Quality and Surface Cleaning Efficacy of Sodium Hypochlorite Products Used in Health Facilities of Jimma Town, Oromia Regional State, Ethiopia

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Faculty of Health Science

School of Pharmacy

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Declaration

This is to certify that Gadisa Chala's thesis entitled "Quality and Surface Cleaning Efficacy of Sodium Hypochlorite Products Used in Health Facilities of Jimma Town, Oromia Reginal State, Ethiopia," has been submitted in partial fulfillment of the requirements for the Degree of Master of Science in Pharmaceutical Quality Assurance and Regulatory Affairs, complies with the Jimma University regulations and meets the standards in terms of originality and quality. All sources of materials used to develop the thesis have been dully acknowledged. Further, I confirm that the part or full contents of the thesis has not been submitted to other higher institutions to earn a similar degree award.

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Abstract

Background: The broader use of sodium hypochlorite in microorganisms control has been challenged by the commercial availability of low-quality products. The problem is more prominent in developing countries where disinfectant regulations are less stringent. Thus, this study aims to determine the quality and surface cleaning efficacy of sodium hypochlorite products used in health facilities of Jimma Town, Oromia Regional State, Ethiopia.

Methods and Materials: A survey-based study was conducted in Jimma town from September 1 to October 31, 2021. Twenty-seven samples were collected randomly from all health facilities based on a joint WHO/FAO guidelines. The samples were categorized into six brands. The chief executive officers of the healthcare facility were informed that the samples are intended only for research purpose. The physicochemical quality and surface cleaning efficacy of the brands were determined using the US Pharmacopeia and EPA standard methods, respectively.

Result: Only one brand (SH 01) out of six had the storage instructions, 'keep in a cool, dry area away from direct sunshine and heat.' Instead of bleaching the red litmus paper, the SH 04 brand turned it blue. Most of the brands differed from the label's claimed contents by a significant percentage (59.6 on average, P = 0.0001). The SH 01 brand had the highest chlorine content (4.64% ± 0.09%), while the SH 05 brand had the lowest (1.09% ± 0.09%). The absence of chlorine was confirmed in the SH 04 brand (0.12% ± 0.02%). Five of the six brands were of poor quality. Low chlorine content was shown to have a weak relationship with pH (r = 0.43, P = 0.025), as well as storage period (r = -0.398, P = 0.040). The mean log reductions (LRs) in *P. aeruginosa* ($LR_{SH\ 01}$ = 4.13, $LR_{SH\ 05}$ =3.17, and p = 0.008) and *S. aureus* ($LR_{SH\ 01}$ = 4.26, $LR_{SH\ 05}$ =3.47, and p = 0.009) varied significantly across the SH 01 and SH 05 brands.

Conclusion and Recommendations: Five of the six brands evaluated were of poor quality. The lowest quality brand was ineffectual at controlling microorganisms. Hence, treating healthcare-associated infections with antibiotics becomes significantly more challenging. Regular manufacturer inspections and a large-scale quality and efficacy evaluation of sodium hypochlorite products are recommended.

Keywords: Sodium hypochlorite, Quality, Surface cleaning efficacy, Assessment, Jimma Town, Ethiopia

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Acronyms and Abbreviations

EFDA	Ethiopian Food and Drug Authority
EPA	Environmental Protection Agency
g/v	Gravitational force
HAIs	Healthcare-associated infections
ICAN	Infection Control Africa Network
NaOCl	Sodium hypochlorite
OECD	Organization for Economic Co-operation and Development
TSA	Tryptic soy agar
TSB	Tryptic soya broth
w/v	Weight by volume

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1. Introduction

1.1. Background

Sodium hypochlorite$_{(NaOCl)}$ products are broadly used in resource-constrained settings to control different microorganisms on healthcare surfaces and floors(1,2). Nevertheless, the broader use of sodium hypochlorite in microorganisms control has been challenged by the commercial availability of low-quality products and inherent chemical instability. The problem is more prominent in developing countries where disinfectant regulations are less stringent(3,4).

In recent years, environmental cleaning, particularly of surfaces in the patient zone, has been recognized as a critical intervention in infection prevention and control (IPC)(5). However, several microorganisms were isolated from the healthcare surface treated with sodium hypochlorite products containing insufficient chlorine(6). The survival and isolation of these microorganisms have intensified the transmission of healthcare-associated infections (HAIs)(7). Notably, the transmission of HAIs in resource-limited countries would have devastating consequences considering the health infrastructure, low-quality sodium hypochlorite commercial availability, and overall resource constraints observed in such countries(8–11).

The treatment of HAIs with currently available antimicrobials is more challenging and, in some cases, impossible. These infections increase mortality and morbidity, which raises healthcare costs significantly(12–15). Hence, the quality and efficacy of sodium hypochlorite products that are routinely used should be assessed(16,17).

In Ethiopia, the national infection prevention guidelines recommend using 0.5 % (w/v) sodium hypochlorite products on healthcare surfaces(18). These products are obtained from the Ethiopian Pharmaceutical Supply Agency (EPSA) and commercially available bleaches. The Ethiopian Food and Drug Authority (EFDA) is mandated to register antiseptics and disinfectants and undertake regulatory assessments(19). However, according to a recent study, antiseptics sold in Addis Ababa fail to meet the regulatory requirements(20). Furthermore, the survival of resistant microorganisms on healthcare surfaces has intensified healthcare-associated infections (HAIs) in Ethiopia(21,22).

A 2016 study evaluated the microbiological features and resistance profiles of bacterial pathogens of HAIs at Jimma University Medical Center (JUMC). The overall prevalence of HAIs at the hospital was 19.41%.(23). Despite the prevalence of HAIs in JUMC, no one had assessed the quality and efficacy of commonly used disinfection products. Hence, it is necessary to ensure that the sodium hypochlorite products used in the health facilities of Jimma Town are of standard quality and efficacy.

1.2. Statement of the problem

Chlorine, which can exist in two forms in solution depending on temperature and pH: hypochlorite ion$_{(OCl^-)}$ and hypochlorous acid$_{(HOCl)}$, is the active ingredient in sodium hypochlorite products(24,25). Both forms of chlorine are essential for successfully cleaning healthcare surfaces(26). In resource-constrained healthcare settings, commercially available bleaches are a primary source of sodium hypochlorite products(7). However, the chlorine content in commercially available bleaches varied significantly, with most samples containing less available chlorine than the label claimed(8–10,24).

According to a study conducted in the Netherlands, out of 84 samples of sodium hypochlorite, 90% contained less chlorine than the label claimed, indicating low-quality sodium hypochlorite products(24). Like in the Netherlands study, the chlorine content of 32 bleaches collected from 12 developing countries, including Ethiopia, varied by 35% from the label claimed contents(8). Furthermore, 85% of the studied samples of commercially available bleaches contained less than 5.5% w/v chlorine content, the Libyan standard limit for sodium hypochlorite(9). Therefore, ensuring that the sodium hypochlorite products used in health facilities meet quality standards is necessary.

Likewise, several microorganisms were isolated from the healthcare surface treated with sodium hypochlorite products containing insufficient chlorine, showing ineffectual products(13). Furthermore, according to a study conducted in Egypt, sodium hypochlorite products at lower-than-optimal concentrations resulted in the development of antimicrobial-resistant (AMR) microorganisms(15). As a result, routine healthcare surface cleaning with a random chlorine content is ineffectual in controlling microorganisms, necessitating the evaluation of sodium hypochlorite product efficacy(27).

Low-quality and ineffective sodium hypochlorite products are the result of several factors. However, the main factor is the inherent chemical instability of sodium hypochlorite products, which results in chlorine degradation(28). The degradation kinetics are influenced by pH, hypochlorite content, temperature, presence of trace elements, and light exposure(29,30). As a result of these factors not being adequately monitored, the chlorine content, quality, and efficacy may have been significantly reduced(12,25,26,31).

Although frequent quality reviews are recommended for products with stability issues, many national regulatory authorities prioritize pharmaceutical quality over disinfectant quality, especially those in developing countries (8,20). In addition, quality control systems and strict manufacturing standards are rarely implemented to ensure sodium hypochlorite product quality and efficacy(8). Subsequently, commercially available bleaches are either unregulated or subject to less strict regulations. As a result, substandard products might be present on the market(8,20).

Due to low-quality and ineffective sodium hypochlorite products, microorganism control in health facilities remains difficult(32). In addition, resistance to antibiotics and chemical disinfectants is becoming a significant impediment in the fight against multidrug-resistant (MDR) infections, i.e., healthcare-associated infections (HAIs)(6,33). HAIs, in turn, cost the patient, the healthcare system, and the extra government resources(4,5).

According to a 2009 study, the chlorine content of commercially available bleaches in Ethiopia differed by 22% from the label claimed content(8). Like in other developing countries, the Ethiopian food and drug administration (EFDA) prioritizes pharmaceutical quality over disinfectant quality(19). In addition, according to a recent study, antiseptics sold in Addis Ababa failed to meet the regulatory requirements. These findings could reflect that disinfectants like sodium hypochlorite products are subject to less strict regulations as antiseptics in Ethiopia(20).

