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# Stability and Efficacy of Chlorinated Disinfectants in Beninese Hospitals: Issues for the Prevention and Control of Infections and Antibiotic Resistance

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Article

# Stability and Efficacy of Chlorinated Disinfectants in Beninese Hospitals: Issues for the Prevention and Control of Infections and Antibiotic Resistance

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## Abstract

In hospitals with limited resources, chlorine solutions are commonly used for biocleaning. The effectiveness of these solutions depends on the concentration of active chlorine and how they are prepared and stored. A study conducted in six University Hospitals in Benin from March 10 to July 11, 2025, aimed to evaluate the stability of active chlorine and the bactericidal efficacy of chlorine solutions used for disinfecting hospital environments. A total of 103 samples were analyzed using iodometric titration following AFNOR standard NF EN ISO 7393-3 (2000) and WHO recommendations. Bactericidal activity was tested on multi-resistant hospital strains using the germ carrier method based on standard NF T72-281. The study found that 88.4% of the solutions had inadequate active chlorine concentrations. Overall, the bactericidal efficacy was low at 14.6%, particularly ineffective against Gram-negative bacilli (79.6%) and Gram-positive cocci (84.5%). There was a significant association between compliance with active chlorine levels and bactericidal efficacy (OR = 42.5;  $p < 0.000001$ ). Factors contributing to inefficiency included storage without light protection, use of transparent containers, storage for more than two days, inadequate active chlorine concentration, and incorrect pH levels. These issues compromise hospital disinfection and contribute to the persistence of multi-resistant bacteria in the hospital environment.

**Keywords:** chlorine solutions; bactericidal efficacy; infection prevention and control; university hospitals; Benin

## 1. Background

Healthcare-associated infections (HAIs) are a significant global public health concern. They pose a threat to patient safety and contribute to higher rates of illness and death in hospitals. The World Health Organization (WHO) reports that 7-10% of patients in low- and middle-income countries develop HAIs during their hospital stay, a much higher rate than in developed countries [1]. In sub-Saharan Africa, the average prevalence of Healthcare-Associated Infections (HAIs) ranges from 12% to 15%, with higher rates observed in surgical, neonatal, and intensive care units [2,3]. Infections are often caused by lapses in infection prevention and control (IPC) practices, such as insufficient disinfection of surfaces and medical devices.

In many African settings, chlorinated solutions are commonly used as the primary disinfectant due to their broad antimicrobial coverage, affordability, and easy availability. Gallandat et al. (2020) showed that in resource-limited epidemic scenarios, chlorinated solutions are one of the most

accessible and effective agents against a variety of pathogens [4]. Similarly, a study in 2024 reported that it is possible to produce chlorinated solutions locally with minimal cost while respecting microbiological efficacy [5]. However, several studies have shown that the efficacy of chlorine solutions is highly dependent on active chlorine concentration, pH, preparation method, and storage conditions [6,7]. The chemical instability of sodium or calcium hypochlorite, accentuated by heat, light, and time, frequently leads to rapid loss of disinfectant activity, reducing their bactericidal and virucidal efficacy [8]. However, several studies have shown that their effectiveness is highly dependent on active chlorine concentration, pH, preparation, and storage conditions [6,7]. The chemical instability of sodium or calcium hypochlorite, accentuated by heat, light, and time, frequently leads to rapid loss of disinfectant activity, reducing their bactericidal and virucidal efficacy [8].

In Benin, despite improvements in infection prevention and control (IPC), healthcare-associated infections (HAIs) remain a significant challenge. Surveys conducted at University Hospitals (CHU) have revealed concerning rates of surgical site infections and neonatal infections, particularly at CNHU-HKM and CHUD-Borgou [9,10]. Additionally, multidrug-resistant bacteria, primarily Gram-negative bacilli like *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*, persist in the hospital environment in Benin [11]. These opportunistic pathogens are often found on surfaces, equipment, and water sources, indicating inadequate or irregular disinfection practices. The potential inefficacy of disinfectants may indirectly contribute to the selection and spread of multidrug-resistant strains through microbial adaptation mechanisms.

Studies have shown that repeated exposure to sublethal concentrations of chlorinated disinfectants can lead to the overexpression of Resistance-Nodulation-Division (RND) efflux pumps, resulting in cross-resistance to antibiotics [12,13]. Recent experiments have confirmed that insufficiently dosed sodium hypochlorite can trigger the production of reactive oxygen species and the development of adaptive mutations that lead to stable multidrug resistance in bacteria [14,15].

The effectiveness of chlorine solutions depends on the quality of the product and adherence to good preparation, labeling, and storage practices. The Africa CDC and WHO recommend monitoring active chlorine levels in hospitals to ensure continuous disinfection [16,17]. Stability and bactericidal efficacy of disinfectant solutions are crucial for assessing infection prevention and control measures.

This study aimed to evaluate the stability of active chlorine and the bactericidal efficacy of chlorine solutions used in Benin's University Hospitals and identify associated factors. It provides current data on compliance with practices and factors influencing disinfectant performance, guiding strategies to enhance infection prevention and control quality.

## 2. Materials and Methods

### 2.1. Study Design

The study was carried out in six University Hospital Centers (CHU) in Benin (CNHU-HKM, CHU-MEL, CHUD-Borgou, CHUD-Ouémé, CHUZ-Abomey-Calavi, and CHUZ-Sourou-Léré), spread from the south to the north of the country. It is part of an approach to assess the quality of chlorinated solutions used for biocleaning in healthcare departments, as part of infection prevention and control.

### 2.2. Study Design and Period

This study was a descriptive and analytical cross-sectional study conducted over four months from March 10 to July 11, 2025.

### 2.3. Study Population

The study focused on ready-to-use disinfectant solutions containing sodium or calcium hypochlorite for surface or medical device disinfection. Samples were collected at the start of the biocleaning process.

