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Assessment of the bacteriological quality and efficacy of two hand sanitizers sold within Ilishan-Remo Community of Ogun State, Nigeria

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Abstract

Background. Hand sanitizers have been recognized as an effective means of reducing bacterial load and transmission. It is needful to periodically assess the bacteriological status of individual products due to batch variation. **Aim**. This study was designed to assess the bacteriological quality and efficacy of two hand sanitizers sold within the Ilishan-Remo community of Ogun State, Nigeria, amidst the COVID-19 pandemic. **Methodology**. Samples of two brands of hand sanitizers were procured and assessed using standard bacteriological methods, including Sterility test, Surface viable count, Gram-stain, Motility test, Biochemical tests, Quantitative suspension test, and Agar diffusion test. Data were analyzed with paired-samples T-Test using Statistical Package for the Social Sciences -Version 20.0 (SPSS-20.0) to assess for significant variation between the effectiveness of the two hand sanitizers. P-values $\leq$0.05 was considered significant. **Results**. The study's outcome showed the satisfactory bacteriological quality of both hand sanitizers tested. However, the mean bacterial load was not significantly reduced after sterilization using both hand sanitizers. The hand sanitizers' bactericidal activity was also considered unsatisfactory since the Log reduction was less than 5. Brand B hand sanitizer proved to be more potent than Brand A at the contact time. Each of the products displayed varying inhibitory activities against the bacterial isolates. **Conclusion**. The study highlighted the need to periodically assess the bacteriological quality and efficacy of hand sanitizers to guarantee the general safety of the end users and ensure proper infection control.

Key word: *Bacteriological quality, Efficacy, Hand sanitizer, Ogun State, Nigeria.*

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Introduction

The hands are commonly implicated in spreading harmful pathogens (such as bacteria and viruses) in health care and community settings. Contamination of the hands usually occurs when an individual comes in contact with contaminated surfaces daily. These pathogens may be spread directly by shaking someone's hands or indirectly by touching an object that others have already touched. As a result, the hands may accumulate many microorganisms, which can be passed from surface to surface or person to person unintentionally(1).

Since the Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2) began, infection control and prevention policy has strongly emphasized hand hygiene and respiratory safety to prevent the virus from spreading(2). Thus, the maintenance of proper hand hygiene is considered to be the key component of the prevention and control of infections both in community and healthcare settings. It is of the utmost importance in reducing the colonization and infection transmission between persons(3).

Hand hygiene practices encompass hand washing with water and soap, antiseptic hand washing with antiseptic detergent and water, and antiseptic hand sanitization with antiseptic hand rubs(4). Hand washing involves using water, friction, and soap to eliminate dirt and microorganisms from the hands. In recent times, the availability of hand sanitizers for use when soap and water are not provided has increased(5). Antiseptic hand rubs, also known as Hand sanitizers, are agents applied to the hands to eliminate common pathogens(6). The effectiveness of hand sanitizers in reducing infection rates is well documented and is generally

applicable, especially where there is restricted access to water, and they have become an essential commodity in everyday life in hospitals and the community(7).

Prior to the COVID-19 pandemic, hand sanitizers had gained popularity in Nigeria during the 2014 outbreak of Ebola Virus Disease (EVD), which had claimed numerous lives and then received worldwide attention as a public health measure to prevent the morbidity of the SARS-CoV-2 as with preceding contagious pathogens. As a result, hand sanitizers have been developed in different forms (such as liquid, gel, and foam), with varied mixtures of ingredients and modes of delivery. Given the attractiveness of hand sanitizers during this pandemic(8), the use of alcohol-based hand sanitizers was specially recommended as an effective hand sanitizer against the morbidity of the virus, and due to this development, the demand for alcohol-based hand sanitizers in the market has drastically skyrocketed(9).