Even though healthcare-associated infections (HAIs) were prevalent at Jimma University Medical Center in a 2016 study, no one had evaluated the quality and efficacy of commonly used disinfection products in the Jimma area(23). Therefore, it is necessary to ensure that the sodium hypochlorite products used in the health facilities of Jimma Town are of the required quality and efficacy standards. The research questions below were employed to design the appropriate methodology for this study.

1. Is there a quality problem with the sodium hypochlorite products used in the health facilities of Jimma Town?
2. Are the sodium hypochlorite products used in the health facilities of Jimma Town efficacious against microorganisms?

1.3. Significance of study

The findings of this study are expected to be used as preliminary information by the regulatory authorities in the quality assurance of disinfectant products. The health facilities use disinfectant solutions whose quality and efficacy have been evaluated and guaranteed. As a result, disinfectant solutions of guaranteed quality and effectiveness improve health facilities' infection prevention and control (IPC) practices. Patients and society are no longer at risk of acquiring HAIs.

Furthermore, substandard disinfectant products in circulation can only be detected through routine post-market surveillance. As a result, the regulatory authorities and health facilities will use the findings of this study to take early action if substandard disinfectant products are reported. The current study's findings could also be used as feedback for suppliers and manufacturers on the quality and efficacy of the disinfectant.

Last but not least, this research will benefit Jimma University by ensuring the quality and surface cleaning efficacy of sodium hypochlorite products. This will contribute to resolving the community's problem, as HAIs are a primary public concern. It could also serve as a starting point for future researchers interested in investigating the quality and efficacy of sodium hypochlorite products.

2. Literature Review

2.1. Quality of sodium hypochlorite

Independent of its eventual purpose, sodium hypochlorite is produced by the electrolysis of a sodium chloride solution. The electrolysis process yields 15% w/v sodium hypochlorite products with a pH of 12–14, subsequently diluted with deionized water to produce the finished product for use in health facilities(17).

The chlorine content, an essential quality indicator parameter(26), varied significantly in commercially available bleaches. Most samples evaluated for quality had less chlorine than the label claimed(8,9,24). According to a study by Van der Waal *et al*(24) on the quality evaluation of sodium hypochlorite products, the contents of chlorine in 84 samples collected from dental clinics across the Netherlands differed by 27.2% from the label claimed contents ($P<0.001$). In addition, out of 84 samples, 90% contained less chlorine than the label claimed, indicating low-quality sodium hypochlorite products(24).

Similarly, Lantagne(8) reported that the chlorine content of 32 bleaches collected from 12 developing countries, including Ethiopia, varied by 35% from the label claimed contents ($P\leq001$). According to these findings, there is more variation than the value reported by the Netherlands study. On the other hand, Ahmida *et al*(9) determined the chlorine contents in 20 samples of bleach products purchased at random from Benghazi markets, Libya. They reported that 17 bleach products (or 85% of the investigated samples) contained less than 5.5% w/v, the Libyan standard limit for sodium hypochlorite.

Likewise, sodium hypochlorite products are inherently unstable, decomposing into chlorate (ClO_3^-) and chloride (Cl^-) ions depending on pH, hypochlorite content, temperature, and light exposure(28–30). According to Jewson(34), the stability of chlorine in sodium hypochlorite products was directly related to the pH of the solution, with pH seven being the most unstable. In addition, the stability of chlorine was related to hypochlorite content, with the 8% (w/v) solution being the most unstable among the 1%, 2.5%, 5.25%, and 8% sodium hypochlorite solution concentrations(29). Further, Dash *et al*(25) reported that a sodium hypochlorite solution subjected to a higher temperature reduced the chlorine content by 58.33% compared to the baseline value.

Finally, containers that did not adequately prevent light from passing through decreased chlorine content, which is uncommon(26).

However, despite the chlorine stability in sodium hypochlorite products, the chlorine content continues to decrease from the baseline content over the storage period(25). As a result, a sodium hypochlorite solution with less than 6% accessible chlorine and a pH of 11 held at room temperature offers adequate stability and good quality, according to Frais *et al*(30).

Similar to other studies on the quality of sodium hypochlorite, Wachira(10) and Marong *et al*(11) evaluated the quality of commercially available bleaches in Nairobi and Gambia, respectively. Accordingly, all samples failed to meet the content standards (4-6 % w/v). In addition, Wachira reported that all samples fell short of the standard; however, Marong *et al* reported that all samples exceeded it. As a result, using commercial bleach without regular quality control testing is not suggested due to the significant differences in the contents ($P \leq 0.001$)(8). Moreover, sodium hypochlorite products are rarely manufactured following high manufacturing standards or quality control systems(8).

Proclamation No. 1112/2019 mandates the Ethiopian Food and Drug Authority (EFDA) to register antiseptics and disinfectants and conduct regulatory assessments(19). However, according to a recent study by Muluken(20), antiseptics sold in Addis Ababa fail to meet the regulatory requirements. In addition, the EFDA did not conduct frequent inspections of manufacturers or assess the quality of antiseptics on the market(20). As a result, disinfectants like sodium hypochlorite products are subject to less strict regulation as antiseptics in Ethiopia.

In summary, chlorine contents in analyzed samples differed from the label claimed contents by a more significant percentage (the Netherlands study, 27.2%, and the developing countries study, 35%). In addition, sodium hypochlorite products are inherently unstable, affecting product quality. Moreover, sodium hypochlorite products are rarely produced to high manufacturing standards or quality control systems, especially in developing countries. As a result, a quality evaluation of sodium hypochlorite products in health facilities is required.

2.2. Efficacy of sodium hypochlorite

Given the significant differences in chlorine contents in analyzed samples, are commercially available sodium hypochlorite products effective against microorganisms that cause healthcare-associated infections (HAIs)?

Rutala *et al*(26) studied the efficacy of low chlorine contents in controlling microorganisms. They reported that 100 parts per million (ppm) or 0.01% (w/v) was the lowest content that successfully inactivated the test microorganisms. Kohler *et al*(9), however, reported a higher range than Rutala *et al*, which was 2000 ppm or 0.2% (w/v) for controlling multidrug-resistant (MDR) gram-negative bacteria. As a result, the success of disseminating healthcare surfaces is determined by the content of chlorine, microorganism intrinsic resistance, and the presence of dirt and biological fluid spills(26).

Furthermore, Almatroudi *et al*(8) evaluated the efficacy of ten different concentrations of chlorine: 10, 50, 100, 200, 500, 1000, 2000, 5000, 10,000, and 20,000 ppm for 10-minute contact periods against *S. aureus* dry-surface biofilms. As a result, chlorine concentrations ranging from 1000 ppm to 20,000 ppm resulted in a more than 7 log reduction in the number of *S. aureus* bacteria immediately after treatment. Despite being exposed to 20,000 ppm chlorine, the biofilm recovered and released planktonic *S. aureus* after a lengthy incubation period.

On the other hand, Amal *et al*(15) studied the antibiotic susceptibility of *P. aeruginosa* at sub-inhibitory hypochlorite concentrations. As a result, utilizing lower-than-optimal concentrations can cause antibiotic-resistant *Pseudomonas* to evolve. In addition, routine cleaning using random chlorine content in health facilities has been ineffective in controlling harmful microbes(27). Accordingly, for environmental cleaning and disinfection in health facilities, the WHO and the Center for Disease Control (CDC) suggest a minimum chlorine content of 5000 ppm or 0.5% (w/v) for a 10-minute exposure time(2,3).

In Ethiopia, Daniel *et al*(35) studied the efficiency of bleach, which is commonly used in health facilities, against *Mycobacterium tuberculosis* isolates. Accordingly, 0.1% (w/v) bleaches for 15 minutes and 0.5% (w/v) for 10 minutes were tuberculocidal without any organic load. Hence, it is vital to guarantee the quality and efficacy of sodium hypochlorite products used in health facilities using a simple, cost-effective, precise, and rapid analysis method.

2.3. Quality and efficacy test methods

Several methods for determining the chlorine content in sodium hypochlorite products have been reported in the literature, including titrimetric, chromatographic, and spectrophotometric methods. The titrimetric (iodometric titration) method is the most widely used because it is simple, precise, cost-effective, and quick(36). In the iodometric titration method, sodium hypochlorite is added to excess iodide ion (I^-) to produce tri-iodide ion (I_3^-), which is then titrated with sodium thiosulfate ($Na_2S_2O_3$) solution using starch as an indicator(37).

Similarly, several methods for determining the efficacy of sodium hypochlorite products have been reported(38)—suspension, surface or carrier challenge, and in situ tests(38). The most widely used official method is the Organization for Economic Co-operation and Development (OECD), a carrier-based procedure recommended by the US Environmental Protection Agency (EPA). According to EPA efficacy criteria, healthcare disinfectants such as sodium hypochlorite products must be shown to be effective against two healthcare-associated pathogens, *S. aureus*, and *P. aeruginosa.* The OECD is a quantitative and carrier-based method that mimics a hard, non-porous inanimate surface(39).

In conclusion, Table 1 summarizes some poor reported quality and ineffectual sodium hypochlorite products and their analysis method.

