had hands contaminated with GNB despite prior hand hygiene performance. The majority of the isolated GNB (171/186,

91.94%) were multidrug-resistant organisms. Hand hygiene compliance was significantly associated with sex ($p = 0.013$), hospital department ($p = 0.031$), and the type of hand hygiene commonly practiced ($p < 0.001$). The presence of GNB on healthcare workers' hands was significantly associated with age group, professional category, and the type of hand hygiene practiced. **Conclusion:** These findings highlight the persistence of a high level of microbiological contamination of healthcare workers' hands and emphasize the need for regular microbiological surveillance, particularly targeting high-risk professional categories such as nurses. Strengthening hand hygiene compliance appears essential to improving the quality of care and reducing the risk of healthcare-associated infections in hospitals in Benin.

Keywords

Hand Hygiene, Gram-Negative Bacilli, Healthcare Workers, Benin

1. Introduction

Healthcare-associated infections (HAIs) are a central indicator of quality of care and patient safety in healthcare facilities. They represent a substantial burden on health systems worldwide due to their high frequency, clinical impact, and economic cost [1]-[4]. This issue is particularly acute in low- and middle-income countries, where limited resources, overcrowded hospital infrastructures, and organizational constraints facilitate the transmission of infectious agents [5]. Cross-transmission of microorganisms in hospital settings largely relies on contamination of healthcare workers' hands. Hands constitute the critical interface between patients, the healthcare environment, and medical devices, and represent the primary vector for the dissemination of pathogens, whether susceptible or multidrug-resistant [6] [7]. Transient hand colonization frequently occurs during patient care, particularly when hand hygiene practices are insufficient or inadequately performed, thereby exposing patients to an increased risk of HAIs [8]-[10]. This transmission dynamic is currently exacerbated by the alarming rise in multidrug-resistant bacteria within healthcare facilities. Gram-negative bacilli occupy a prominent position in this context due to their ability to persist in the hospital environment, colonize surfaces over prolonged periods, and develop complex mechanisms of antimicrobial resistance [11]. Their increasing involvement in HAIs is associated with high morbidity, increased mortality, and a significant reduction in available therapeutic options [3]. In response to this threat, hand hygiene is universally recognized as the most effective, simplest, and most cost-effective measure for preventing the transmission of infectious agents in healthcare settings [6] [8]. The World Health Organization (WHO) has structured this approach through a standardized definition of HAIs [1] [12] and the introduction, in 2009, of the "Five Moments for Hand Hygiene" concept, which now constitutes the international reference framework for hand hygiene practices [13]. However,

despite the widespread dissemination of these recommendations and recognition of their effectiveness, hand hygiene compliance among healthcare workers remains insufficient worldwide [14] [15]. Available data indicate that average compliance rates remain below 40%, with considerable variability according to professional category, clinical department, and care setting [14]. In high-risk units, such as intensive care units, compliance rates may fall below 10% in low-income countries, severely compromising the effectiveness of prevention programs [16]. Although numerous studies have focused on self-reported or directly observed hand hygiene compliance, few have incorporated an objective microbiological assessment of the actual contamination of healthcare workers' hands. This methodological limitation restricts understanding of the effective role of hands in the transmission of multidrug-resistant bacteria, particularly Gram-negative bacilli. This gap is even more pronounced in sub-Saharan Africa, where data remain scarce despite a high prevalence of HAIs and documented circulation of multidrug-resistant bacteria in hospitals [5]. In Benin, CHUs serve as referral centers providing specialized care, initial and continuing training of healthcare personnel, and management of complex medical conditions. However, scientific data regarding hand contamination of healthcare workers by Gram-negative bacilli and the factors associated with such contamination remain limited, even though these facilities concentrate high-risk patients and frequent invasive procedures. In this context, the present study aims to determine the prevalence of Gram-negative bacilli contamination of healthcare workers' hands and to identify associated factors in six CHUs in Benin. By combining the assessment of hand hygiene compliance with microbiological analysis of hand samples, this study proposes an integrated approach to better understand transmission mechanisms in hospital settings and to generate evidence-based data essential for strengthening infection prevention and control strategies, as well as efforts to combat antimicrobial resistance.

2. Methods

2.1. Study Design

This was a cross-sectional analytical study conducted from September 2024 to June 2025 in six CHUs in Benin: the National University Hospital Hubert Koutoukou Maga (CNHU-HKM) in Cotonou, the Mother and Child Lagoon University Hospital (CHU-MEL), the Ouémé Departmental University Hospital (CHUD-Ouémé), the Borgou Departmental University Hospital (CHUD-Borgou), the Suru-Léré Zone University Hospital (CHUZ-Suru-Léré), and the Abomey-Calavi/Sô-Ava Zone University Hospital (CHUZ-Abomey-Calavi). These institutions are the country's main referral hospitals, providing tertiary healthcare services as well as teaching and research activities.

2.2. Participant Selection Criteria

The study involved healthcare workers directly engaged in patient care, including physicians, nurses, and nursing assistants.

- The physician category included general practitioners and medical specialists.
- The nurse category comprised state-registered nurses, midwives, anesthetists, surgical instrument technicians, and emergency nurses.
- Nursing assistants referred to support staff involved in direct patient care without full paramedical training.