However, most hand sanitizers have been deemed relatively ineffective against bacterial spores, non-enveloped viruses such as the norovirus, and encysted parasites such as Giardia spp. The proper use of hand sanitizer does not require water; less time compared to hand washing, and does not require hand drying with possibly tainted or contaminated surfaces. In order to obtain the expected effect of pathogen control, sanitizers with the ability to effectively eliminate microbes must be correctly used(10). Although, according to the Centres for Disease Control and Prevention(11), hand washing is a more desirable method of hand hygiene as hand sanitizers are not reliable in situations where the hands are greasy or visibly soiled, as well as hands contaminated with chemicals such as pesticides or heavy metals like lead.

The use of hand sanitizers gained more popularity during the Coronavirus pandemic and was highlighted as one of the recommended control measures for reducing viral transmission. This led to a worldwide shortage of suitable hand sanitizer products. As a result, various formulations were formulated and sold in the market to meet the shortage of hand sanitizers. Hand sanitizers have become more popular because of their ease of use, proven efficiency, and increased accessibility. However, several products marketed to the public as antimicrobial hand sanitizers are ineffective in reducing bacterial counts on the hands despite the claim of reducing harmful bacteria by 99.9%. While the production and sales of standardized hand sanitizers are being encouraged by the World Health Organization(12), due to disparity in the quality and potency of different batches of the same hand sanitizer, as well as the emergence and re-emergence of mutants, the need to periodically verify and re-verify the bacteriological quality and efficacy of individual product cannot be overemphasized. The study aims to evaluate the bacteriological quality and efficacy of two selected hand sanitizers sold within the Ilishan-Remo Community, Ogun State, Nigeria, amidst the COVID-19 pandemic.

Materials and Methods

Study Design

This prospective, observational and analytical study was carried out at the Department of Medical Laboratory Science, Babcock University, Ilishan-Remo, Ogun State, Nigeria, for the period of April-June, 2021.

Test Hand Sanitizers, Control, Neutralizer, and Diluent

Two different brands of hand sanitizers were procured from vendors in the Ilishan-Remo Community of Ogun State and were transported to the Medical Microbiology and Parasitology Unit of the Department of Medical Laboratory Science Babcock University, Ilishan-Remo, Ogun State. Sterile distilled water was used as a control. The neutralizer comprised of a mixture of equal volumes of 1% Sodium thiosulphate and 0.1% Tween 80, while sterile saline was used as a diluent.

Physical Examination of the Test Hand Sanitizers

Upon procurement of the test hand sanitizers, the products were physically inspected for the following: Manufacturer details, product composition, active ingredients, volume, physical evidence of deterioration, e.g., color and odor, expiry date, batch number, National Agency for Food and Drug Administration and Control (NAFDAC) registration number, etc. and recorded before analysis of the test hand sanitizers.

Assessment of Bacteriological Quality of Hand Sanitizers

Sterility Test

The method described by Maurer(13) was used to identify the presence of bacterial contaminants in the hand sanitizers. A 0.1 ml sample of each hand sanitizer was added to a 0.9 ml sterile diluent, which also contained a 0.1 ml neutralizer to neutralize the residual activity of the hand sanitizers. About 0.02 ml of the diluted sample was placed on each prepared nutrient agar (NA) plate. The NA plate was incubated in the incubator (Uniscope Laboratory incubator, Surhifriend Medicals, England) at 37°C for three days. Five or more colonies on the NA plates indicate contamination of the hand sanitizers. Bacterial isolates were identified using standard bacteriological methods.

Sample Size and Collection

Twenty (20) Student-volunteers consisting of 10 males and ten females from diverse ethnic, religious, and cultural experiences were recruited for the study and grouped into different categories according to the Brand of hand sanitizers designated as Hand sanitizer A and Hand sanitizer B.

Pre-sterilization Phase

A swab sample from each student's hands (palms

and fingers) was collected in duplicates aseptically with the aid of sterile swab sticks moistened with sterile normal saline solution before the application of the hand sanitizers. The swab sticks were used to rub on the hands and palms of the participants to enable sample collection. The swab sticks were decapitated and heads were placed in screw-capped tubes containing 3 ml of Tryptic Soy Broth (TSB) medium.