Table 1: Summary of some of the poor reported quality and ineffectual sodium hypochlorite products

Aim	Study area	Method	Findings	Ref
Quality assessment of sodium hypochlorite products	Amsterdam, The Netherlands	Iodometric titration	90% of the samples had less chlorine than the labeled value	(24)
Investigation of the effects of temperature, concentration, and time on the chemical stability of sodium hypochlorite products	US	Iodometric titration	The stability of sodium hypochlorite is influenced by the storage temperature, solution concentration, and storage time.	(12)
The quality analysis of commercially available bleaches	In 12 developing countries	Iodometric titration	The significant differences between labeled and measured contents were found in 24 of the 32 samples.	(8)
The effect of exposure to sub-inhibitory concentrations of hypochlorite on antimicrobial susceptibility of *P. aeruginosa*	Egypt	Dilution method	Antibiotic-resistant *Pseudomonas* bacteria developed due to the use of lower-than-optimal concentrations	(15)

2.4. Conceptual framework

Finally, based on a review of related literature, the conceptual framework for this study is shown in Figure 1. The graph depicts the relationship between variables.

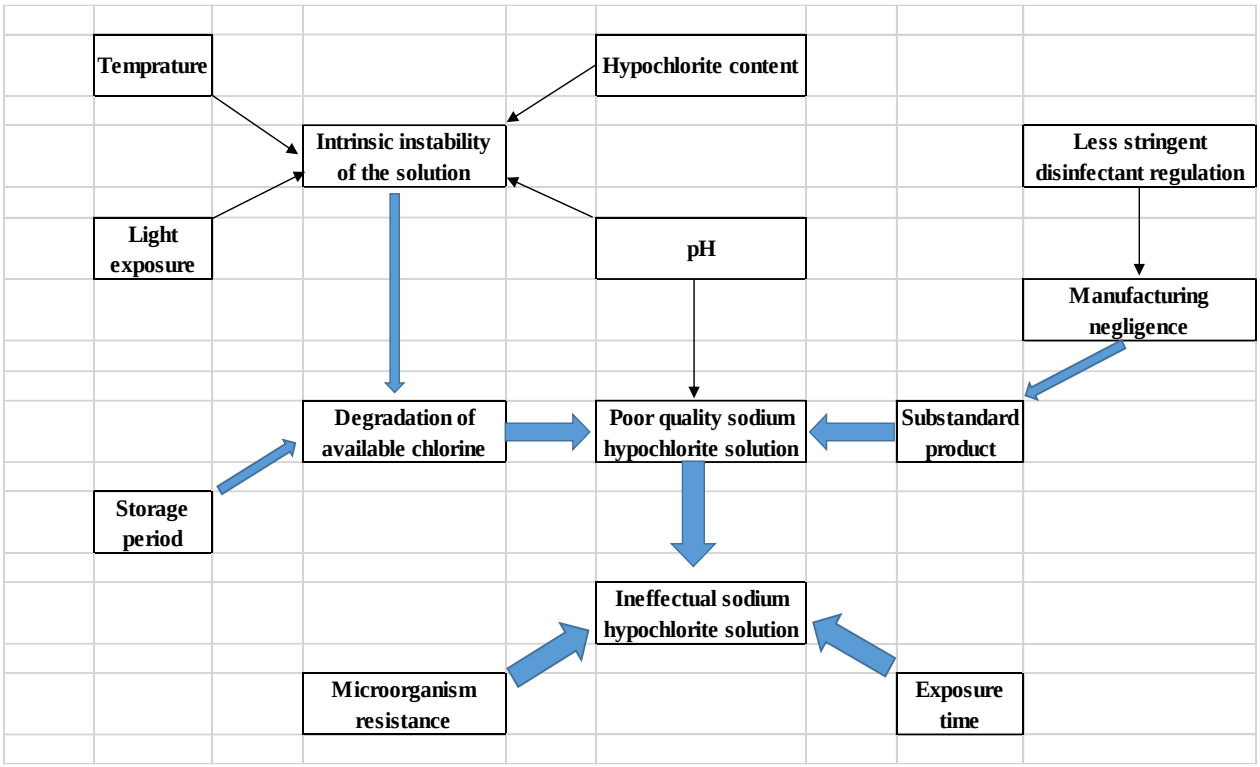

Figure 1: The conceptual framework for the quality and surface cleaning efficacy assessment of sodium hypochlorite products used in the health facilities of Jimma Town, Oromia, Ethiopia.

3. Objective

3.1. General objective

The main objective is to determine the quality and surface cleaning efficacy of sodium hypochlorite products used in health facilities of Jimma Town, Oromia Regional State, Ethiopia.

3.2. Specific objectives

- To determine the physicochemical quality (visual inspection, identification, chlorine content/assay, and pH) of sodium hypochlorite products.
- To determine the surface cleaning efficacy of sodium hypochlorite products (highest and lowest quality brands).

4. Method and Materials

4.1. Study area and period

The study was conducted in Jimma town between September 1 and October 31, 2021. Jimma town is located in southwest Ethiopia, 352 kilometers from the capital Addis Ababa. According to the Jimma Town health administration office, five hospitals, four health centers, and twenty clinics provide healthcare services to an estimated 224,565 population.

4.2. Study design

A survey-based study design was used in the present study. Simple random sampling was used to collect representative samples from all health facilities in Jimma Town. The Jimma University Institutional Review Board approved the proposed study and provided letter of support for facilitating the study and sample collection for the laboratory analysis.

The physicochemical quality of sodium hypochlorite was assessed using a checklist based on WHO/ Food and Agriculture Organization (FAO) guidelines (visual inspection) and standard protocols recommended by the US Pharmacopeia (identification, chlorine content/assay, and pH). The surface cleaning efficacy of sodium hypochlorite was evaluated using the standard protocol recommended by the US Environmental Protection Agency (EPA). The experimental work was carried out at the analytical chemistry and medical microbiology laboratories of Jimma University.

4.3. Sample collection

All health facilities in Jimma Town were included as sample collection sites. The samples were collected randomly from each health facility using a joint WHO/FAO guidelines for pesticide specifications(40) (Annex I). Collection was done by the principal investigator following the submission of the support letter from Jimma University and providing information to the chief executive officers of the healthcare facility that the samples are intended only for research purpose. Twenty-seven samples (n = 27) of sodium hypochlorite products were collected between August 1 and August 31, 2021 (Annex II). The samples were categorized into six groups based on their brands: SH 01 (n = 6), SH 02 (n = 8), SH 03 (n = 3), SH 04 (n = 3), SH 05 (n = 5) and SH 06 (n = 2), where SH represents sodium hypochlorite (Table 2).

Table 2: Samples of 5% w/v sodium hypochlorite products collected from health facilities in Jimma Town between August 1 and August 31, 2021.

Brand codes	Sample collection sites	Net content (L)	n	Batch no.	Manufacture date*	Expiry date*
		0.8	3	000O13	3/10/2021	9/15/2022
SH 01	Shenen Gibe General Hospital	5	3	N/A	3/20/2021	9/25/2022
	Awetu Primary Hospital	1	2	N/A	11/7/2020	11/6/2022
	Jimma Higher 2 Health Center	1	3	N/A	7/24/2020	7/23/2022
SH 02	Jimma Higher 1 Health Center	1	3	N/A	11/7/2020	11/6/2022
SH 03	Oda Hulle Primary Hospital	1	3	N/A	1/1/2021	1/31/2024
SH 04	Firomsis Primary Hospital	1	3	N/A	8/1/2020	8/31/2022
SH 05	Jimma University Medical Center	0.8	5	N/A	5/1/2021	11/30/2023
	Becho Bore Health Center	5	1	N/A	6/1/2021	6/30/2025
SH 06	Mendara Kochi Health Center	5	1	N/A	6/1/2021	6/30/2025
Total			**27**			

Abbreviations: * the date is month/date/year; N/A, not available; n, number of samples collected.

The adequate bulk and laboratory samples for subsequent laboratory analysis were obtained using the WHO/FAO pesticide specifications protocol. A bulk sample for the smaller containers (0.8 L or 1L) is obtained by combining 200 mL of solution from each sample unit per brand in one apparatus. In the case of a 5L container, each bulk sample should include material from the container's top, middle, and bottom. A laboratory sample is obtained by taking 200 ml of solution from each bulk sample (Figure 2).