#### 2.4. Eligibility Criteria

The study included chlorine-based solutions used for disinfecting surfaces, equipment, or premises. This included solutions prepared locally or from diluted commercial products. All clinical departments that used chlorine-based disinfectant solutions at the time of the survey were eligible, including sites where chlorine solutions were produced or reconstituted within each facility.

This study does not include chlorinated solutions with unidentified chemical composition and those lacking minimal traceability

#### 2.5. Sampling Method

A non-probability sampling approach was employed, encompassing all clinical departments utilizing chlorine-based solutions and the production or reconstitution sites within each CHU. This method allowed for the thorough examination of practices related to the preparation, storage, and utilization of chlorine-based disinfectants.

#### 2.6. Data Collection

Chlorine solutions were collected in sterile opaque vials and transported in a cooler with frozen cold accumulators to the Public Health Laboratory of the Clinique Universitaire d'Hygiène Hospitalière at CNHU-HKM. A standardized collection form was used to document the origin, active ingredient, date of preparation, storage conditions, and traceability of each sample.

#### 2.7. Laboratory Analysis

##### 2.7.1. Physico-Chemical Analysis

###### pH Measurement

The initial pH of the chlorine solutions was measured directly in the field using a ProfiLine Multi 3320 SET 1 multiparameter handheld meter, fitted with the SenTix® 41 probe dedicated to pH measurement. The instrument was calibrated in accordance with the manufacturer's recommendations, guaranteeing reliable measurements. Two interpretation zones were defined according to the following classifications:

- pH < 8.5 ≥ 12: non-compliant, corresponding to an unstable zone where active chlorine degrades more rapidly.
- pH ≥ 8.5 < 12: compliant, corresponding to an optimum disinfectant efficiency zone.

###### Dosing of Active Chlorine

The active chlorine concentration of hypochlorite solutions was determined by iodometric titration, in accordance with AFNOR standard NF EN ISO 7393-3 (2000) [18] and WHO recommendations (2017) [19]. This method is based on the release of iodine in an acid medium, followed by titration with sodium thiosulfate and a starch indicator.

A reagent blank assay was carried out at the beginning of each series of manipulations under the same experimental conditions. Each determination was carried out in duplicate, and the active chlorine concentration was calculated according to the formula [18]:

$$\text{Active Cl} = \frac{N * V * MCl * 2}{V_{\text{sample}}}$$

where:

- N = normality of sodium thiosulfate (Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub>);
- V = volume of thiosulfate consumed;
- MCl = molar mass of chlorine;
- V sample = sample volume of chlorine solution used.

##### 2.7.2. Assessment of Bactericidal Activity

Bactericidal efficacy was assessed using the germ carrier method, inspired by AFNOR standard NF T72-281 [20]. Carriers were contaminated with hospital bacterial strains (*Klebsiella pneumoniae*, *Escherichia coli*, *Enterobacter cloacae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Enterococcus faecalis*), then treated with chlorinated solutions under real-life conditions.

After 30 minutes of contact, the germ carriers were swabbed, incubated in nutrient broth at 37°C for 24 to 48 hours, and then seeded on selective media.

Positive controls, sterility checks, and neutralization tests were systematically carried out to guarantee the reliability of the results.

## 2.8. Study Variables

### 2.8.1. Dependent Variables

Two dependent variables were selected to assess the quality of the chlorine solutions used:

#### ▪ **Active chlorine concentration**

This variable reflects the actual oxidizing agent content in each solution tested. It enables us to assess the compliance of the solutions with the thresholds required for effective disinfection. Active chlorine concentrations were therefore interpreted in relation to the WHO-recommended reference value of 0.5% for biocleaning of surfaces and medical devices. A tolerance margin of  $\pm 0.1\%$  was tolerated. Concentrations were characterized as follows:

- < 0.4%: Insufficient: non-compliant
- 0.4 - 0.6%: Adequate: compliant
- 0.6%: acceptable but must be monitored

#### ▪ **Bactericidal quality**

This variable defines the ability of chlorinated solutions to inactivate hospital bacterial strains under actual conditions of use. Its interpretation is based on the appearance of the broth and the presence or absence of bacterial growth:

- Clear broth and no growth: efficacy confirmed
- Cloudy broth and bacterial growth: bactericidal ineffectiveness

### 2.8.2. Independent Variables

The independent variables were chosen for their epidemiological significance and their ability to impact the effectiveness of chlorine solutions in hospital settings. These variables include the type of health facility, the specific hospital service, the kind of disinfectant, temperature, exposure to light, shelf life, container material, type of container, presence of a hermetic cap, and pH level.

## 2.9. Statistical Analyses

Statistical analysis was conducted using Epi Info™ version 7.2.6.0 and Microsoft Excel 2019 software. Data were checked for consistency and completeness.

Descriptive analysis was used to characterize chlorine solution samples based on their institutional origins, storage arrangements, physicochemical properties, and bactericidal efficacy. Qualitative variables were presented as frequencies and percentages, while quantitative variables were summarized using mean, standard deviation, median, quartiles, minimum, maximum, and mode.

Bivariate analysis was performed to explore associations between sample characteristics and two primary outcomes: non-compliance of active chlorine concentration and bactericidal ineffectiveness. Chi<sup>2</sup> or Fisher tests were used for categorical variables, and crude Odds Ratios (OR) with 95% confidence intervals (CI95) and p-values were calculated. Variables with a significant association ( $p < 0.05$ ) were included in multivariate analysis. Two logistic regression models were developed to identify factors independently associated with insufficient active chlorine concentration and bactericidal ineffectiveness. Variable selection was based on statistical significance, coefficient

stability, and epidemiological relevance. Results were presented as adjusted ORs with IC95 and p-values, with a significance threshold of 5%.