Sterilization Phase

About 3ml of the hand sanitizer was applied on both palms of the subject to ensure proper coverage of both hands. They were instructed to rub the assigned Brand of hand sanitizer all over the surface of their palms and fingers until both hands became dry. In addition, they were also instructed to avoid touching any contaminated surface until the second sample was collected.

Post-Sterilization Phase

Another sterile swab stick was used to collect the second sample from the sterilized hands 10 minutes post-sterilization of the hands. The time between the application of the hand sanitizer and swabbing the hand, quantity of hand sanitizer used, application technique, application time, and the bacterial count method used were kept constant throughout the study.

Each swab stick was streaked directly on the plates containing blood agar (BA) medium, MacConkey Agar medium and Mannitol Salt Agar medium already prepared and sterilized (using Vertical pressure steam sterilizer SM-1000, Microfiedl instrument, England) according to the manufacturer's instruction. Afterward, the specimens were transported to the laboratory in a tight, sealed case for immediate bacteriological examination.

Sample Culture

To culture the samples, the screwed-cap tubes enclosing each sample were manually vortex vigorously for appropriate mixing, and spillage was avoided. Then 0.002 ml of the sample was inoculated into plates containing Blood agar (BA) medium and MacConkey agar (MCA) medium with a sterile calibrated wire loop. This was incubated at 37°C for 24 hours, as described by Mokhtari et al.(14).

Determination of Population Density Pre- and Post-sterilization

To determine the bacterial load before and after sterilization of the hand, viable surface count as described by Miles and Misra(15) was carried out. The population density (CFU/ml) was calculated using the formula: *Mean no of colonies x no of drops/ml x dilution factor*. In contrast, the surface reduction rate of the hand sanitizer was calculated by subtracting Log10 post-sterilization count from Log10 pre-sterilization count.

Isolation of Pure Cultures

Pure cultures of isolate within a mixed bacterial population were attained using the streak plate technique as described by Ochei and Kolhatkar(16). Aseptic streaking of the inoculum with the help of a wire loop results in continuous dilution of the inoculum to give well-separated surface colonies.

Identification of Bacterial Isolates

After incubation, plates containing cultured samples were examined, and colonies of bacteria were recognized by Gram-stain (Viewed under the microscope Olympus CX23 binocular microscope) using X100 objective lenses with oil immersion), motility test, and routine biochemical tests such as determining the fermentation of glucose, lactose and sucrose in the triple sugar iron (TSI) medium, urea hydrolysis, producing indole from tryptophan, use of citrate, producing hydrogen sulfide, oxidase, catalase, and coagulase production as described by Cheesbrough(17). The results of the above tests were entered into IDENTAX bacterial identifier (a free software developed using Sun Microsystems' Java Technology) for taxonomically identifying bacteria isolates using phenotypical characteristics.

Evaluation of the Efficacy of Hand Sanitizer

Evaluation of the efficacy of the hand sanitizers against each bacterial isolate was determined using the quantitative suspension test (QST) as described by Merap et al.(18) and Abban et al.(19).

Standardization of Organism

A single isolated colony of bacteria was removed from tryptic soy agar (TSA) plates and grown separately in 10 ml of tryptic soy broth (TSB) for 24 hours at 37°C. After incubation, the 24-hour broth culture was filtered with a saline pre-wet filter paper in order to remove slime and centrifuged for 20 minutes at 2000 rpm with a rotor centrifuge (Centrifuge 80-2, Medfield equipment and scientific, England). Afterward, the cell pellets were washed with 10 ml of TSB. Then the population density of the bacterial suspensions in the TSB (about 10^7 CFU/ml) was tuned to match that of 0.5 McFarland Standard (10^5 CFU/ml) by making a dilution of 1:100 in sterile TSB.