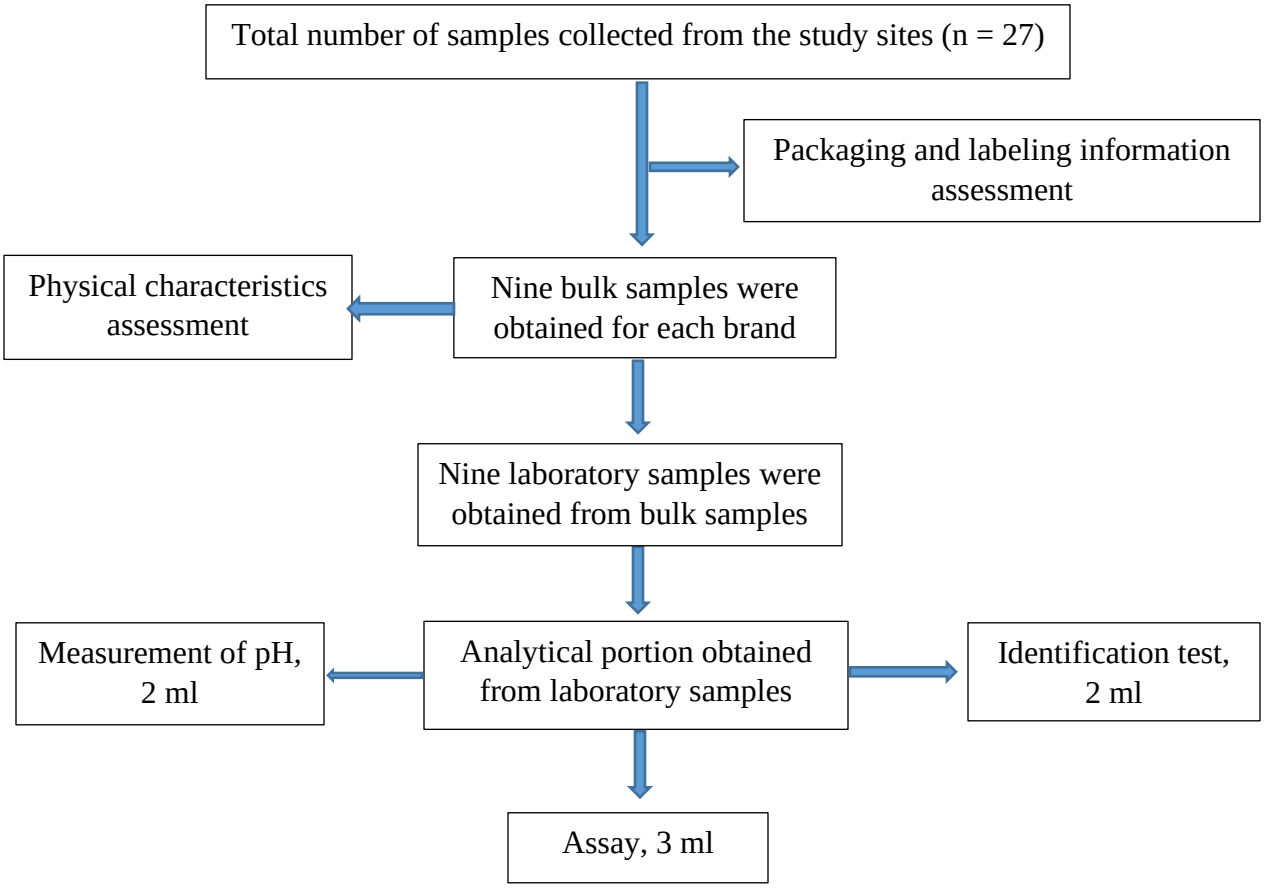

Figure 2: The general approach for appropriate subsampling bulk and laboratory samples from collected samples for laboratory analysis.

4.4. Materials

4.4.1. Chemicals and reagents

Sodium bicarbonate (Reidel-de Haen, Germany; Batch No. 304056), sodium thiosulphate pentahydrate (Reidel-de Haen, Germany; Batch No. 403145), sodium carbonate (Reidel-de Haen, Germany), potassium dichromate (UNI-CHEM Chemical Reagents, USA; Batch No. 05037), potassium iodide (UNI-CHEM Chemical Reagents, USA; Batch No. AVIS/462/47), and glacial acetic acid (Neolab Life Sciences, Germany; Batch No. 010717), and hydrochloric acid (Neolab Life Sciences, Germany; Batch No. 020827). All chemicals used were of analytical grade.

4.4.2. Test microorganisms and media

P. aeruginosa (#ATCC 27853), *S. aureus* (#ATCC 25923), Bacto TM Trypticase soy Broth (Becton, Dickinson and Company, USA; Batch No. 8141955), tryptic soya agar (HiMedia Laboratories, India; Batch No. 0000405556) and phosphate buffer.

4.4.3. Equipment

Incubator plus Series (Gallenkamp Laboratories, UK), colony counter (Gallenkamp Laboratories, UK), Bante pH meter (Bante Instruments Inc., USA), Textile electronic balance (A & E Co., Ltd, UK), Olympus CX23 microscope (Olympus Corporation, Japan), and Maxi Mix II sonicator (Thermolyne, US).

4.5. Test methods

4.5.1. Physicochemical quality of sodium hypochlorite

The physicochemical quality (visual inspection, identification, chlorine content/assay, and pH) were determined for all six brands of sodium hypochlorite products collected from health facilities in Jimma town.

4.5.1.1. Visual inspection

All six brands were visually inspected for packaging (opaque, tight, and plastic container), labeling information (product name and active ingredient content, net content of the pack, batch number, storage advice, manufacturing company, expiry date, and manufacturing date), and physical

characteristics (clear solution and free of particulate matter) using a checklist based on 2015 FAO/WHO guidelines(41) (Annex III).

4.5.1.2. Identification test

A sample solution of 2 mL was accurately measured and transferred into a beaker. The red litmus paper was then immersed in the sample solution. The color change was then visually assessed and compared to the US pharmacopeia requirements for sodium hypochlorite identification tests(37).

According to the US Pharmacopeia, the sample solution must turn red litmus paper blue, then white to pass the identification test.

4.5.1.3. Chlorine content assay

4.5.1.3.1. Preparation of 0.1 N sodium thiosulphate solution

Anhydrous sodium thiosulphate (26 gm) and sodium carbonate (200 mg) were accurately weighed and transferred in a 1000 mL glass-stoppered flask. The flask was then filled with a few portions (20 ml) of boiled and cooled water and thoroughly swirled until the solids were entirely dissolved. Finally, the flask was filled with boiled and cooled water to a volume of 1000 ml.

4.5.1.3.2. Standardization of 0.1N sodium thiosulphate solution

In a 500 mL glass-stoppered flask, 210 mg of primary standard potassium dichromate, previously pulverized and dried at 120°c for 4 hours, was accurately weighed. A flask was then filled with 100 mL of water and swirled until the solids were entirely dissolved. The stoppered was removed, and 3gm of potassium iodide, 2gm of sodium bicarbonate, and 5 ml of hydrochloric acid were quickly added to the flask. For ten minutes, the flask was left in the dark. After 10 minutes, the stopper and inner walls of the flask were rinsed with water. The iodine liberated from the reaction was titrated with sodium thiosulphate solution (0.1 N) until the solution was yellowish-green in color. Finally, 3 mL of starch test solution was added to the solution, and the titration was continued until the discharge of blue color.

The factor, N, of 0.1N $Na_2S_2O_3$ solution versus standardization was calculated using the chemical reactions and formula below [**Equations 4.1, 4.2,** and **4.3**].

$$K_2Cr_2O_7 + 6KI + 14HCl \leftrightarrow 2CrCl_3 + 8KCl + 7H_2O + 3I_2 \quad \textbf{(4.1)}$$

$$I_3^- + 2S_2O_3^{-2} \leftrightarrow 3I^- + S_4O_6^{-2} \quad \textbf{(4.2)}$$

$$\mathbf{N} = \frac{\textbf{mg of potassium dichromate}}{\mathbf{49.033}\ \mathrm{X}\ \textbf{ml of sodium thiosulphate}} \quad (\mathbf{4.3})$$

The standardized concentration of $Na_2S_2O_3$ solution used to determine chlorine content in samples was 0.1060 ± 0.0003N.

4.5.1.3.3. Determination of chlorine content

In a 500 mL glass-stoppered flask, 3 ml of sample solution was accurately weighed and diluted with 50 ml of distilled water. The stopper was removed, and 2 gm of potassium iodide and 10 ml of 6 N acetic acid were quickly added to the flask. For ten minutes, the flask was left in the dark. After 10 minutes, the stopper and inner walls of the flask were rinsed with water. The iodine liberated from the reaction was titrated with sodium thiosulphate solution (0.1060 ± 0.0003N) until the solution was yellowish-green in color. Finally, 3 mL of starch test solution was added to the solution, and the titration was continued until the discharge of blue color(37).

Two moles (n) of sodium thiosulphate were reacted with one mole of iodine (I_2) liberated from one mole of sodium hypochlorite solution (**Equations 4.4** and **4.5**).

$$NaOCl + 2CH_3COOH + 2KI \rightarrow NaCl + 2CH_3COOK + H_2O + I_2 \quad \textbf{(4.4)}$$

$$I_2 + 2Na_2S_2O_3 \rightarrow 2NaI + Na_2S_4O_6 \quad \textbf{(4.5)}$$

The formula below (**Equation 4.6**) was used to calculate the chlorine content in the samples. Each ml of sodium thiosulphate (0.1060N) consumed was equivalent to 3.930 mg sodium hypochlorite or 3.744 mg chlorine.

$$\textbf{Content of chlorine}\ (\%\frac{\mathrm{w}}{\mathrm{v}}) = \frac{\textbf{ml of thiosulphate}\ \mathrm{X}\ \mathbf{0.106}\ (\mathrm{N})}{\mathbf{2}\ \mathrm{X}\ \textbf{Sample volume}\ (\mathrm{ml})} \times \mathbf{100} \qquad (\mathbf{4.6})$$

According to the US Pharmacopeia, 5% (w/v) sodium hypochlorite must contain not less than 4% and not more than 6%, by weight, of sodium hypochlorite.