Participants included healthcare workers present in the wards at the time of the survey and actively involved in patient care. Administrative or non-clinical staff, healthcare workers who were absent or on leave, and those who refused to participate were excluded. Participants who were initially included and observed but did not consent to microbiological sampling were also excluded. Anonymity was ensured, and observations were conducted discreetly to minimize the *Hawthorne* effect.

2.3. Sampling Method and Sample Size

A stratified proportional probability sampling method was used. Primary stratification was based on the six CHUs, while secondary stratification was based on professional categories within each hospital (physicians, nurses, and nursing assistants). The sample size was calculated using Cochran's formula for estimating a proportion:

$$n_0 = \frac{z^2 p(1-p)}{d^2} = 384$$

where z = 1.96 (95% confidence interval), p = 0.5 (conservative estimate in the absence of reliable local data), and d = 0.05.

Given that the total study population consisted of 1982 healthcare workers across the six CHUs, a finite population correction was applied:

$$n = \frac{n_0}{1 + \frac{n_0 - 1}{N}} = 322 \quad \text{with } n_0 = 384 \text{ and } N = 1982$$

Participants were then proportionally allocated by professional category and by CHU according to staff size. To ensure representativeness at the departmental level, each CHU quota was distributed among the relevant departments. Within each department, allocation across professional categories was proportional to their respective workforce size. In total, 339 healthcare workers actively involved in patient care were randomly observed and sampled during routine care activities. The study was conducted in accordance with the Declaration of Helsinki [17] and ethical guidelines. Required authorizations were obtained, including institutional approval from each CHU and approval from the local biomedical research ethics committee (564/2024/*CLERB-UP/P/SPμ/R/SA*).

2.4. Assessment of Hand Hygiene Compliance

Hand hygiene compliance was assessed through direct non-participant observation, using the standard World Health Organization (WHO) observation tool, based on the "Five Moments for Hand Hygiene" framework. Each hand hygiene

opportunity was classified as compliant or non-compliant with WHO recommendations. For each healthcare worker, ten consecutive hand hygiene opportunities were observed during the assessment period, in accordance with WHO methodological guidelines, in order to minimize observation bias and ensure comparability between participants and hospital units [18]. To observe practices as close as possible to routine behavior, healthcare workers were not informed of the observation phase at the time of assessment. The investigator introduced himself only after completion of the observation phase, in order to explain the objectives of the study and to obtain informed consent for subsequent microbiological sampling.

2.5. Hand Sampling and Microbiological Analysis

To avoid any behavioral modification related to anticipation of sampling, microbiological hand sampling was performed only after a hand hygiene action (simple handwashing, alcohol-based hand rub, or handwashing followed by alcohol-based hand rub) spontaneously carried out by the healthcare worker during patient care, without any solicitation or indication from the investigator. This procedure aimed to ensure that the samples reflected actual hand hygiene practices. Swabbing was performed on both hands, including the palms, dorsum of the hands, interdigital spaces, and periungual areas, using a sterile swab moistened with sterile brain-heart infusion (BHI) broth. The swabs were then transported at 4˚C in a cold chain to the Public Health Laboratory of the University Hospital Hygiene Clinic of CNHU-HKM, Cotonou, for microbiological analysis. Upon arrival at the laboratory, samples were immediately inoculated into sterile brain-heart infusion broth used as an enrichment medium and incubated at 37˚C for 18 - 24 h. After the enrichment phase, the broths were subcultured onto appropriate solid culture media, followed by incubation at 37˚C for 24 h, for the isolation and identification of Gram-negative bacilli (GNB). The detection of Gram-negative bacilli (GNB) was performed for all collected samples. Antimicrobial susceptibility testing was systematically carried out for all isolated GNB. Antibiotic resistance profiles were determined using the Kirby-Bauer disk diffusion method, in accordance with the recommendations of the Antibiogram Committee of the French Society of Microbiology (CA-SFM/EUCAST 2024). An isolate was classified as multidrug-resistant (MDR) when it exhibited resistance to at least one agent in three different antimicrobial classes, and as pandrug-resistant (PDR) when it showed resistance to all tested antibiotics, in accordance with the criteria defined by Magiorakos *et al.* [19]. Phenotypic detection of extended-spectrum β-lactamase (ESBL) production was performed using the synergy test between amoxicillin-clavulanic acid and third-generation cephalosporins (cefotaxime, ceftazidime, ceftriaxone). AmpC β-lactamase production was suspected in cases of cefoxitin resistance and phenotypically confirmed using an inhibitor-based test on Mueller-Hinton agar supplemented with cloxacillin (200 µg/mL), combined with cephalosporin disks (cefotaxime). Carbapenemase production was investigated using the modified Hodge test. To ensure analytical quality, internal quality controls were performed for

each series of analyses using reference strains such as Escherichia coli ATCC 25922 and Pseudomonas aeruginosa ATCC 27853. Swabs, culture media, and laboratory equipment were handled and monitored in accordance with good microbiological practices.