Quantitative Suspension Test (QST)

Briefly, 0.1ml of the homogeneous bacterial suspension was added to 0.9 ml of the hand sanitizer solutions and mixed gently at room temperature for the contact times of 0, 1, 3, 5 and 10 minutes. The timer was started when the test bacterial suspension and hand sanitizer were combined. Then at Time X, the specified contact time, 0.1 ml of the hand sanitizer-organism mixture was removed and transferred to a tube containing 0.9 ml of neutralizer (the 100 designated as Tube A) and mixed thoroughly. Within 5 minutes of the transfer to the neutralizer tube, three additional ten-fold dilutions in saline blanks were made to achieve 10^{-1}, 10^{-2}, and 10^{-3} dilutions

(designated Tube B, Tube C, and Tube D, respectively). 0.1 ml of each dilution was inoculated onto nutrient agar plates in duplicate by the spread-plate technique and incubated at 37°C for 24 hours. The TSA plates were observed for any visible growth after incubation. The surviving bacterial colonies were enumerated, multiplied by a factor of a hundred (100), and expressed as colony-forming units per milliliter (CFU/ml). Controls were put up for all the test organisms to show the activity of the neutralizer. For control, 0.1 ml of 0.5 McFarland broth of each test organism was vortex with 0.9 ml of neutralizer in separate tubes and then transferred to TSB, as the procedure described with hand sanitizers. Subsequently, all the controls were streaked onto TSA plates. Incidence of growth indicates that the neutralizer is not inhibiting the bacterial isolates tested. In the same way, 0.1 ml of each hand sanitizer was mixed with 0.9ml of neutralizer, then 0.1 ml suspension of the test organism (0.5 McFarland standard) was added to each tube, later directly transferred and incubated in TSB and streaked on TSA plates. Growth on TSA plates shows effective neutralization of the hand sanitizer activity.

Determination of Bactericidal Effect of the Hand sanitizers

The logarithm reduction factor (The bactericidal effect) of the hand sanitizers was determined by subtracting the logarithm of the survivors after contact with hand sanitizer from the logarithm of the original inoculum in control plates using the following formula:

Logarithmic Reduction Factor (RF) = Log Nc – Log Nd

Where:
Nc = Number of colonies from control plates (No hand sanitizer)
Nd = Number of colonies from test plates (after contact with hand sanitizer)

Log10 reductions of 5 or more were used as an indication of satisfactory bactericidal activity, *i.e.*, at least 99.99% of the organisms killed.

Determination of the Killing Rate of the Hand sanitizers

The killing rate of the hand sanitizers, on the other hand, was calculated by plotting the logarithms of surviving cells (CFU/ml) against the exposure time (min) of the hand sanitizer as described by Kelsey and Maurer(20).

Agar Diffusion Test

Agar diffusion test using the punch-hole method described by slack(21) was used to determine the susceptibility of the test isolates to the hand sanitizers. Sterile semi-solid Nutrient Agar (NA) plates were prepared. 1ml of 24-hour old standardized cultures of bacteria broths were used to flood the surface of the NA plates. The plates were swirled, allowing the inoculums to spread on the surface of the agar, and the excess was drained off, in a disinfectant jar. With a sterile cork borer of 6mm diameter, six ditches (wells) were bored at equal distances around the plates. The bottom of each well was sealed with one drop of sterile molten nutrient agar to prevent diffusion of the hand sanitizers under the agar. Zero point one milliliter (0.1 ml) of each Brand of hand sanitizer was aseptically dropped into each appropriately labeled well on the plate (wells 1-4). The 5th and 6th wells functioned as negative and positive controls and were filled with sterile distilled water and Ciprofloxacin (used at tissue concentration- 10l/ml), respectively. The inoculated plates were left on the table for 1 hour to allow pre-diffusion of the hand sanitizers into the agar. The NA plates were incubated aerobically at 37°C for 24 hours. The resulting zone diameter of inhibition was measured using a ruler calibrated in millimeters (mm). The susceptibility of the test isolates was demonstrated by inhibitions which were specified by a clear zone around the wells to which the hand sanitizers had been added.