4.5.1.4. Determination of pH value

The pH and temperature of the samples were measured using a calibrated pH meter (Bante Instruments Inc., USA). The pH meter electrodes and beaker were rinsed three times using 2 ml of distilled water and sample solution. A sample solution of 2 mL was then accurately measured and transferred into a beaker. The pH and temperature sensors were then wholly immersed in the sample solution. The pH meter was left in the beaker for a sufficient time until the measurements were stabilized(37). Finally, the sample solution pH and temperature values were calculated using three readings. The pH values of all samples were measured at an average solution temperature of 20.98 ^{0}C ± 0.28 ^{0}C.

4.5.2. Surface cleaning efficacy of sodium hypochlorite

The surface cleaning efficacy of sodium hypochlorite solution was evaluated using the Organization for Economic Co-operation and Development (OECD) method(39).

4.5.2.1. Samples selected for efficacy testing

The samples selected for the surface cleaning efficacy evaluation were based on the results of the physicochemical quality. As a result, the highest (SH 01) and the lowest (SH 05) quality brands were selected to assess the surface cleaning efficacy of sodium hypochlorite. The SH 04 brand was excluded from efficacy testing because the brand failed both the identification and assay tests.

4.5.2.2. Efficacy testing procedure

4.5.2.2.1. Preparation and sterilization of carriers

The surfaces of the carriers were visually checked for abnormalities. After being visually inspected, the carriers were cleaned with a suitable detergent solution and distilled water. The carriers were then placed in Petri dishes and were sterilized by autoclaving for 45 minutes at 121°C. After sterilization, the carriers were aseptically transferred to sterile Petri dishes for inoculation.

4.5.2.2.2. Preparation of media

The tryptic soya broth (TSB) was prepared according to the manufacturer's instructions (Becton, Dickinson and Company, USA). TSB powder (30 gm) was dissolved in 1L of purified water and was then thoroughly mixed. The broth medium was then slightly warmed to dissolve the powder completely. Finally, the broth medium was sterilized by autoclaving for 15 min at 121°C.

Similarly, the tryptic soya agar (TSA) was prepared according to the manufacturer's instruction (HiMedia Laboratories, India). TSA powder (40 gm) was measured and dissolved in 1L of purified water. The medium was then heated to boiling to dissolve the powder completely. The medium was then sterilized by autoclaving for 15 min at 121°C. Finally, the medium was cooled to 45-50°C, and 20 ml of the medium was poured into sterile 90 mm Petri dishes.

4.5.2.2.3. Preparation of microorganism test suspension

A cryovial (at -80±5°C) of *P. aeruginosa* (#ATCC 27853) and *S. aureus* (#ATCC 25923) stains were defrosted at room temperature. Then 100 µL of the defrosted stock culture of each strain was added to 10 ml of tryptic soya broth (TSB) in a test tube. The test tube was then vortexed briefly before being incubated at 36 ± 1°C for 24 hours. The 24 hours broth culture for each strain was then transferred into a 15 mL centrifuge tube. The broth culture was then centrifuged at 5,000g/v for 20 minutes. The supernatant broth culture was then removed and re-suspended in 10 mL of phosphate-buffered saline (PBS), yielding an average of 5 logs colony-forming unit (CFU) per carrier. In addition, for culture isolation, characterization, and purity testing, the 24 hours broth culture was inoculated on tryptic soya agar (TSA) and incubated at 36 ± 1°C for 24 hours. After purity checking, 250 µL of final test suspension was prepared for each test microorganism by combining 170 µL of each microbial test suspension and 80 µL PBS. The final test suspension was used within 30 min of preparation at room temperature, 22±2°C, to inoculate carriers.

4.5.2.2.4. Inoculation and drying of carriers

The final test suspension for each microorganism was vortexed for 10 seconds before the inoculation of carriers. For each brand of sodium hypochlorite product, 24 carriers were inoculated (6 controls and 18 treated). A 10 µL final test suspension of each microorganism (*P. aeruginosa* and *S. aureus*) was placed in each carrier's center. Then, the inoculated carriers were dried inside

a Petri dish (90 mm diameter) in the biological safety cabinet for 50 min. The dried inoculated carriers were used within 24 hours of inoculation.

4.5.2.2.5. Enumeration of viable microorganisms from control carriers

One dried inoculum was aseptically placed into a test tube containing 10 ml of tryptic soya broth (TSB). The contents of a test tube (10^0 dilution) were then mixed briefly, and a serial ten-fold dilution was made in a test tube containing 9 mL of TSB (10^{-1} dilution). Then, 0.1 ml of aliquots each dilution was plated in duplicate on tryptic soya agar (TSA) and incubated at 36 ± 1°C for 48 hours. Finally, the colonies were counted, yielding an average of 5 logs CFU per carrier.

4.5.2.2.6. Exposure of the dried inoculum to disinfectant solution

The disinfectant sample solution (highest and lowest quality brands) was diluted by ten from its initial concentration. In a timed fashion, 50 µL of sample solution (equilibrated to 22±2°C) was deposited over the dried inoculum on each test carrier, ensuring complete coverage. The test carriers were exposed for 10 minutes. Similarly, each control carrier received 50 µL of phosphate-buffered saline (PBS), equilibrated to 22±2°C, instead of sodium hypochlorite solution. The control carriers were also exposed for 10 minutes.

4.5.2.2.7. Neutralization and colony counting

The carriers were removed from the disinfectant sample solution and placed into a test tube containing 10 ml of neutralizing medium (phosphate-buffered saline with 0.1% (w/v) sodium thiosulfate) after the exposure time. The neutralized test tube containing the carrier was recorded as the 10^0 dilution. After 30 minutes of neutralization, a serial ten-fold dilution was made in a test tube containing 9 mL of phosphate-buffered saline (PBS) (10^{-1} dilution). The contents of each test tube (10^0 and 10^{-1}) were then filtered using 0.2 µm membrane filters. The membrane filters were then incubated (inverted) on 20 ml tryptic soya agar (TSA) at 36 ± 1°C for 72 hours and counted the number of colonies. For control carries, the membrane filters were incubated (inverted) at 36 ± 1°C for 48 hours.

The sample solution's CFU, log density, and log reduction are calculated using the formula below (**Equations 4.7, 4.8,** and **4.9**).

$$\mathbf{CFU} = \frac{\mathbf{CFU}\ (\mathbf{10-y}) + \boldsymbol{CFU}\ (\mathbf{10-}\boldsymbol{z})}{(\mathbf{9\ ml\ x\ 10-y}) + (\mathbf{10}\ \boldsymbol{ml}\ \boldsymbol{x}\ \mathbf{10-}\boldsymbol{z})}\ \mathbf{x\ 10\ ml} \qquad (\mathbf{4.7}),$$

where 10^{-y} and 10^{-z} are the dilution filtered and CFU is colony forming unit.

$$\mathbf{LD = log\ of\ CFU} \qquad (\mathbf{4.8}),$$

where LD is log density, a log is a logarithm, and CFU is a colony-forming unit.

$$\mathbf{LR = LD\ of\ control - LD\ of\ a\ sample} \qquad (\mathbf{4.9}),$$

where LR is log reduction and LD is log density.

4.6. Statistical analysis

The data were entered into Microsoft Excel 2013 before being exported to IBM statistical package for the social sciences (SPSS) statistics for Windows (version 20; IBM Corp, USA) for analysis. The descriptive statistical analysis was conducted using Microsoft Excel 2013. The variance in chlorine content among brands was analyzed using IBM SPSS statistics non-parametric tests (the Kruskal Wallis test and Dunn's test post hoc analysis). The Pearson correlation test determined the correlations between chlorine content and pH/storage period. The efficacy of the highest and lowest quality brands was analyzed using the independent-samples T-test. The differences were deemed statistically significant at the probability level of $p < 0.05$. Graphs, tables, and numerical summary measures presented the findings.

4.7. Operational definitions

- Chemical disinfectants are chemicals only used for disinfection after all organic matter and soil have been removed by a cleaning product.
- A bulk sample comprises all of the primary samples from each brand.
- Laboratory samples are a portion of material obtained from a bulk sample following the specified sampling technique and submitted to the laboratory for testing.

5. Results

5.1. Physicochemical quality of sodium hypochlorite

5.1.1. Visual inspection

All brands did not provide all the minimal required information on the labels. Only one brand (SH 01) out of six had the storage instructions ('keep in a cool, dry area away from direct sunshine and heat'). The SH 03 and SH 06 brands had more than two years of shelf-life. Similarly, no brands provided usage instructions, dilution factors, first-aid instructions, or medical advice. Furthermore, none of the brands had warnings on the labels ('do not get in the eyes, on the skin, or clothing') (Table 3).

Table 3: The visual inspection of sodium hypochlorite products collected from healthcare facilities in Jimma Town (n = 27).

Visual inspection	Brand code (n)					
	SH 01 (6)	SH 02 (8)	SH 03 (3)	SH 04 (3)	SH 05 (5)	SH 06 (2)
Packaging (√ or x)						
Opaque tight plastic container	√	√	√	√	√	√
Labeling information (√ or x)						
Product name, net content of the pack & supplier information	√	√	√	√	√	√
Batch number	√[a]	x	x	x	x	x
Precautionary statements or warnings						
Keep out of the reach of children	√	√	√	√	√	√
Do not get in the eyes, skin, or clothing	x	x	x	x	x	x
Wear relevant PPE when handling	√	x	x	x	x	x
Store in a cool, dry area, away from direct sunlight and heat	√	x	x	x	x	x
Release and expiry date of the product	√	√	√	√	√	√
Shelf-life	√[b]	√[b]	√[c]	√[b]	√[b]	√[c]
Direction for use, first aid & medical advice	x	x	x	x	x	x
Physical characteristics (√ or x)						
Clear solution & free of particulate matter	√	√	√	√	√	√

Abbreviations: [a], only 3 samples had a batch number; √, yes; x, no; [b], less than or equal to 2 years shelf-life; [c], greater than 2 years shelf-life; PPE, personal protective equipment.