2.6. Statistical Analysis

The dependent variables included the level of hand hygiene compliance, categorized as good (>80%), moderate (50% - 80%), and poor (<50%), as well as the microbiological quality of hands, defined as contaminated or non-contaminated with Gram-negative bacilli (GNB). The independent variables comprised the CHU, clinical department, professional category, sex, age, type of hand hygiene practice routinely performed, and years of professional experience, defined as the total duration, expressed in complete years, between the date of entry into clinical practice and the date of survey administration. Results were presented as frequencies, means, and standard deviations. Comparisons of proportions for identifying factors associated with hand hygiene compliance were performed using the Chi-square test, with the statistical significance threshold set at $p < 0.05$. Variables associated with the presence of GNB on healthcare workers' hands were entered into a manual stepwise backward logistic regression model. All variables significant at the 20% threshold ($p < 0.20$) in univariate analysis were included in the initial model. Adjusted odds ratios (aORs) were calculated with their 95% confidence intervals (95% CI). Appropriate diagnostic tests were performed to assess the goodness-of-fit and validity of the final model.

3. Results

3.1. Characteristics of the Surveyed Healthcare Workers

A total of 339 healthcare workers were included from the six CHUs in Benin. Table 1 presents the characteristics of the healthcare workers surveyed in this study. Participants were mainly nurses (56.3%), followed by physicians (23.3%) and nursing assistants (20.4%), with a sex ratio of 1.13 in favor of males (53.1% men versus 46.9% women). The majority of participants were aged between 26 and 54 years (90.8%) and had 6 - 10 years of professional experience (47.8%).

Table 1. Characteristics of the study population.

Variables	n (%)
Professional category	
Nurse	191 (56.3)
Physician	79 (23.3)
Nursing assistant	69 (20.4)
Years of experience	
<5	53 (15.6)
6 - 10	162 (47.8)

Continued

11 - 20	86 (25.4)
>20	38 (11.2)
Sex	
Male	180 (53.1)
Female	159 (46.9)
Age (years)	
<25	11 (3.2)
26 - 34	111 (32.7)
35 - 44	122 (36)
45 - 54	75 (22.1)
>54	20 (6)

3.2. Hand Hygiene Compliance among Healthcare Workers

Out of the 3390 hand hygiene opportunities observed among the surveyed healthcare workers, only ,038 were performed, corresponding to an overall compliance rate of 30.62%. On average, 3.07 ± 1.9 opportunities were performed out of the 10 consecutive opportunities observed per healthcare worker. Figure 1 illustrates the distribution of healthcare workers according to their level of hand hygiene compliance across the six CHUs in Benin.

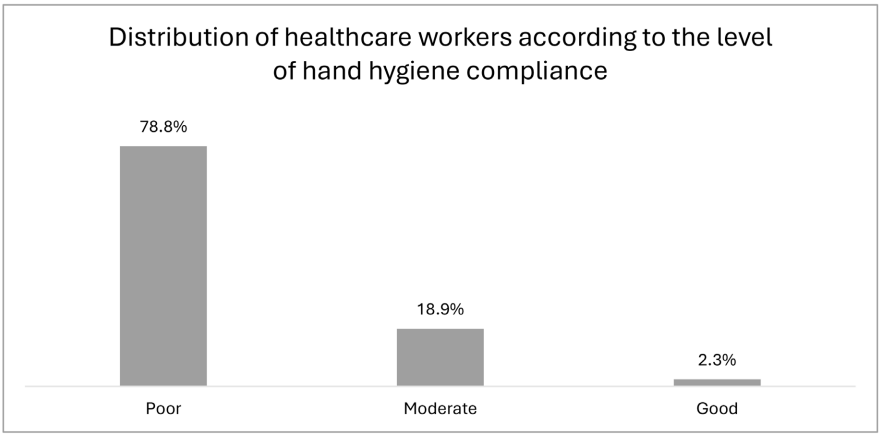

Figure 1. Distribution of healthcare workers according to hand hygiene compliance level.

The vast majority of healthcare workers (78.76%) demonstrated poor hand hygiene compliance, while only 2.36% achieved a good level of compliance and 18.88% had a moderate level. These findings highlight substantial gaps in routine hand hygiene practices.

3.3. Microbiological Quality of Healthcare Workers' Hands

Microbiological analysis of hand samples collected after hand hygiene practice revealed the presence of Gram-negative bacilli (GNB) in more than half (54.9%) of the surveyed healthcare workers, corresponding to 186 out of 339 participants.

Antibiotic susceptibility testing enabled characterization of the resistance profiles of the isolated organisms. Figure 2 and Figure 3 present the antimicrobial resistance profiles of the isolated GNB and the detection of multidrug-resistant Gram-negative bacilli (MDR-GNB), respectively. Extended-spectrum β-lactamase (ESBL), cephalosporinase or AmpC β-lactamase, and carbapenemase production were observed in 26.3%, 47.9%, and 26.3% of the isolated GNB, respectively (Figure 2).