### 3. Results

Below are the results obtained, along with the corresponding statistical analyses, to emphasize the factors influencing the stability of active chlorine and the bactericidal efficacy of chlorine solutions used in the university hospitals of Benin

#### 3.1. Characteristics of Chlorine Solution Samples Collected

A total of 103 chlorine solution samples were collected from Benin's University Hospitals (CHU) and characterized. CNHU-HKM was the most represented with (20 samples; 19.4%), followed by CHUD-Ouémé (19 samples; 18.5%) and CHUD-Borgou (17 samples; 16.5%). These samples were taken in various clinical departments, notably: medicine (25.2%), followed by pediatrics (13.6%), maternity (12.6%), and neonatology (10.7%). Sodium hypochlorite is the most widely used type of chlorine solution (72.8%), while calcium hypochlorite accounts for 27.2%. 79.6% (82) of these solutions were produced by health facilities, and 20.4% (21) by external suppliers (Table 1).

**Table 1.** Characteristics of chlorinated water solution samples (n = 103).

Variables	Frequency	Percentage
<b>Hospitals</b>		
CNHU/CHU-MEL	36	35.0
CHUD_Borg/Ouémé	36	35.0
CHUZ_Ab-Calavi/Sou-Léré	31	30.1
<b>Hospital services</b>		
Technical platforms/interventions	32	31.1
General care/maintenance	33	32.0
Mother/child	38	36.9
<b>Type of disinfectant</b>		
Sodium hypochlorite	75	72.8
Calcium hypochlorite	28	27.2

#### 3.1.1. Extrinsic Characteristics of Chlorine Solution Samples

Table 2 shows that the majority of chlorine solutions used in Benin's university hospitals are exposed to light (72.8%), 67.96% are stored at unsuitable temperatures ( $\geq 25^{\circ}\text{C}$ ), and 76.7% are kept for inappropriate lengths of time ( $> 2$  days). Although most containers are fitted with hermetic caps (78.6%), almost half are made of transparent plastic.

**Table 2.** Storage and preservation characteristics of chlorinated solutions (n = 103).

Variables	Frequency	Percentage
<b>Store away from light.</b>		
Yes	28	27.2
No	75	72.8
<b>Temperature &lt; 25 °C</b>		
Yes	33	32.0
No	70	68.0
<b>Storage container</b>		
Seal	49	47.6
Can	54	52.4
<b>Container material</b>		
Transparent plastic	50	48.5

Opaque plastic	53	51.5
<b>Presence of an airtight cap</b>		
Yes	81	78.6
No	22	21.4
<b>Storage duration</b>		
Compliant	24	23.3
Not compliant	79	76.7

### 3.1.2. Intrinsic Characteristics of Chlorine Solution Samples

Analysis of the data reveals significant shortcomings in the physico-chemical and bactericidal quality of chlorine solutions used in Benin's university hospitals. Of the 103 samples studied, only 12 (11.7%) had a compliant active chlorine concentration ( $\geq 0.5\% \pm 0.1$ ), while 91 (88.4%) were insufficiently concentrated, resulting in a non-compliance prevalence rate of 88.4%. About solution pH, 38.8% complied (pH between 8.5 and 11) with recommended standards, compared with 61.2% non-compliant. Effective bactericidal quality was observed in 15 (14.7%) samples, while 88% of the bacterial strains tested were ineffective, resulting in a prevalence rate of non-effective bactericidal quality of 85.4% (Table 3).

**Table 3.** Physico-chemical and bactericidal characteristics of chlorine solution samples (n = 103).

Variables	Frequency	Percentage
<b>Active chlorine concentration</b>		
Adequate - compliant	12	11.7
Insufficient - non-compliant	91	88.4
<b>Solution pH</b>		
Compliant	40	38.8
Non-compliant	63	61.2
<b>Bactericidal quality</b>		
Effective	15	14.6
Non-effective	88	85.4

### 3.2. General Distribution of Active Chlorine Concentrations

The mean active ingredient concentration of the chlorine solutions analyzed was 0.1680%, with a standard deviation of 0.156. The minimum concentration observed was 0.0120%, while the maximum was 0.6000%. The median was 0.1000%, and the mode was 0.0130%.

Quartiles indicate that 25% of concentrations were below 0.0400%, while 75% remained below 0.2600%. The distribution was asymmetrical, with a significant spread towards the lower values.

### 3.3. Bactericidal Activity by Type of Chlorine Solution

Bacterial growth was observed in 85.4% of broth cultures. This trend was observed for both sodium hypochlorite (86.7%) and calcium hypochlorite (82.1%). Statistical analysis ( $\chi^2 = 0.0024$ ;  $p = 0.96$ ; OR = 0.97; 95% CI [0.28 - 3.34]) showed no significant difference between the efficacy of sodium hypochlorite and calcium hypochlorite solutions (Table 4).

**Table 4.** Efficiency rates by type of chlorine solution.

Type of disinfectant	Development of bacteria (%)		
	No	Yes	Total
Calcium hypochlorite	5 (17.9)	23 (82.1)	28 (27.2)
Sodium hypochlorite	10 (13.3)	65 (86.7)	75 (72.8)
<b>Total</b>	<b>15 (14.6)</b>	<b>88 (85.4)</b>	<b>103 (100.0)</b>

### 3.4. Efficacy of Chlorine Solutions on the Bacterial Groups Tested

The bactericidal efficacy of the chlorinated solutions measured was 20.4% on Gram-negative bacilli (GNB) and 15.5% on Gram-positive cocci (PGC) (Table 5).