5.1.2. Identification test

Instead of bleaching the red litmus paper, the SH 04 brand turned it blue (Annex IV), indicating that it is the only one without chlorine (Table 4).

Table 4: The results of the identification test of sodium hypochlorite brands

Parameter	Brand codes					
	SH 01	SH 02	SH 03	SH 04	SH 05	SH 06
Litmus paper test (+/-)						
Chlorine	+	+	+	-	+	+

Abbreviations: +, present; -, absent.

5.1.3. Chlorine content assay

Most of the brands differed from the label's claimed contents by a significant percentage (59.6 on average). The SH 01 brand had the highest chlorine content (4.64% ± 0.09%), while the SH 05 brand had the lowest (1.09% ± 0.09%). Further, the absence of chlorine was confirmed in the SH 04 brand (0.12% ± 0.02%) (Table 5).

Table 5: Summary of chlorine content assay results, contents in percentage (% w/v)

Brand code	Label content (% w/v)	Chlorine content, mean (%) ± SD	Percentage error, mean %
SH 01	5	4.64 ± 0.09	7.22
SH 02	5	1.37 ± 0.12	72.70
SH 03	5	2.28 ± 0.05	54.41
SH 04	5	0.12 ± 0.02	97.52
SH 05	5	1.09 ± 0.01	78.22
SH 06	5	1.33 ± 0.01	73.43
Total		2.02 ± 1.52	59.57
Measurements are mean ± Standard deviation, n = 3			

Abbreviations: n = triplicate analysis

5.1.3.1. Quality categories of sodium hypochlorite brands

Most of the sodium hypochlorite brands used at healthcare facilities in Jimma Town, 83.33%, failed to meet the US Pharmacopeia standard for chlorine content. Five of the six brands were of poor quality (Table 6).

Table 6: Summary of quality categories of sodium hypochlorite brands based on chlorine content.

The label claimed content (% w/v)	USP requirement (% w/v)	Quality, descriptive evaluation	n (%)
5	$4 \geq x \leq 6$	Good	1 (16.67)
	$x < 4$	Poor	5 (83.33)
	$x > 6$	Poor	0 (0)

Abbreviations: USP = United States pharmacopeia; x = mean content of chlorine; n = number of brands in each USP quality category

5.1.3.2. Result of statistical analysis

The chlorine content of all six sodium hypochlorite brands used in the health facilities of Jimma Town varied significantly (Kruskal-Wallis test, P 0.0001). The SH 01 and SH 05 brands were of the highest and lowest quality, respectively (Figure 3).

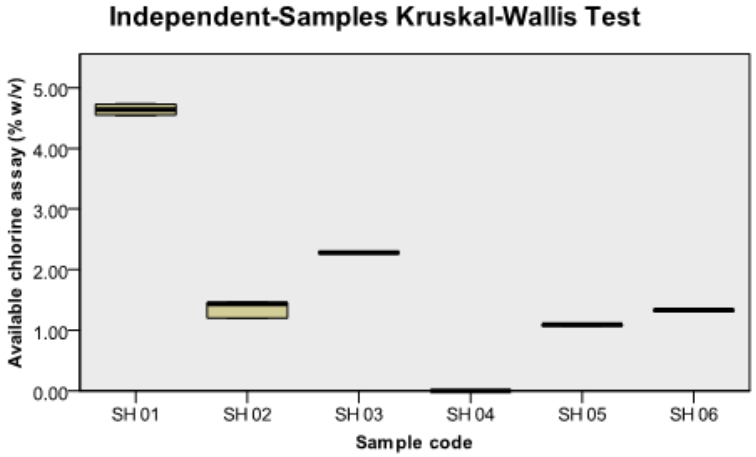

Figure 3: Quality ranking of sodium hypochlorite brands.

5.1.4. The pH value of samples

The pH values of all brands ranged from 12.41 to 13.18 (at 20.98 ^{0}C on average) (Figure 4).

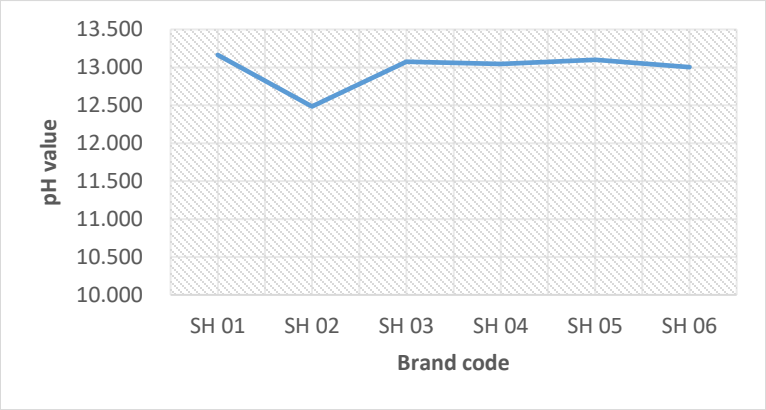

Figure 4: The pH values of sodium hypochlorite brands obtained at a mean solution temperature of 20.98 ^{0}C.

5.1.5. Combined physicochemical quality analysis of samples

Factors associated with low chlorine content in sodium hypochlorite brands were evaluated in the present study.

5.1.5.1. Observed pH value and chlorine content

A weak association was found between the low chlorine content and the pH value (r = 0.43, P = 0.025) (Figure 5).

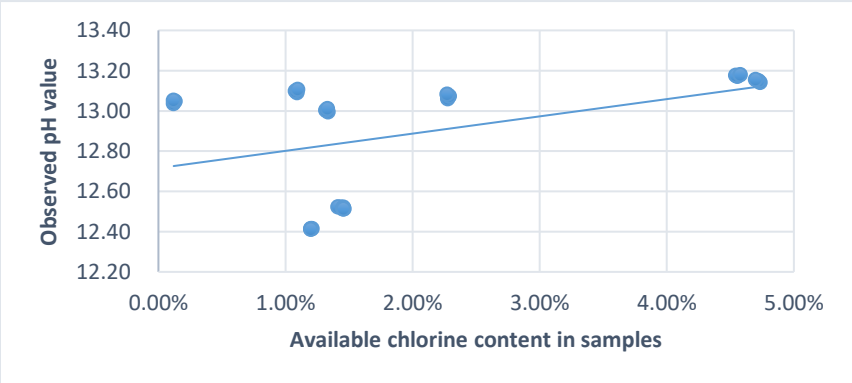

Figure 5: Correlation (r = 0.43) of the observed pH value of brands with chlorine content, showing a weak association between low pH and low chlorine content.

5.1.5.2. Storage period and chlorine content

The storage period was the time (in months) between the product manufacture and the experimental work (September, 2021). A weak relationship was observed between the low chlorine content and the storage period (r = -0.398, P = 0.040) (Figure 6).

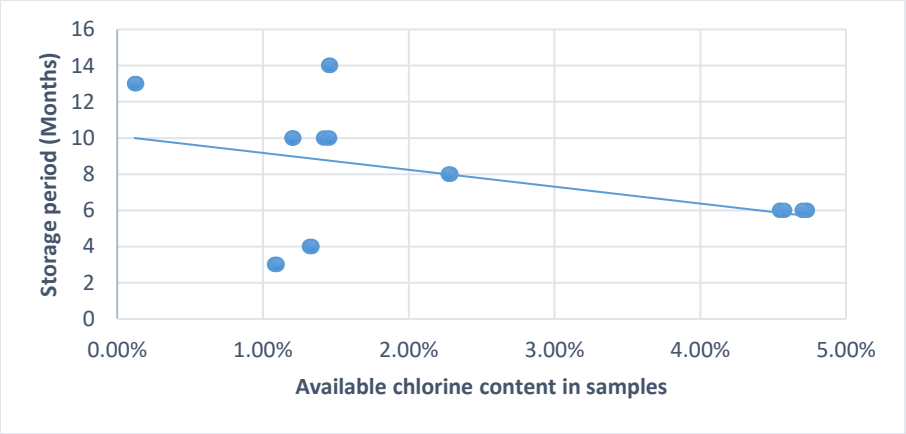

Figure 6: Correlation (r = - 0.39) of the storage periods of brands with chlorine content, showing a weak association between more extended storage periods and low chlorine content.

5.1.5.3. Sodium hypochlorite price and chlorine content

Compared to more expensive sodium hypochlorite brands, cheaper brands had less chlorine content (Table 7).