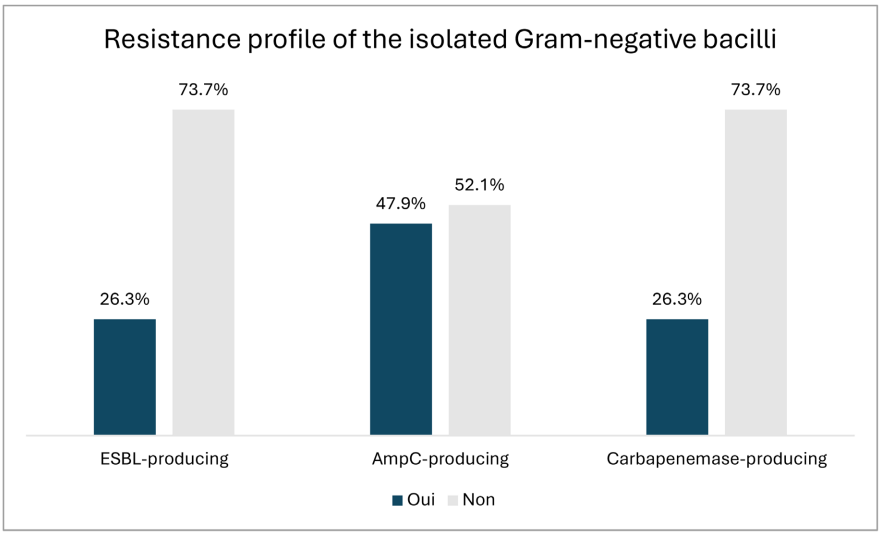

Figure 2. Antimicrobial resistance profiles of isolated Gram-negative bacilli.

Nearly all (91.9%) of the isolated GNB were multidrug-resistant organisms (Figure 3).

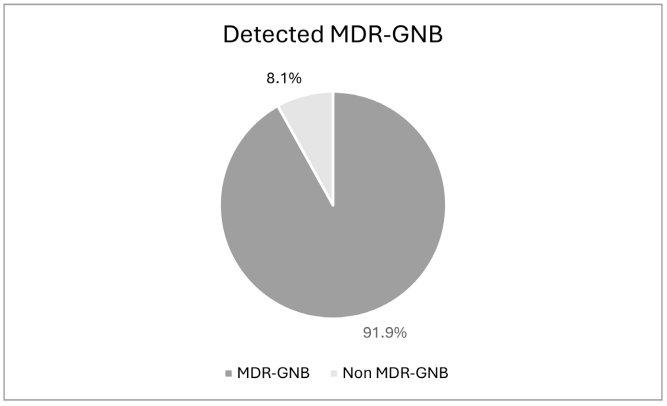

Figure 3. Detection of multidrug-resistant Gram-negative bacilli.

3.4. Factors Associated with Hand Hygiene Compliance among Healthcare Workers

3.4.1. Bivariate Analysis

The bivariate analysis showed that sex, hospital department, and the type of hand hygiene performed were significantly associated with the level of hand hygiene

compliance among healthcare workers (Table 2). A significant association was observed with sex (χ^2 = 8.65; df = 2; p = 0.013) and with department (χ^2 = 19.86; df = 10; p = 0.031). The type of hand hygiene performed was strongly associated with the level of compliance (χ^2 = 28.02; df = 4; p < 0.001), with higher compliance levels observed among healthcare workers who primarily used alcohol-based hand rub compared to those who mainly relied on simple handwashing. In contrast, no statistically significant association was found between the level of hand hygiene compliance and professional category (χ^2 = 6.74; df = 4; p = 0.150) or years of professional experience (χ^2 = 4.20; df = 6; p = 0.649).

Table 2. Bivariate analysis of factors associated with the level of hand hygiene compliance among healthcare workers.

Variables	Poor n (%)	Moderate n (%)	Good n (%)	Total	χ^2 (df)	p-value
Age (years)						0.471
<25	11 (100)	0 (0.0)	0 (0.0)	11		
26 - 34	87 (78.4)	22 (19.8)	2 (1.8)	111		
35 - 44	93 (76.2)	27 (22.1)	2 (1.7)	122		
45 - 54	62 (82.7)	10 (13.3)	3 (4)	75		
≥55	14 (70)	5 (25)	1 (5)	20		
Sex					8.65 (2)	0.013*
Male	143 (79.4)	29 (16.2)	8 (4.4)	180		
Female	124 (78)	35 (22)	0 (0)	159		
Professional category					6.74 (4)	0.150
Nurse	141 (73.8)	45 (23.6)	5 (2.6)	191		
Physician	67 (84.8)	10 (12.7)	2 (2.5)	79		
Nursing assistant	59 (85.5)	9 (13)	1 (1.5)	69		
Department					19.86 (10)	0.031*
Surgery	70 (78.7)	17 (19.1)	2 (2.2)	89		
Intensive care	42 (65.6)	22 (34.4)	0 (0)	64		
Neonatology	46 (78)	10 (17)	3 (5)	59		
Internal medicine	39 (88.6)	5 (11.4)	0 (0)	44		
Maternity ICU	37 (88.1)	4 (9.5)	1 (2.4)	42		
Pediatric ICU	33 (80.5)	6 (14.6)	2 (4.9)	41		
Years of experience					4.20 (6)	0.649
<5	44 (83)	9 (17)	0 (0)	53		
6 - 10	126 (77.8)	34 (21)	2 (1.2)	162		
11 - 20	74 (86)	12 (14)	0 (0)	86		
>20	30 (79)	8 (21)	0 (0)	38		
Type of hand hygiene performed					28.02 (4)	<0.001*
Alcohol-based hand rub	73 (70.9)	23 (22.3)	7 (6.8)	103		
Handwashing with soap	172 (86.4)	27 (13.6)	0 (0)	199		
Handwashing + ABHR	22 (59.5)	14 (37.8)	1 (2.7)	37		

Note: Pearson's χ^2 test. $p < 0.05$ *statistically significant.*

Although the bivariate analysis identified several factors significantly associated with hand hygiene compliance, these associations did not account for potential interactions between explanatory variables. To identify factors independently associated with poor hand hygiene compliance and to control for confounding effects, a multivariate analysis was performed. For this purpose, hand hygiene compliance was dichotomized into poor compliance versus acceptable compliance (moderate/good). Variables with a p-value ≤ 0.20 in the bivariate analysis, as well as variables considered epidemiologically relevant, were included in a multivariate logistic regression model.