**Table 5.** Bactericidal activity of chlorine solutions on the bacterial strains tested.

Disinfectant effect	Gram-negative bacilli		Gram-positive cocci	
	n	%	n	%
Effective	21	20,4	16	15,5
Ineffective	82	79,6	87	84,5

### 3.5. Factors Associated with Insufficient Active Chlorine Concentration

#### 3.5.1. Bivariate Analysis

Bivariate analysis showed that several basic sample characteristics were significantly associated with insufficient active chlorine concentration. Chlorinated solutions stored at a temperature  $\geq 25^{\circ}\text{C}$  (OR = 4.3; IC95% = [1.3-14.1]), exposed to light (OR = 6.2; IC95% = [1.7-22.4]), stored in transparent plastic containers (OR = 8.4; CI95% = [1.9-36.7]), with a storage duration  $> 2$  days (OR = 6.9; CI95% = [1.4-33.8]) and with a pH non-compliant present an increased risk of non-compliance. These variables all showed high Odds Ratios and significant p-values, confirming their role in the degradation of active chlorine.

Other variables such as health facility (CHU), hospital departments, type of chlorine solution, container labeling, type of storage container, and presence of a cap showed no statistically significant association ( $p > 0.05$ ) with the observed deficiency of active chlorine concentration in chlorine solutions (Table 6).

**Table 6.** Association between insufficient active chlorine concentration in chlorine solutions and basic sample characteristics.

Variables	Insufficient active chlorine concentration			
	Not compliant %	Crude OR	95 % CI	p-value
<b>Health facilities</b>				
CHUD_Borg/Ouémé	88.2 %	1.0	—	—
CNHU/CHU-MEL	94.6 %	2.1	0.6 – 7.3	0.239
CHUZ_Ab-Calavi/Sou-Léré	93.5 %	1.9	0.5 – 7.1	0.312
<b>Services</b>				
Labeling,	91.1 %	1.0	—	—
Mother and child	88.9 %	0.8	0.2 – 3.2	0.744
Technical platforms/interventions	94.4 %	1.7	0.4 – 7.3	0.459
<b>Type of disinfectant</b>				
Calcium hypochlorite	88.2 %	1.0	—	—
Sodium hypochlorite	93.3 %	1.9	0.5 – 6.9	0.319
<b>Storage protected from light</b>				
Yes	74.2 %	1.0	—	—
No	94.4 %	6.2	1.7 – 22.4	<b>0.006</b>
<b>Temperature <math>&lt; 25^{\circ}\text{C}</math></b>				
Yes	75.9	1.0	—	—
No	93.2	4.3	1.3 – 14.1	<b>0.015</b>
<b>Container correctly labeled</b>				
Yes	92.0	1.0	—	—
No	93.8	1.3	0.3 – 5.7	0.739
<b>Storage container</b>				

Can	91.7	1.0	—	—
Seal	93.3	1.2	0.3 – 5.0	0.803
<b>Container material</b>				
Opaque	77.3	1.0	—	—
Transparent	96.6	8.4	1.9 – 36.7	<b>0.003</b>
<b>Presence of hermetic cap</b>				
Yes	91.5	1.0	—	—
No	94.4	1.6	0.4 – 6.7	0.518
<b>Storage duration</b>				
Compliant	75.0	1	—	—
Non-compliant	95.1	6.9	1.4 – 33.8	<b>0.017</b>
<b>pH value</b>				
Compliant	66.7	1	—	—
Non-compliant	94.7	8.2	1.6 – 41.3	<b>0.011</b>

### 3.5.2. Multivariate Analysis

The results of the multivariate analysis, summarized in Table 7, show the five factors associated with insufficient active chlorine concentration. Storage not protected from light (OR = 6.2; IC95% = [1.7 - 22.4]), storage temperature  $\geq 25^{\circ}\text{C}$  (OR = 4.3; IC95% = [1.3 - 14.1]), use of transparent containers (OR = 8.4; IC95% = [1.9 - 36.7]), storage time > 2 days (OR = 6.9; IC95% = [1.4 - 33.8]), as well as non-compliant pH (OR = 8.2; IC95% = [1.6 - 41.3]) significantly increased the risk of non-compliance.

**Table 7.** Factors associated with insufficient active chlorine concentration.

Variables	Adjusted OR	95% CI	p-value
<b>Storage protected from light</b>			
Yes	1		
No	6.2	1.7 – 22.4	0.006
<b>Temperature &lt; 25°C</b>			
Yes	1		
No	4.3	1.3 – 14.1	0.015
<b>Container material</b>			
Opaque	1		
Transparent	8.4	1.9 – 36.7	0.003
<b>Storage duration</b>			
Compliant	1		
Non-compliant	6.9	1.4 – 33.8	0.017
<b>pH value</b>			
Compliant	1		
Non-compliant	8.2	1.6 – 41.3	0.011

### 3.6. Factors Associated with Bactericidal Ineffectiveness

#### 3.6.1. Bivariate Analysis

In bivariate analysis, five sample characteristics were significantly associated with ineffective bactericidal quality. Solutions stored without protection against light ( $p = 0.038$ ), in non-opaque containers ( $p = 0.025$ ), with inadequate storage time ( $p = 0.037$ ), inadequate pH ( $p = 0.036$ ), or insufficient active chlorine concentration ( $p = 0.011$ ) were at greater risk of bactericidal ineffectiveness. No statistically significant association was observed between institutional variables (university hospital, hospital department), type of disinfectant, type of storage container, presence of hermetic cap, container labeling, or storage temperature and the bactericidal quality of chlorine solutions ( $p > 0.05$ ) (Table 8).