Table 7: Comparison of the cost of six brands of sodium hypochlorite with their chlorine content

Brand code	Label content (% w/v)	Average unit price (ETB)*	Chlorine content, mean (%) ± SD
SH 01	5	48	4.64 ± 0.09
SH 02	5	27	1.37 ± 0.12
SH 03	5	36	2.28 ± 0.05
SH 04	5	25	0.12 ± 0.02
SH 05	5	23	1.09 ± 0.01
SH 06	5	29	1.33 ± 0.01

Abbreviations: *, average unit price of 1L sodium hypochlorite during the sample collection period

5.2. Surface cleaning efficacy of sodium hypochlorite

On average, 5 logs of colony-forming unit (CFU) were inoculated into the surface carriers. The mean log reductions (LRs) in *P. aeruginosa* ($LR_{SH\ 01}$ = 4.13, $LR_{SH\ 05}$ =3.17, and p = 0.008) and *S. aureus* ($LR_{SH\ 01}$ = 4.26, $LR_{SH\ 05}$ =3.47, and p = 0.009) varied significantly across the SH 01 and SH 05 brands (Table 8).

Table 8: The log reduction of the highest and lowest quality brands of sodium hypochlorite against *P. aeruginosa* and *S. aureus*.

Brand Code	**Quality category (Chlorine content)**	**Observed log reduction Mean (Values)[a]**	
		P. aeruginosa	***S. aureus***
SH 01	Highest (4.64% w/v)	4.13 (4.4, 4.1, 3.9)	4.26 (4.5, 4.2 4.1)
SH 05	Lowest (1.09% w/v)	3.17 (3.4, 3.1, 3.0)	3.47 (3.6, 3.4, 3.4)

Abbreviations: [a] Individual value data are presented for the three replicate analyses

6. Discussion

Commercially available bleaches are a primary source of sodium hypochlorite products in resource-constrained healthcare settings. However, the chlorine content of those bleaches is insufficient to disinfect microorganisms on healthcare surfaces successfully(8,9,24). Sodium hypochlorite products are also inherently unstable, reducing the chlorine content and quality(12). Consequently, multidrug-resistant strains were isolated from healthcare surfaces treated with sodium hypochlorite products containing insufficient chlorine, increasing the prevalence of healthcare-associated infections, particularly in developing countries(27). Therefore, this study is aimed to assess the quality and surface cleaning efficacy of sodium hypochlorite products used in health facilities of Jimma Town.

The physicochemical quality of six brands of sodium hypochlorite products used in Jimma Town health facilities was assessed using internationally recognized compendia, the World Health Organization/Food and Agricultural Organization guidelines (visual inspection)(41), and the United States Pharmacopoeia (identification test, chlorine content assay, and pH)(37). The efficacy of surface cleaning was then evaluated using the highest and lowest quality brands, with physicochemical quality data considered(39).

All six brands collected from Jimma town health facilities were visually inspected for packaging, labeling information, and physical attributes using the checklist provided in Annex III. However, as shown in Table 3, most brands did not provide all the minimal information on their labeling. These findings could indicate that crucial product information was not correctly presented to users to ensure the stability and quality of sodium hypochlorite(41).

According to previous studies, increased temperatures and light exposure cause the chlorine in sodium hypochlorite products to degrade(12,26). Accordingly, sodium hypochlorite products should be stored in a cool, dry area away from direct sunlight and heat. Unfortunately, only one (SH 01) of the six brands assessed provided storage instructions on the labels. Sodium hypochlorite products used in health facilities of Jimma town are likely to be unstable due to storage conditions(12,26).

Regardless of storage conditions, the chlorine content in sodium hypochlorite products decreases over time, emphasizing the importance of using these products within two years(25). The SH 03 and SH 06 brands had more than two years of shelf-life in this study. Chlorine in these brands may degrade before expiration. As a result, sodium hypochlorite products with insufficient chlorine content can be utilized in health facilities as though they had more time before expiration.

Besides, Racioppi *et al*(42) reported that sodium hypochlorite exposure necessitates prompt medical intervention, underlining the importance of cautionary remarks or warning statements on product labels. However, none of the brands had warnings on the labels ('do not get in the eyes, on the skin, or clothing') (Table 3). This finding indicates that the sodium hypochlorite products used in health facilities of Jimma Town may pose both safety and quality concerns.

According to the Ethiopian Food and Drug Authority (EFDA) guideline for registration of antiseptics and disinfectants, all of the minimal required information must be provided on the labels(19). However, most sodium hypochlorite suppliers do not adhere to EFDA antiseptic and disinfectant regulation standards. These suppliers are also not regularly inspected by the EFDA(20). The visual inspection findings of the study support the notion that sodium hypochlorite products used in health facilities, particularly in developing countries, are of poor quality(8). Besides, these findings are consistent with a study conducted in Kenya, which reported that some samples did not meet the Kenya Bureau of standards requirements for sodium hypochlorite(10).

The United States Pharmacopeia identification test standards for sodium hypochlorite include turning red litmus paper blue and then bleaching it(37). Instead of bleaching the red litmus paper, the SH 04 brand turned it blue, indicating that it is the only one without chlorine (Table 4, Annex IV). This finding could imply that sodium hydroxide is being produced instead of sodium hypochlorite on purpose or that part of the available chlorine has already been lost due to the instability of the solution(17). Given the lack of chlorine in the sodium hypochlorite solution, microorganism control in health facilities may be challenging, putting patients at risk of healthcare-associated infections(1).

According to the United States Pharmacopeia, the 5% w/v sodium hypochlorite assay must produce a chlorine content range from 4% to 6% w/v(37). However, most of the brands analyzed differed from the label's claimed chlorine contents by a significant percentage (59.6 on average).

Only one of the six brands fulfilled the United States Pharmacopoeia requirements (Table 5, P 0.004). The lower chlorine content in the brands of sodium hypochlorite products could be attributed to the fact that important product information was not presented to ensure product stability throughout storage and usage(29,30). As a result, the chlorine content in brands may be insufficient to control microorganisms in health facilities, potentially leading to antimicrobial resistance strains(15,27). The assay results confirmed the absence of chlorine in the SH 04 brand, which showed a content of 0.12 % ± 0.02 % chlorine (Table 5). This finding indicates that sodium hypochlorite product with no chlorine is used in health facilities, resulting in ineffective cleaning(27).

Depending on the dilution factor, the dilution of sodium hypochlorite product decreased chlorine content compared to the initial value. According to WHO, sodium hypochlorite products be applied to wet surfaces at a concentration of no less than 0.5% during a 10-minute exposure time. Accordingly, 5 % w/v sodium hypochlorite products are diluted by 10 before being utilized to control microorganisms(2,3). Most importantly, all Jimma Town health facilities followed the WHO-recommended sodium hypochlorite preparation procedures.

In all health facilities of Jimma Town, the dilution factor for sodium hypochlorite preparation was solely based on the product label claim content. In this study, most of the brands analyzed differed from the label's claimed chlorine contents by 59.6% on average. Given this, dilution considerably impacts the content of chlorine and microorganism control(30). As a result, sodium hypochlorite products with less than 0.5% w/v concentration were used as though they contained 0.5% w/v.

Moreover, the findings of the chlorine content assay of this study are consistent with those of Costa *et al*(31) and Van der Waal *et al*(24). They reported that none of the sodium hypochlorite products analyzed met the label claimed contents. This, however, contradicts the findings of Marong *et al*(11), who reported that the chlorine contents in most samples were higher than the label claimed contents.

The pH of the sodium hypochlorite product determines the rate of chlorine degradation, affecting disinfection activity. The sodium hypochlorite products are most stable at pH values greater than 11. However, the degradation rate of chlorine rapidly increases from pH 11 to 7, with a peak rate at pH 7. Hence, a sodium hypochlorite product with less than 6% w/v chlorine content and a pH

of 11 held at room temperature offers adequate stability and quality(30). In this study, the pH of all brands was kept above 11 (Figure 4), resulting in a slower loss of chlorine content and more stable brands(17). This shows that the large percentage (59.6) difference between the chlorine contents of the brands and their label claimed contents is not attributable to pH-induced solution instability. Additionally, there was no significant link between lower-than-the-label claimed chlorine contents and a reduction in pH below 11 ($r = 0.43$, $P = 0.025$) (Figure 5). This finding is consistent with a prior study that reported that, despite pH stability, chlorine was decreased from the baseline contents(8,25).

Given that the pH of the solution(43) was not the reason for the lower chlorine contents in all brands, other probable causes were addressed in this study. Previous research has shown that the chlorine content in a solution decreases over time, indicating a negative link between the storage period and chlorine content(12,30). In this study, the storage period was the time (in months) between the product manufacture and the experimental work (September, 2021). There was no strong indirect relationship between sample storage period and chlorine contents ($r = - 0.398$, $P = 0.040$) (Figure 6). In addition, the brand (SH 05) that was stored for the shortest period (3 months) lost more chlorine (Figure 6, percentage error = 78.22). This indicates early depleted chlorine in certain brands (SH 02 and SH 05). Nevertheless, the cause of the lowest chlorine content could be related to the storage period (13 months) in the case of the SH 04 brand (0.12 % ± 0.02 %).

Even though the statistical significance is not usually related to the clinical activity of the product, only one (SH 01) of six brands was determined to be of good quality using the independent samples Kruskal-Wallis test (P 0.0001) (Figure 3). This statistical finding indicates that most sodium hypochlorite products used in health facilities of Jimma Town are of low quality.

According to this study, possible causes for low-quality sodium hypochlorite products include; valuable product information not being presented to users to ensure product stability and quality, less stringent disinfectant regulation, ineffective quality operations during the manufacturing process, and a deliberate attempt to lower the chlorine content.