3.4.2. Multivariate Analysis

After adjustment for sex, department, and type of hand hygiene performed, only one factor remained independently associated with poor hand hygiene compliance, as shown in Table 3. Healthcare workers who mainly practiced simple handwashing had a significantly higher likelihood of poor compliance compared to those using alcohol-based hand rub (adjusted OR = 3.03; 95% CI [1.56 - 5.90]; p = 0.001). No statistically significant association was observed between poor hand hygiene compliance and sex (adjusted OR = 1.04; 95% CI [0.60 - 1.79]; p = 0.885). Similarly, after adjustment, department was no longer significantly associated with poor compliance, although trends were observed for internal medicine (adjusted OR = 2.75; p = 0.071) and maternity intensive care units (adjusted OR = 2.65; p = 0.083). Alcohol-based hand rub thus emerged as the central and independent determinant of hand hygiene compliance, confirming its pivotal role in strategies for the prevention of healthcare-associated infections.

Table 3. Factors independently associated with poor hand hygiene compliance among healthcare workers (multivariate logistic regression).

Variables	Adjusted OR	95% CI	p-value
Sex			
Female	1	–	–
Male	1.04	[0.60 - 1.79]	0.885
Department			
Surgery	1	–	–
Internal medicine	2.75	[0.92 - 8.24]	0.071
Neonatology	1.68	[0.69 - 4.06]	0.251
Intensive care	1.14	[0.46 - 2.81]	0.774
Maternity ICU	2.65	[0.88 - 7.97]	0.083
Pediatric ICU	2.08	[0.75 - 5.77]	0.161
Type of hand hygiene performed			
Alcohol-based hand rub	1	–	–
Handwashing with soap	3.03	[1.56 - 5.90]	0.001
Handwashing + ABHR	0.78	[0.32 - 1.91]	0.586

Note: ORa = adjusted Odds Ratio; CI = 95% Confidence Interval.

3.5. Factors Associated with the Microbiological Quality of Healthcare Workers' Hands

3.5.1. Bivariate Analysis

The bivariate analysis showed that professional category was strongly associated with hand contamination (χ^2 = 57.06; df = 2; p < 0.001), suggesting differential exposure according to professional role, with a higher probability of Gram-negative bacilli (GNB) contamination among nurses (72.8%). Hospital department was also significantly associated with hand contamination (χ^2 = 29.07; df = 5; p < 0.001), reflecting the influence of the clinical setting and the level of infectious risk specific to each department. The type of hand hygiene performed was the factor most strongly associated with bacterial isolation (χ^2 = 87.48; df = 2; p < 0.001), indicating that inadequate or incomplete practices significantly increase the risk of hand contamination. Professional experience was significantly associated with bacterial isolation (χ^2 = 9.26; df = 3; p = 0.026), suggesting that years of practice influence actual infection prevention behaviors.

Hand hygiene compliance level was significantly associated with hand contamination by GNB (χ^2 = 11.19; df = 2; p = 0.003). Poor compliance levels were associated with a higher frequency of bacterial isolation, confirming the central role of adherence to best practices in preventing microbial transmission.

Sex (p = 0.071) and age (p = 0.078) were not significantly associated with hand contamination by Gram-negative bacilli (Table 4).

Table 4. Bivariate analysis of factors associated with hand contamination among healthcare workers (n = 339).

Variables	Non contami-nation n (%)	Contami-nation n (%)	Total	χ^2	df	*p-value*
Age (years)				8.40	4	0.078
<25	7 (63.6)	4 (36.4)	11			
25 - 34	46 (41.4)	65 (58.6)	111			
35 - 44	48 (39.3)	74 (60.7)	122			
45 - 54	39 (52.0)	36 (48.0)	75			
≥55	13 (65.0)	7 (35.0)	20			
Sex				3.26	1	0.071
Female	90 (50.0)	90 (50.0)	180			
Male	63 (39.6)	96 (60.4)	159			
Professional category				57.06	2	<0.001*
Nurse	52 (27.2)	139 (72.8)	191			
Physician	52 (65.8)	27 (34.2)	79			
Nursing assistant	49 (71.0)	20 (29.0)	69			
Department				29.07	5	<0.001*
Surgery	43 (48.3)	46 (51.7)	89			
Intensive care	24 (37.5)	40 (62.5)	64			
Neonatology	28 (47.5)	31 (52.5)	59			
Internal medicine	16 (36.4)	28 (63.6)	44			