**Table 8.** Bivariate analysis of factors associated with the bactericidal ineffectiveness of chlorine solutions.

Variables	Inefficacy (%)	OR brut	IC à 95 %	p-value
<b>Health facilities</b>				
CHUD_Borg/Ouémé	37.8	1	-	-
CNHU/CHU-MEL	33.3	0.8	0.3 – 2.3	0.697
CHUZ_Ab-Calavi/Sou-Léré	42.9	1.3	0.4 – 4.2	0.647
<b>Services</b>				
Labeling,	38.9	1	-	-
Mother and child	33.3	0.8	0.2 – 2.9	0.762
Technical platforms/interventions	41.7	1.1	0.3 – 4.2	0.878
<b>Type of disinfectant</b>				
Calcium hypochlorite	38.5	1	-	-
Sodium hypochlorite	37.5	1.0	0.4 – 2.6	0.964
<b>Storage protected from light</b>				
No	40.3	1	-	-
Yes	17.2	0.3	0.10 – 0.94	0.038
<b>Température &lt; 25°C</b>				
No	37.8	1	-	-
Yes	17.2	0.4	0.11 – 1.09	0.071
<b>Contenant opaque</b>				
No	41.0	1	-	-
Yes	16.7	0.3	0.10 – 0.86	0.025
<b>Storage duration</b>				
Non-compliant	38.5	1	-	-
Compliant	14.3	0.3	0.08 – 0.93	0.037
<b>Container correctly labeled</b>				
No	40.0	1	-	-
Yes	36.4	0.9	0.3 – 2.6	0.838
<b>Container type</b>				
Seal	40.0	1	-	-
Can	36.4	0.9	0.3 – 2.6	0.838
<b>Presence of an airtight cap</b>				
No	40.0	1	-	-
Yes	36.4	0.9	0.3 – 2.6	0.838
<b>pH interpretation</b>				
Non-compliant	41.7	1	-	-
Compliant	16.7	0.3	0.09 – 0.93	0.036
<b>Active chlorine concentration</b>				
Non-compliant	39.6	1	-	-
Compliant	8.3	0.1	0.03 – 0.63	0.011

### 3.6.2. Multivariate Logistic Analysis

At the end of the bivariate analysis, the five variables that showed a statistically significant association with the bactericidal ineffectiveness of chlorine solutions were: unprotected storage from light, use of non-opaque containers, non-compliant storage time, non-compliant pH, and insufficient active chlorine concentration. To identify the factors associated with this inefficiency, these variables were included in a multivariate logistic regression model. The multivariate analysis identified five factors associated with the ineffective bactericidal quality of chlorine solutions. Solutions stored without protection against light (OR = 4.6; IC95%= [1.2 - 17.9]), in transparent containers (OR = 5.1; IC95%= [1.3 - 20.2]), with inadequate storage time (OR = 4.4; ; IC95%= [1.1 - 17.2]), non-compliant pH (OR = 4.7; ; IC95%= [1.2 - 18.3]) and insufficient active chlorine concentration (OR = 7.2; ; IC95%= [1.5 - 34.1]) had a significantly increased risk of bactericidal ineffectiveness.

**Table 9.** Factors associated with ineffective bactericidal quality of chlorine solutions.

Variables	Adjusted OR	IC 95 %	p-value
<b>Storage protected from light</b>			
Yes	1		
No	4.6	1.2 – 17.9	0.026
<b>Container material</b>			
Opaque	1		
Transparent	5.1	1.3 – 20.2	0.021
<b>Storage duration</b>			
Compliant	1		
Non-compliant	4.4	1.1 – 17.2	0.035
<b>pH value</b>			
Compliant	1		
Non-compliant	4.7	1.2 – 18.3	0.030
<b>Active chlorine concentration</b>			
Compliant	1		
Non-compliant	7.2	1.5 – 34.1	0.011

#### 4. Discussion

A study conducted in six University Hospitals (CHU) in Benin found significant deficiencies in the quality of chlorine solutions used for hospital cleaning. Out of 103 samples analyzed, only 11.7% had the correct active chlorine concentration, and 14.7% showed satisfactory bactericidal efficacy. These results indicate a high prevalence of non-compliance, with 88.4% failing physico-chemical standards and 85.4% failing microbiological standards, highlighting concerns about healthcare safety.

The study found that a concerning 88.4% of disinfectants in hospitals had insufficient active chlorine content. This raises significant concerns about the quality of biocleaning practices. Inadequate chlorine levels not only render the disinfectants ineffective but also promote the survival of bacteria in sublethal conditions. This can lead to the development of adaptive mechanisms that result in bacterial tolerance and multi-resistance.

Repeated or prolonged exposure of microorganisms to low doses of chlorinated disinfectants, such as sodium hypochlorite, can cause the development of adaptive mechanisms that may result in cross-resistance to antibiotics. In a study by Nam et al. (2024), it was demonstrated that exposure to low doses of sodium hypochlorite can cause an increase in the expression of RND (Resistance-Nodulation-Division) efflux pumps in *Pseudomonas aeruginosa*. This overexpression results in decreased susceptibility to imipenem and other  $\beta$ -lactam antibiotics [12]. Efflux pumps, which are linked to reduced membrane permeability, are a crucial mechanism for biocide tolerance and contribute to the spread of multidrug-resistant bacteria [15]. Similarly, Aljuwayd et al. (2024) demonstrated that sublethal exposure to chlorine causes increased production of reactive oxygen species (ROS), leading to adaptive genetic mutations and secondary antibiotic resistance in *Salmonella* [14]. Wu-Chen et al. (2023) also confirm that prolonged exposure to food-grade disinfectants promotes stable cross-resistance to several classes of antibiotics, including fluoroquinolones and  $\beta$ -lactams [21].

These observations support Pereira and Tagkopoulos' (2021) synthesis, indicating that inadequately dosed biocides can create selective pressure similar to antibiotics. This pressure can trigger bacterial adaptive responses, including the activation of efflux systems, modification of intracellular targets, and DNA repair mechanisms [13]. Improper use or insufficient application of disinfectants in hospitals may undermine the efficacy of biocleaning and ultimately promote the development and persistence of multidrug-resistant strains in the hospital setting.

Our findings indicate that while chlorine solution remains the predominant disinfectant in university hospitals, its efficacy is highly dependent on how it is prepared and applied. The

connection between inadequate disinfection and bacterial resistance is indirect but well-documented: using insufficient disinfectant can create a selective environment that promotes the development of more resistant bacterial strains.