Data Analyses

Microsoft Excel was used for data entry. Statistical analysis was carried out with Paired-Samples T-Test using Statistical Package for the Social Sciences - Version 20.0 (SPSS-20.0) to test for significant differences between the efficacies of the hand sanitizers. P-values 0.05 were designated significant(22).

Results

Two brands of hand sanitizers (designated as Brand A and Brand B) sold in Ilishan-Remo, Ogun State, Nigeria, were assessed for their bacteriological quality and efficacy. A total of 10 samples (5 samples per Brand) were purchased from local vendors.

The detail of the hand sanitizers is presented in Table 1. The Brand A Hand Sanitizer comes in a 500ml capacity container. It is colorless, transparent, and slightly fragrant odor, with a gel-like texture. Active ingredients include aqua, ethanol 70% v/v, glycerine, propylene glycol, neutralizer, carbopol, and fragrance. Brand B Hand Sanitizer, on the other hand, comes in a 100ml capacity container. It is also colorless, transparent, and has a slightly fragrant odor, with a gel-like texture. Active ingredients include carbomer, cetrimide, PEG-100 monostearate, ethyl alcohol, 2-amino-2-methyl-1-propanol, water, propylene glycol, glycerin, and fragrance.

Five (5) samples of each Brand were assessed for their bacteriological quality and efficacy among student-volunteers of Babcock University. The socio-demographic Characteristics of the study subjects are presented in Table 2. A total of 20 volunteers (16-25 years) Babcock University Students were recruited for the study. Ten (10, 50%) of them were males, while the remaining 10 (50%) were females. 17 (85%) of them were Christians by religion, while the remaining 3 (15%) were Muslims. Eight (8, 40%) of them were Yoruba, 6 (30%) were Igbo, and the remaining 6 (30%) belonged to other tribal groups.

Table 1
Details of Test Hand Sanitizers

Parameters	Brand A	Brand B
Active Ingredients	Aqua, Ethanol 70% v/v, Glycerine, Propylene glycol, Neutralizer, Carbopol and Fragrance.	Carbomer, Cetrimide, PEG- 100 Monostearate, Ethyl Alcohol, 2- Amino-2-Methyl-1-Propanol, Water, Propylene glycol, Glycerin, Fragrance.
Size (Volume)	500ml	100ml
Physical Appearance, Texture and Scent	Colorless, Transparent, Slightly Fragrant, With a gel-like texture.	Colorless, Transparent, Slightly fragrant, With gel-like texture.
Manufacture Date	December 2020	November 2020
Expiry Date	November 2022	October 2022

Table 2
Socio-demographic characteristics of the study subjects

Characteristics	Category	Frequency	Percent
Gender	Male	10	50
	Female	10	50
	Total	20	100
Age Group (Years)	16-20	10	50
	21-25	10	50
	Total	20	100
Religion	Christianity	17	85
	Islam	3	15
	Others	0	0
	Total	20	100
Marital status	Single	20	100
	Married	0	0
	Total	20	100
Tribe	Yoruba	8	40
	Igbo	6	30
	Hausa	0	0
	Others	6	30
	Total	20	100

The use of hand sanitizer and hand hygiene practices of the study subjects is presented in Table 3. All of the subjects (100%) were aware that hand hygiene is necessary for day-to-day life. With regard to how they maintain hand hygiene, half of the participants (50%) indicated washing their hands with both soap and water, together with the use of hand sanitizers, as their preferred hand hygiene practice, 7(35%) chose washing their hands with water and soap only, and the remaining 3 (15%) use hand sanitizers only.