Continued

Maternity ICU	18 (42.9)	24 (57.1)	42			
Pediatric ICU	24 (58.5)	17 (41.5)	41			
Years of experience				9.26	3	0.026*
<5	17 (32.1)	36 (67.9)	53			
6 - 10	69 (42.6)	93 (57.4)	162			
11 - 20	50 (58.1)	36 (41.9)	86			
>20	17 (44.7)	21 (55.3)	38			
Type of hand hygiene performed				87.48	2	< 0.001*
Alcohol-based hand rub	74 (71.8)	29 (28.2)	103			
Handwashing with soap	48 (24.1)	151 (75.9)	199			
Handwashing + ABHR	31 (83.8)	6 (16.2)	37			
Hand hygiene compliance level				11.19	2	0.004*
Poor	113 (42.3)	154 (57.7)	267			
Moderate	32 (50.0)	32 (50.0)	64			
Good	8 (100.0)	0 (0.0)	8			

Statistically significant association ($p < 0.05$).

3.5.2. Multivariate Analysis

After adjustment for sex, professional category, department, and type of hand hygiene performed, nursing profession and type of hand hygiene remained significantly associated with bacterial isolation.

Nurses had a significantly higher risk of bacterial isolation compared to nursing assistants (adjusted OR = 17.47; 95% CI [7.41 - 41.19]; $p < 0.001$). Similarly, reliance on simple handwashing was strongly associated with bacterial isolation compared to alcohol-based hand rub (adjusted OR = 39.55; $p < 0.001$). However, after adjustment, no statistically significant association was observed between bacterial isolation and sex, department, or years of professional experience ($p > 0.05$) (Table 5).

Table 5. Factors associated with hand contamination among healthcare workers—multivariate logistic regression.

Variables	Adjusted OR	95% CI	p-value
Sex			
Female	Ref	–	–
Male	0.69	[0.37 - 1.28]	0.243
Profession			
Nursing assistant	Ref	–	–
Nurse	17.47	[7.41 - 41.19]	<0.001
Physician	1.56	[0.65 - 3.75]	0.319
Department			
Surgery	Ref	–	–
Internal medicine	0.62	[0.24 - 1.63]	0.331

Continued

Neonatology	1.70	[0.65 - 4.48]	0.282
Intensive care	0.74	[0.26 - 2.16]	0.586
Type of hand hygiene			
Alcohol-based hand rub	Ref	–	–
Handwashing with soap	39.55	[>1 - >10]	<0.001
Handwashing + ABHR	1.68	[0.66 - 4.26]	0.259

4. Discussion

4.1. Prevalence of Hand Contamination by Gram-Negative Bacilli in University Hospitals in Benin: A Strong Signal of Dissemination of Multidrug-Resistant Bacteria

In this multicenter study conducted in six CHUs in Benin, more than half of healthcare workers (54.87%) had Gram-negative bacilli (GNB) detected on their hands after performing hand hygiene, reflecting a concerning persistence of GNB on healthcare workers' hands. This finding suggests that, in a substantial proportion of situations, the procedures performed do not allow effective elimination of potentially pathogenic transient flora. Recent studies have shown that certain hand hygiene products themselves may be contaminated with Gram-negative bacilli, including multidrug-resistant strains, thereby compromising the effectiveness of hand hygiene practices in healthcare settings [20]. Of particular concern, 91.94% of the isolated GNB were multidrug-resistant, involving major resistance mechanisms such as extended-spectrum β-lactamase (ESBL) production, cephalosporinases, and carbapenemases. This situation highlights the active circulation of highly resistant strains within the clinical environment studied. These findings are consistent with reports indicating that a non-negligible proportion of hygiene-related resources (water, soap) in hospitals in resource-limited settings may harbor multidrug-resistant GNB, including New Delhi Metallo-β-Lactamase (NDM) producers [21]. Assessing contamination after hand hygiene confers substantial added value to our approach by simultaneously integrating the behavioral dimension (compliance) and the actual microbiological effectiveness of the gesture. This methodology allows a more accurate assessment of the risk of dissemination of multidrug-resistant GNB beyond conventional compliance indicators measured through audits. The high prevalence of multidrug-resistant GNB on healthcare workers' hands observed in this study is consistent with recent international data identifying healthcare workers' hands as a key reservoir and vector of multidrug-resistant bacteria, particularly in settings characterized by high antibiotic pressure and suboptimal implementation of infection prevention and control (IPC) measures [20] [22] [23].

4.2. Resistance Profile of Gram-Negative Bacilli Isolated from Healthcare Workers' Hands: A Key Indicator of Antimicrobial Resistance Emergence