Several factors contribute to these non-conformities, including exposure to light, storage temperature of 25°C or higher, use of transparent containers, prolonged storage exceeding 2 days, and non-compliant pH levels. These factors were found to be statistically associated with the degradation of active chlorine and reduced bactericidal effectiveness. This highlights the importance of proper storage conditions in maintaining the stability and efficacy of disinfectant solutions. Similar findings have been reported in hospitals in Uganda, Tanzania, and Nigeria, where non-compliance rates ranging from 60% to 90% have been observed [7,22,23].

A multivariate analysis identified several factors linked to insufficient active chlorine concentration in chlorine solutions used in Benin University hospitals. These factors included inadequate light protection during storage, high storage temperatures ( $\geq 25^\circ\text{C}$ ), use of transparent containers, prolonged storage times ( $> 2$  days), and incorrect pH levels. All of these variables were found to be statistically significant, highlighting the influence of storage conditions and physico-chemical properties on active chlorine stability. These findings are consistent with previous studies that have demonstrated the rapid degradation of active chlorine due to photodegradation and oxidation of hypochlorite [6,24,25]. In the tropical conditions of Benin, high temperatures and frequent exposure to light exacerbate these phenomena, reducing the active life of disinfectant solutions [26]. In hot and humid conditions like those found in Benin's university hospitals, ready-to-use solutions should not be stored for more than 24 hours, following WHO guidelines.

The pH imbalance in some solutions is a key factor in their instability. Research indicates that sodium hypochlorite breaks down faster when the pH is below 8.5 or above 12, resulting in a rapid decline in active chlorine and the creation of less effective by-products [28]. To extend the stability and bactericidal effectiveness of chlorine solutions, it is essential to adjust the pH and carefully manage storage conditions [26].

Out of the solutions tested, only 14.6% showed satisfactory bactericidal efficacy based on the WHO's defined thresholds. This finding is similar to a study conducted in Uganda in 2024 [7], where most locally-prepared solutions lost their microbicidal activity after a few days of storage.

A strong correlation was found between the concentration of active chlorine and the effectiveness of chlorinated solutions in killing bacteria ( $p < 0.001$ ).

Solutions with sufficient active chlorine concentration showed a bactericidal efficacy of 75%, while those with insufficient concentration only had a 6.6% efficacy. The Odds Ratio (OR) of 42.5 with a 95% confidence interval [9.05-199.6] indicates that compliant solutions were over 40 times more likely to be bactericidal compared to non-compliant ones. These findings underscore the importance of maintaining the right level of active chlorine in disinfectant solutions, aligning with recommendations from the WHO and CDC for regular monitoring of concentrations to ensure optimal performance of oxidizing disinfectants. Fabrizio et al. (2024) demonstrated that concentrations below 0.5% of sodium hypochlorite are ineffective against biofilms of *P. aeruginosa* and *A. baumannii*, with a significant decrease in bactericidal activity below 0.4% [29].

Similarly, String et al. (2020) showed that an improperly dosed chlorine solution can lose up to 90% of its effectiveness on contaminated surfaces. This is especially true in cases of excessive dilution or prolonged storage [30].

The study findings reveal issues that extend beyond just the technical aspects of chlorine solution quality. In Benin's University Hospitals, the main obstacle to infection prevention and control (IPC) is not just the availability of equipment but also the need for strict adherence to protocols, a culture of accountability, and raising awareness among all staff members.

The study found that 85.44% of the chlorine solutions analyzed were not effective at killing bacteria, posing a significant risk of incomplete disinfection of surfaces and medical devices. This could potentially lead to the development of multi-resistant bacteria. In Benin, there is a high prevalence of healthcare-associated infections (HAIs) in referral hospitals. A national survey

conducted by Ahoyo et al. in 2014 reported an overall HAI prevalence of 19.1%, with urinary, pulmonary, and surgical site infections being the most common types [10].

A study conducted at CNHU-HKM in Cotonou by Dégbey et al. (2021) found a 7.81% prevalence of surgical site infections, which was closely associated with sepsis and disinfection practices [9].

Several African studies have highlighted the importance of biocleaning in preventing neonatal infections. For instance, a multicenter study in sub-Saharan Africa by Nakibuuka et al. (2025) found that confirmed neonatal infection rates were between 28% and 35%, mainly due to insufficient cleaning practices and the use of improperly disinfected shared equipment [31]. A study conducted in nine public hospitals in Benin found that standard hospital hygiene precautions are not adequately followed, leading to an increase in neonatal infections, especially in intensive care units [32]. These results highlight the critical importance of strengthening disinfection protocols and providing staff training in these departments. The data, along with our findings, suggest that the ineffectiveness of disinfectants due to chemical and microbiological non-conformity may contribute to the rise of multi-resistant bacteria and the persistence of hospital-acquired infections. This means that hospitals can unknowingly facilitate the spread of diseases when disinfection products fail to work correctly.

While these results are significant, it is essential to acknowledge some methodological limitations and recognize the strengths of this study.

This study is the first national evaluation of the stability and effectiveness of chlorine solutions in Benin's university hospitals. It followed a standardized methodology, including WHO-recommended tests, and was conducted in six representative university hospitals. This comprehensive approach offers an unbiased assessment of disinfectant quality in Benin's healthcare facilities. Additionally, the rigorous statistical analysis identified key factors associated with non-compliance, providing valuable insights for national infection prevention and control strategies.

However, there are limitations to consider for a comprehensive interpretation of the findings. The study was cross-sectional so that it couldn't track the degradation of active chlorine over time. Storage conditions varied among hospitals, and organizational factors like staff training and supervision were not explored. Lastly, while the study demonstrated a loss of bactericidal efficacy, its direct impact on healthcare-associated infections and bacterial resistance was not assessed.