Given that the majority of the analyzed brands were of low quality, the results of the physicochemical quality were complemented by an assessment of the surface cleaning efficacy. The efficacy of the highest quality brand (SH 01) was compared with the lowest quality brand (SH

05) against the most frequent HAI pathogens, *P. aeruginosa* and *S. aureus*(44). The Organization for Economic Co-operation and Development (OECD) quantitative method(39) was used to assess the surface cleaning efficacy of SH 01 (4.64% ± 0.09% w/v chlorine content) and SH 05 (1.09% ± 0.01% w/v chlorine content).

According to the US Environmental Protection Agency (EPA)(44) guidance for efficacy testing, cleaning dried inoculum (5.0-6.0 logs CFU/carrier) with a 0.5% w/v NaOCl solution recovers zero to very few test microorganisms (mean log reduction, LR, ≥ 4.5). However, for *P. aeruginosa* and *S. aureus* dried inoculums, the SH 01 brand achieved mean LRs of 4.13 and 4.26, respectively (Table 8). Similarly, the SH 05 brand achieved mean LRs of 3.17 and 3.47, respectively, with *P. aeruginosa* and *S. aureus* dried inoculums. These findings indicate that the SH 01 brand achieved findings nearly comparable to those required by the EPA, but the SH 05 brand did not(44).

In addition, the log reduction in *P. aeruginosa* ($p = 0.008$) and *S. aureus* ($p = 0.009$) varied significantly across the highest (SH 01) and lowest (SH 05) quality brands. Consequently, the low-quality sodium hypochlorite product was ineffectual at achieving the desired action, which is antibacterial activity(32). These findings could also explain why resistant microorganisms now inhabit healthcare surfaces(33). Moreover, ineffectual surface disinfection caused by low-quality disinfectants can lead to the survival of microbes on healthcare surfaces, leading to the spread of healthcare-associated infections (HAIs)(45).

According to the physicochemical quality findings, only one of the six brands evaluated for chlorine content fulfilled the US Pharmacopoeia requirements, 4% - 6% w/v. In addition, the absence of chlorine in the SH 04 brand was confirmed by both identification and assay tests. Likewise, the lowest quality brand (SH 05) was ineffectual in controlling the most challenging microorganisms in health facilities; *P. aeruginosa* and *S. aureus*. As a result, microorganisms on healthcare surfaces may develop resistance due to the low-quality, ineffective sodium hypochlorite product(6). Consequently, treating multidrug-resistant (MDR) infections with antibiotics in health facilities becomes significantly more challenging(6,33). This, in turn, may exacerbate the spread of HAIs(16,46).

The limitation of the present study includes: (1) the regulatory basis or status of sodium hypochlorite suppliers was not taken into account; (2) due to a lack of resources, the efficacy study was conducted over a month, which may have influenced the concentration of a sample; (3) for surface cleaning efficacy, evaluated only one brand from each good and poor quality sample; (4) the test microbes used in this study are laboratory stains, not clinical isolates; and (5) a robust study design was not used.

Despite these limitations, this study adds to our understanding of and helps to provide evidence for the quality and surface cleaning efficacy of sodium hypochlorite products used in health facilities. Furthermore, the findings of this study will serve as a benchmark for future sodium hypochlorite product quality and efficacy studies in Ethiopia.

7. Conclusion and Recommendations

7.1. Conclusion

The physicochemical quality of sodium hypochlorite products used in Jimma town health facilities was evaluated using World Health Organization/Food and Agricultural Organization guidelines (visual inspection) and the United States pharmacopeia methods (identification, chlorine content assay, and pH). Of most of the brands evaluated, five of the six were of poor quality. Quality problems may arise due to valuable product information not being presented to users to ensure product stability and quality, less stringent disinfectant regulation, ineffective quality operations during manufacturing, and a deliberate attempt to reduce chlorine content.

Similarly, the Organization for Economic Co-operation and Development method was used to evaluate surface cleaning efficacy for the highest and lowest quality brands of sodium hypochlorite products. The log reductions of *Pseudomonas aeruginosa* and *Staphylococcus aureus* varied significantly across the highest and lowest quality brands. The lowest quality brand was ineffectual at controlling microorganisms. Thus, treating multidrug-resistant infections with antibiotics in health facilities becomes significantly more challenging.

7.2. Recommendations

The following recommendations are made based on the findings of the present study:

- The Ethiopian food and Drug Administration (EFDA) should undertake regular manufacturer inspections and quality testing of sodium hypochlorite products.
- The regulatory authority may be alerted to this study's quality and efficacy concerns and take appropriate measures to protect the general public.
- Each healthcare facility should assess the quality of disinfectants before purchasing them to avoid the risks of using low-quality solutions in clinical settings.
- With timely recommendations forwarded to the regulatory body and other relevant stakeholders, a large-scale quality assessment of disinfectants can be conducted.
- Jimma University should provide feedback to the EFDA Jimma branch to take relevant action.

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Annexes

Annex I: Number of samples to be taken randomly from each subgroup, WHO and FAO pesticides specifications

Number of packing units in each subgroup	Number of samples to be taken
< 10	1
11 - 20	2
21 - 40	3
> 40	Three plus 1 for every additional 20 units up to a maximum of 15 sampled units

Annex II: Sample collection form

Brand codes	Name & address of the manufacturer	Sample collection sites	Net content (L)	n	Batch no.	Manufacture date*	Expiry date*
SH 01	Ghion industrial chemical sector, Addis Ababa, Ethiopia	Shenen Gibe General Hospital	0.8	3	000O13	3/10/2021	9/15/2022
			5	3	N/A	3/20/2021	9/25/2022
SH 02	Misky Industries PLC, Addis Ababa, Ethiopia	Awetu Primary Hospital	1	2	N/A	11/7/2020	11/6/2022
		Jimma Higher 2 Health Center	1	3	N/A	7/24/2020	7/23/2022
		Jimma Higher 1 Health Center	1	3	N/A	11/7/2020	11/6/2022
SH 03	Jimmitti Chemical Product, Jimma, Ethiopia	Oda Hulle Primary Hospital	1	3	N/A	1/1/2021	1/31/2024
SH 04	Amen Chemical PLC, Addis Ababa, Ethiopia	Firomsis Primary Hospital	1	3	N/A	8/1/2020	8/31/2022
SH 05	Ocean chemical PLC, Addis Ababa, Ethiopia	Jimma University Medical Center	0.8	5	N/A	5/1/2021	11/30/2023
SH 06	Onel detergent Manufacture PLC, Addis Ababa, Ethiopia	Becho Bore Health Center	5	1	N/A	6/1/2021	6/30/2025
		Mendara Kochi Health Center	5	1	N/A	6/1/2021	6/30/2025
Total				27			

Annex III: Checklist: physical characteristics, packaging, and labeling information

Packaging	Opaque plastic Container (Yes/No)		
	Label (Yes/No)	Product name	
		Active ingredient name	
		The net content of the pack	
		Batch number	
		Precautionary statements or warnings	Keep out of reach of children
			Do not get in the eyes, skin, or clothing.
			Wear PPE when handling
		First aid advice	
		Store in a cool, dry area, away from direct sunlight and heat	
		Release date of the product	
		Expiry date	
		Shelf-life	≤ 2 years
			> 2 years
		Directions for use and dilution factor	
		Supplier identification information	
	Physical characteristics	Clear solution	
		No foreign material	

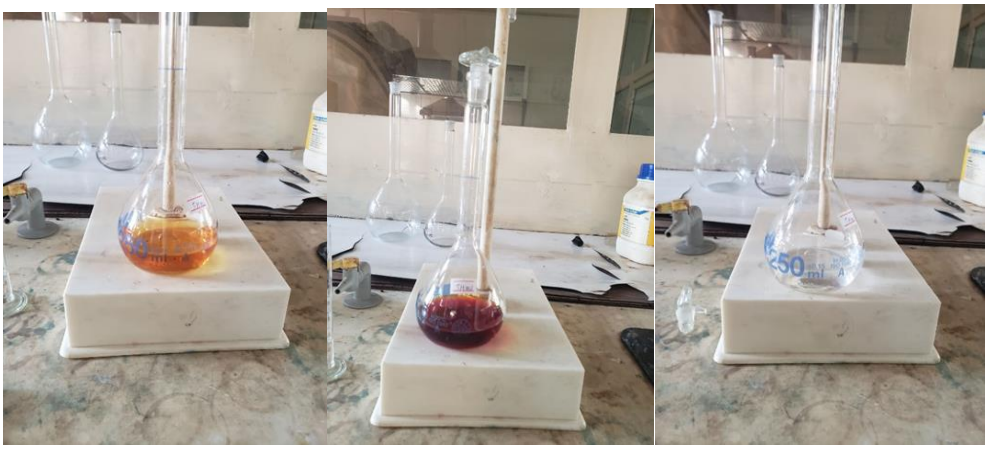
Annex IV: The representative photo of one negative and five positive results for the identification test (Photo courtesy Gadisa Chala)

Annex V: The representative photo of laboratory work (Photo courtesy Gadisa Chala)

Annex V a: Quality analysis of sodium hypochlorite

Measurement of pH Value

Assay



Annex V b: Surface cleaning efficacy of sodium hypochlorite