This study highlights a particularly alarming resistance profile among GNB iso-

lated from healthcare workers' hands, with 91.94% of multidrug-resistant strains among the 186 GNB identified. The detection of cephalosporinases (47.85%), ESBLs (26.34%), and carbapenemases (26.34%) indicates the dissemination of critical resistance mechanisms affecting last-resort antibiotics within the healthcare environment of Benin's CHUs. The presence of these multidrug-resistant GNB on healthcare workers' hands constitutes a direct marker of hand-borne transmission of multidrug-resistant bacteria (MDR), which is especially concerning in hospital settings exposed to high antibiotic pressure and structural constraints related to IPC [20]. Studies conducted in sub-Saharan Africa have shown that healthcare workers' hands and hygiene-related devices can act as secondary reservoirs of multidrug-resistant GNB, thereby undermining the effectiveness of prevention strategies [20] [21]. The isolation of these strains after hand hygiene underscores that compliance alone, whether declared or observed, does not guarantee effective microbiological elimination. The effectiveness of hand hygiene is closely dependent on the quality of the technique, the product used, and the level of environmental contamination, as demonstrated by several studies documenting the persistence of multidrug-resistant GNB in water, liquid soaps, and handwashing infrastructures [20] [21]. These findings are consistent with a recent systematic review conducted in West Africa reporting a high and persistent prevalence of multidrug-resistant bacteria in healthcare settings and confirming the central role of the hospital environment and healthcare workers' hands in the silent dissemination of antimicrobial resistance (AMR) [24].

4.3. Low Hand Hygiene Compliance and Persistence of Microbiological Risk

In this study, 78.76% of healthcare workers exhibited a low level of hand hygiene compliance, with an overall mean compliance rate of 30.6%. This level is comparable to those reported in several hospitals in resource-limited countries, where actual compliance rarely exceeds 40% in daily practice [25] [26]. Recent literature indicates that this low compliance is multifactorial, involving workload overload, insufficient continuing education, lack of supervision, and constraints related to the availability or quality of hygiene products [26] [27]. In our study, low compliance was significantly associated with the presence of GNB on hands (χ^2 = 11.19; df = 2; p = 0.003), confirming that the quality of hand hygiene practices is a major determinant of residual microbiological risk. These results support current evidence indicating that sustained improvement in hand hygiene compliance remains one of the most effective strategies to reduce GNB transmission and limit the dissemination of multidrug-resistant bacteria in healthcare settings [28] [29].

4.4. Factors Associated with Hand Contamination by Gram-Negative Bacilli

Nurses were the professional category most frequently contaminated with GNB,

with a significantly higher risk compared with nursing assistants. This observation is consistent with evidence showing that nurses, due to direct and repeated patient contact, invasive procedures, and frequent handling of medical devices, are particularly exposed to hospital-acquired bacteria, including multidrug-resistant strains [21] [30]. They therefore represent a priority target for interventions aimed at preventing AMR dissemination. Furthermore, healthcare workers who exclusively practiced handwashing with soap had an increased risk of contamination compared with those using alcohol-based hand rub. Recent studies confirm the superiority of alcohol-based hand rub in eliminating transient flora, including multidrug-resistant GNB, when the technique is correctly applied [31] [32]. The possible contamination of certain hand hygiene products with GNB, reported in resource-limited settings, may also contribute to the persistence of these bacteria despite hand hygiene practices [33] [34], highlighting the need for rigorous quality control of the solutions used. Finally, the association observed with intermediate age groups (25 - 44 years) suggests that GNB dissemination is influenced by workload, cumulative exposure to patient care, and organizational factors beyond professional experience alone [35].

4.5. Factors Independently Associated with Hand Contamination by Gram-Negative Bacilli

Multivariate analysis identified professional category and type of hand hygiene as the main factors independently associated with hand contamination by Gram-negative bacilli (GNB). Nurses had a markedly higher risk of hand contamination compared with nursing assistants (adjusted OR = 17.47; 95% CI [7.41 - 41.19]; $p < 0.001$). This finding aligns with data showing that nurses, due to frequent and close patient contact, invasive care activities, and manipulation of medical devices, are particularly exposed to hospital bacteria, including multidrug-resistant strains [21] [30]. They therefore constitute a priority target for AMR prevention interventions. No significant association was observed among physicians (aOR = 1.56; 95% CI [0.65 - 3.75]; $p = 0.319$). Simple handwashing was strongly associated with contamination, with a substantially higher risk compared with alcohol-based hand rub use (aOR = 39.55; 95% CI > 1; $p < 0.001$). Recent studies confirm the superiority of alcohol-based hand rub for eliminating transient flora, particularly multidrug-resistant GNB, when correctly applied [31] [32]. The association with the combined "handwashing + alcohol-based hand rub" practice was not statistically significant (aOR = 1.68; 95% CI [0.66 - 4.26]; $p = 0.259$). No significant association was found with sex (aOR = 0.69; $p = 0.243$) or with any clinical department (internal medicine, neonatology, or intensive care; $p > 0.05$), suggesting widespread dissemination of risk across all departments.

Overall, these findings indicate that hand-borne contamination by GNB in Benin's CHUs is primarily driven by the intensity of professional exposure and the quality of hand hygiene practices, identifying nurses and exclusive handwashing as priority targets for AMR prevention and control strategies.