## 5. Conclusions

This study in Benin's hospitals was the first to assess the stability and bactericidal efficacy of chlorine solutions nationwide. The research revealed significant variations in active chlorine concentration, directly impacting the effectiveness of disinfection. Exposure to sublethal doses of chlorine can lead to the development of adaptive mechanisms in microorganisms, such as increased efflux pump expression, which can contribute to bacterial multi-resistance. These findings emphasize the importance of monitoring active chlorine levels regularly and implementing better practices for preparing, storing, and using disinfectants in hospitals. This is essential to prevent a decrease in disinfection effectiveness and the potential emergence of antimicrobial cross-resistance.

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## Abbreviations

The following abbreviations are used in this manuscript (alphabetically ordered):

AFNOR : Association Française de Normalisation (French Standardization Association)

BMR : Bacteria multirésistantes (Multidrug-Resistant Bacteria)

BGN : Bacilles à Gram négatif (Gram-Negative Bacilli)

CDC : Centers for Disease Control and Prevention

CHL : Chlorine (abbreviation occasionally used in formulas)

CHU : Centre Hospitalier Universitaire (University Teaching Hospital)

CHUD : Centre Hospitalier Universitaire Départemental (Departmental University Teaching Hospital)

CHU-MEL : Centre Hospitalier Universitaire de la Mère et de l'Enfant Lagune (Lagoon Mother and Child University Hospital Center)

CHUZ : Centre Hospitalier Universitaire de Zone (Zonal University Teaching Hospital)

CNHU-HKM : Centre National Hospitalier Universitaire Hubert Koutoukou Maga (Hubert Koutoukou Maga National University Hospital Center)

CLERB-UP : Comité Local d'Éthique pour la Recherche Biomédicale de l'Université de Parakou (Local Ethics Committee for Biomedical Research of the University of Parakou)

CGP : Cocci à Gram positif (Gram-Positive Cocci)

Epi Info™ : Epidemiological analysis software developed by the CDC

HAI : Healthcare-Associated Infection (Infections associées aux soins, IAS)

IC : Confidence Interval

IC95% : 95% Confidence Interval

IPC : Infection Prevention and Control (Prévention et Contrôle des Infections)

NaOCl : Sodium Hypochlorite

NF EN ISO : Norme Française / Européenne / Internationale d'Organisation de Normalisation (French/European/International Standardization Standard)

OR : Odds Ratio

pH : Hydrogen Potential

RR : Risk Ratio

T72-281 : AFNOR Standard related to airborne disinfection processes

WHO : World Health Organization (Organisation mondiale de la santé)

## References

1. World Health Organization (WHO). *Global Report on the Burden of Healthcare-Associated Infections Worldwide*; WHO: Geneva, Switzerland, 2023. Available online: <https://www.who.int/publications/i/item/9789240073715> (accessed on 20 July 2025)
2. Abubakar, U.; Amir, O.; Rodríguez-Baño, J. Healthcare-associated infections in Africa: A systematic review and meta-analysis. *J. Pharm. Policy Pract.* 2022, *15*, 99. <https://doi.org/10.1186/s40545-022-00500-5>

3. Melariri, H.; Freercks, R.; van der Merwe, E.; et al. The burden of hospital-acquired infections in sub-Saharan Africa: A systematic review and meta-analysis. *eClinicalMedicine* 2024, 71, 102571. <https://doi.org/10.1016/j.eclinm.2024.102571>
4. Gallandat, K.; Kolus, R.C.; Julian, T.R.; Lantagne, D.S. A systematic review of chlorine-based surface disinfection efficacy to inform recommendations for low-resource outbreak settings. *Am. J. Infect. Control* 2021, 49(1), 90–103. <https://doi.org/10.1016/j.ajic.2020.05.014>
5. Naranjo-Soledad, A.; Smesrud, L.; Bandaru, S.R.S.; et al. Low-cost, local production of a safe and effective disinfectant for resource-constrained communities. *PLOS Glob. Public Health* 2024, 4(6), e0002213. <https://doi.org/10.1371/journal.pgph.0002213>
6. Gow, C.K.; Weinhouse, C.; O'Brien Johnson, G.; et al. Stability of free available chlorine levels in dilute sodium hypochlorite solutions over a six-week period. *J. Am. Assoc. Lab. Anim. Sci.* 2022, 61(2), 181–187. <https://doi.org/10.30802/AALAS-JAALAS-21-000080>
7. Drolet, A.; Mugumya, T.; Hsu, S.; et al. Performance and acceptability of the STREAM Disinfectant Generator for IPC practices in Ugandan primary health care facilities. *Antimicrob. Resist. Infect. Control* 2024, 13, 77. <https://doi.org/10.1186/s13756-024-01433-1>
8. Brown, L.; Marshall, A.; Conway, L.; Otter, J.; Norville, P.; Clarke, J. Assessing the stability and sporicidal efficacy of oxidizing disinfectants. *J. Hosp. Infect.* 2024, 149, 22–25. <https://doi.org/10.1016/j.jhin.2024.04.010>
9. Dégbey, C.; et al. Prevalence and factors associated with surgical site infections in CNHU-HKM, Benin. *Front. Public Health* 2021, 9, 629351. <https://doi.org/10.3389/fpubh.2021.629351>
10. Ahoyo, T.A.; Bankolé, H.S.; et al. Prevalence of nosocomial infections and anti-infective therapy in Benin hospitals. *Antimicrob. Resist. Infect. Control* 2014, 3, 17. <https://doi.org/10.1186/2047-2994-3-17>
11. Dougnon, T.V.; et al. Environmental contamination by Gram-negative bacilli in Benin's university hospitals. *Microorganisms* 2023, 11(3), 617. <https://doi.org/10.3390/microorganisms11030617>
12. Nam, J.-H.; Yoo, J.-S.; Lee, S.Y.; Kwon, H.J.; et al. Sublethal sodium hypochlorite exposure: Impact on Resistance-Nodulation-Cell Division efflux pump overexpression and cross-resistance to imipenem. *Antibiotics* 2024, 13(9), 828. <https://doi.org/10.3390/antibiotics13090828>
13. Pereira, B.M.P.; Tagkopoulos, I. Biocide-induced emergence of antibiotic resistance in bacteria: A review. *Front. Microbiol.* 2021, 12, 640923. <https://doi.org/10.3389/fmicb.2021.640923>
14. Aljuwayd, M.; Malli, I.A.; Ricke, S.C.; Kwon, Y.M. A sublethal concentration of chlorine induces antibiotic resistance in *Salmonella* via production of reactive oxygen species. *Appl. Microbiol.* 2024, 4(2), 745–752. <https://doi.org/10.3390/applmicrobiol4020051>
15. Maillard, J.Y. Resistance of bacteria to biocides: Active efflux and reduced uptake mechanisms. *Pathogens* 2018, 7(1), 8. <https://doi.org/10.3390/pathogens7010008>
16. Centers for Disease Control and Prevention (CDC). *Best Practices for Environmental Cleaning and Disinfection in Healthcare Settings*; CDC: Atlanta, GA, USA, 2023. Available online: <https://www.cdc.gov/infectioncontrol/guidelines/environmental/index.html> (accessed on 15 March 2025)
17. World Health Organization (WHO). *Implementation Framework for IPC Programmes in Health Facilities*; WHO: Geneva, Switzerland, 2023. Available online: <https://www.who.int/publications/i/item/9789240051164> (accessed on 15 March 2025)
18. Association Française de Normalisation (AFNOR). *NF EN ISO 7393-3:2000 – Water Quality – Determination of Free and Total Chlorine – Part 3: Iodometric Titration Method for Total Chlorine Determination*; AFNOR: Paris, France, 2000. Available online: <https://www.boutique.afnor.org> (accessed on 15 March 2025)
19. World Health Organization (WHO). *Guidelines for Drinking-Water Quality*, 4th ed.; WHO Press: Geneva, Switzerland, 2017.
20. Association Française de Normalisation (AFNOR). *NF T72-281:2014 – Airborne Surface Disinfection Processes – Determination of Bactericidal, Fungicidal, Yeasticidal, Mycobactericidal, Tuberculocidal, Sporicidal and Virucidal Activity*; AFNOR: Paris, France, 2014. Available online: <https://www.boutique.afnor.org/fr-fr/norme/nf-t72281> (accessed on 27 March 2025)
21. Wu-Chen, R.A.; Feng, J.; Elhadidy, M.; Nambiar, R.B.; Liao, X.; Yue, M.; Ding, T. Long-term exposure to food-grade disinfectants causes cross-resistance to antibiotics in *Salmonella enterica* serovar Typhimurium strains. *Antimicrob. Resist. Infect. Control* 2023, 12, 145. <https://doi.org/10.1186/s13756-023-01333-w>

22. Mussa, R. Assessment of environmental cleaning practices and their impacts on infection prevention and control in selected hospitals in Tanzania. *J. Environ. Prot.* 2022, 13, 1045. <https://doi.org/10.4236/jep.2022.1310045>
23. Gon, G.; et al. A better disinfectant for low-resourced hospitals? A multi-period cluster randomised trial comparing hypochlorous acid with sodium hypochlorite in Nigerian hospitals: The EWASH trial. *Microorganisms* 2022, 10(5), 910. <https://doi.org/10.3390/microorganisms10050910>
24. Chaúque, B.J.M.; Rott, M.B. Photolysis of sodium chloride and sodium hypochlorite by ultraviolet light inactivates trophozoites and cysts of *Acanthamoeba castellanii* in the water matrix. *J. Water Health* 2021, 19(1), 190–202. <https://doi.org/10.2166/wh.2020.401>
25. Ahmad, S.; Almeahadi, M.; Janjuhah, H.T.; et al. The effect of mineral ions present in tap water on photodegradation of organic pollutants: Future perspectives. *Water* 2023, 15(1), 175. <https://doi.org/10.3390/w15010175>
26. Brown, L.; et al. Assessing the stability and sporicidal efficacy of oxidizing disinfectants. *J. Hosp. Infect.* 2024, 137, 30–38. <https://doi.org/10.1016/j.jhin.2024.04.010>
27. World Health Organization (WHO). *Cleaning and Disinfection of Environmental Surfaces in Healthcare Settings: Guidance on Chlorine-Based Solutions*; WHO: Geneva, Switzerland, 2023.
28. Sil, T.; Malyshev, D.; Aspholm, M.; et al. Improvement of disinfectant power of hypochlorite by pH modulation. *BMC Microbiol.* 2025, 25, 101. <https://doi.org/10.1186/s12866-025-03831-w>
29. Fabrizio, G.; Sivori, F.; Cavallo, I.; et al. Efficacy of sodium hypochlorite in overcoming antimicrobial resistance and eradicating biofilms in clinical pathogens from pressure ulcers. *Front. Microbiol.* 2024, 15, 1432883. <https://doi.org/10.3389/fmicb.2024.1432883>
30. String, G.M.; Vargas Gutiérrez, E.; Lantagne, D.S. Laboratory efficacy of surface disinfection using chlorine against *Vibrio cholerae*. *J. Water Health* 2020, 18(6), 1009–1019. <https://doi.org/10.2166/wh.2020.199>
31. Nakibuuka, V.; Nampijja, J.; Ajigbotosho, S.O.; et al. Resources to support infection prevention and control in African neonatal units. *J. Afr. Neonatol.* 2025, 3(3), 78–84. Available online: <https://janeonatology.org/index.php/jan/article/view/177> (accessed on 15 October 2025)
32. Bello, C.; Dégbey, C.C.; Baba-Moussa, L. Observance des précautions standards en hygiène hospitalière dans les services de pédiatrie au Bénin. *Rev. Bén. Mal. Infect.* 2024, 3(2), 52. <https://doi.org/10.70699/rev.ben.mal.inf.v3i2.52>

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