4.6. Implications for Infection Prevention and Control and Antimicrobial Resistance Containment in Benin's CHUs

The high prevalence of hand contamination by multidrug-resistant GNB observed in this study constitutes a major warning signal for IPC and AMR control programs in Benin's CHUs. It confirms that healthcare workers' hands represent a central vector of silent dissemination of multidrug-resistant GNB, potentially contributing to the persistence of difficult-to-treat healthcare-associated infections [20]. ESBL- and carbapenemase-producing GNB are now among the most critical public health priorities due to their impact on morbidity, mortality, and healthcare costs, particularly in resource-limited countries [36]. When IPC measures are incompletely implemented, hand-borne transmission remains one of the main mechanisms of spread of these bacteria [20] [21]. Our results indicate that promoting hand hygiene, although fundamental, is insufficient if not integrated into a multimodal approach. According to the World Health Organization, sustainable hand hygiene effectiveness relies on the combination of continuous training, audit with feedback, availability of quality products, and strong institutional commitment to patient safety [35]. The high proportion of multidrug-resistant GNB (91.94%) also highlights the close interdependence between IPC and antimicrobial stewardship. Hand-borne transmission promotes severe healthcare-associated infections, leading to increased use of last-resort antibiotics and reinforcing selective pressure in a self-perpetuating cycle of AMR [29] [36].

In this context, our findings support the strengthening of integrated IPC-AMR programs within university hospital centers in Benin, incorporating targeted microbiological surveillance of healthcare workers' hands, in addition to routine clinical and environmental surveillance, with particular emphasis on the systematic integration of microbiological monitoring of water and hand hygiene products. Such strategies have demonstrated their effectiveness in hospitals in low- and middle-income countries, by reducing the transmission of multidrug-resistant bacteria and improving patient safety [21] [29]. This study therefore provides essential local evidence to inform hospital-level and national policies aimed at combating antimicrobial resistance (AMR).

4.7. Study Limitations

Despite the methodological efforts undertaken to ensure the robustness and quality of the collected data, this study has several limitations. First, the cross-sectional design does not allow the establishment of causal relationships between hand hygiene compliance, contamination of healthcare workers' hands, and the dissemination of multidrug-resistant Gram-negative bacilli (MDR-GNB). The findings represent a snapshot at a given point in time and do not allow assessment of temporal trends in contamination or evaluation of the direct impact of potential preventive interventions. Second, the use of an enrichment broth prior to inoculation onto solid culture media constitutes a methodological limitation. While this approach enhances the detection of GNB, including those present at low inoculum

levels, it does not allow quantification of the bacterial load, expressed as colony-forming units (CFU). Third, the analysis of bacterial resistance relied primarily on phenotypic methods. Although these methods are appropriate for the context of university hospital centers in Benin and are relevant for routine surveillance, the absence of molecular analyses limits precise identification of genetic resistance mechanisms and the exploration of clonal transmission dynamics. Nevertheless, this approach accurately reflects the real-world conditions of microbiological diagnostics in many hospitals in resource-limited settings. Finally, the study did not account for certain organizational factors that may influence hand hygiene practices and the risk of contamination, such as workload, healthcare worker-to-patient ratios, continuous availability of hand hygiene products, or the level of institutional supervision. The absence of these variables restricts a more in-depth analysis of the systemic determinants involved in the dissemination of multidrug-resistant bacteria.

Despite these limitations, this study provides original, multicenter, and context-specific data, offering valuable insights into the role of healthcare workers' hands in the dissemination of multidrug-resistant Gram-negative bacilli within university hospital centers in Benin. It constitutes a solid foundation for the development of longitudinal studies, molecular investigations, and integrated interventions in infection prevention and control (IPC) and antimicrobial resistance (AMR) containment.

5. Conclusion

This multicenter study conducted in six University Hospital Centers in Benin highlights a high prevalence of hand contamination among healthcare workers with multidrug-resistant Gram-negative bacilli, observed after the performance of hand hygiene. The substantial proportion of multidrug-resistant isolates reflects active hand-mediated dissemination of multidrug-resistant bacteria within the hospital environments investigated. Hand contamination was associated with the intensity of occupational exposure, hand hygiene practices, and the type of method used, with a particularly high risk observed among nurses and when handwashing alone was exclusively used. These findings indicate that hand hygiene compliance, whether self-reported or directly observed, does not necessarily guarantee optimal microbiological effectiveness. Beyond behavioral determinants, our data also suggest that extrinsic factors, notably the microbiological quality of water and products used for hand hygiene, may contribute to the persistence of contamination following hand hygiene practices. Consequently, the prevention of multidrug-resistant bacteria dissemination cannot rely solely on individual healthcare worker behavior. Overall, these findings underscore the need for integrated infection prevention and control strategies, combining qualitative improvement of hand hygiene practices, systematic promotion of alcohol-based hand rub, and regular microbiological surveillance of healthcare workers' hands and the resources used for hand hygiene. Such an approach is essential to enhance

patient safety and limit the transmission of multidrug-resistant bacteria within university hospital centers in Benin.

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Contributions

Conceptualization: SESD and CCD; Methodology: SESD and CCD; Validation: CCD and HSB; Investigation and data collection: SESD, DAA, YRSB, CUD and LH; Laboratory sample analysis: SESD, CCD, and DAA; Statistical analyses: SESD, CCD and CUD; Data curation: SESD and CCD; Writing of the original version: SESD; Coursework and validation: CCD, DAA and HSB. All authors have read and approved the final version of the manuscript.

Declarations

Ethics Approval and Consent to Participate

The research protocol was approved by the Local Ethics Committee for Biomedical Research of the University of Parakou (CLERB-UP). Reference number: 564/2024/CLERB-UP/P/SP/R/SA.

Conflicts of Interest

The authors declare that there are no conflicts of interest related to this article.

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