Table 3
Use of hand sanitizer and hand hygiene practices of the study participants

Characteristics	Responses	No. of participants N (%)
Is hand hygiene necessaryin day-to-day life?	Yes	20 (100)
	No	0 (0)
How do you maintain hand hygiene?	Wash with water only	0 (0)
	Wash with both soap and water	7 (35)
	Use Hand sanitizers c	3 (15)
	All of the above	10 (50)
Which type of hand sanitizer do you prefer?	Gel	14 (70)
	Spray	4 (20)
	Liquid	2 (10)
Attributes searched for in hand sanitizers	Packaging	1 (5)
	Effectiveness	12 (60)
	Ingredients	1 (5)
	Effectiveness and Packing	1 (5)
	Effectiveness and Ingredients	2 (10)
	Effectiveness and Fragrance	3 (15)
Do you check the expir date before purchasing hand sanitizers?	Yes	15 (75)
	No	5 (25)
Do you think hand cleansing is achieved more rapidly using hand sanitizer than hand washing?	Yes	11 (55)
	No	9 (45)
Are sanitizers more effective against microbes than hand washing?	Yes	10 (50)
	No	10 (50)
How often do you use hand sanitizer in a day?	Once a day	5 (25)
	2-4 intervals in a day	11 (55)
	5-7 intervals in a day	2 (10)
	More than seven intervals in a day	2 (10)
Where do you use hand sanitizers mostly?	At home	0 (0)
	At School	8 (40)
	At public places	12 (60)
Do you carry pocket hand sanitizer around?	Yes	15 (75)
	No	5 (25)
How often do you have access to hand sanitizer in public places?	Rarely	1 (5)
	Sometimes	6 (30)
	Often	11 (55)
	Always	2 (10)
Does COVID-19 have an effect on your usage of hand sanitizers?	Yes	18 (90)
	No	2 (10)
To what extent has COVID-19 affected your usage of hand sanitizers?	Immense	3 (15)
	Considerable	11 (55)
	Minor	4 (20)
	Never	2 (10)

With regards to the preference for hand sanitizers, 14 (70%) of the participants indicated that they preferred gel hand sanitizers, while 4 (20%) and 2 (10%) of the subjects indicated that they preferred spray and liquid hand sanitizers, respectively. Effectiveness and ingredients were the most identified attribute that the participants (12, 60%) search for in hand sanitizers. 15 (75%) of them check the expiry date of hand sanitizers before making purchases. 11 (55%) believe hand cleansing is achieved more rapidly using hand sanitizers than hand washing. Meanwhile, only 10 (50%) indicated hand sanitizers are more effective against microbes than hand washing. While most of the participants (11, 55%) indicated that they sanitize their hands 2-4 times a day, 5 (25%) use hand sanitizers Once daily, 2 (10%) use hand sanitizers 5-7 times daily, and the remaining 2 (10%) use hand sanitizers more than seven times daily. The majority of them (12, 60%) indicated that they mostly use hand sanitizers in public places, and 8 (40%) indicated that they mostly use hand sanitizers at school. 15 (75%) of the participants indicated that they carry a pocket hand sanitizer around. While only 11 (55%) indicated that they often have access to hand sanitizers in public places, 6 (30%) indicated that they sometimes have access to hand sanitizers in public places, 2 (10%) indicated that they always have access to hand sanitizers in public places, while only 1 (5%) of the participants indicated that they rarely have access to hand sanitizers in public places. A large proportion of 18 (90%) indicated that COVID-19 had an effect on their usage of hand sanitizer to a varied extent as follows: Immense (15%), Considerable (55%), Minor (20%), and Never (10%).

All the samples of the two brands of hand sanitizers examined were found to be sterile as there was no growth on the culture plates (Negative culture). The percentage occurrence of contamination in both brands of hand sanitizers was zero (0%). None of the five batches of Brand A and Brand B hand sanitizers tested had bacterial growth after the appropriate days of incubation on Nutrient Agar plates.

Table 4

Bactericidal activities of Brand A hand sanitizer on selected test isolates

Isolates	Mean Zone Diameter of Inhibition (mm)						
	Batch 1	Batch 2	Batch 3	Batch 4	Batch 5	+C	-C
Escherichia coli	0	7	0	12	0	25	0
Pseudomonas aeruginosa	0	15	0	10	0	24	0
Klebsiella pneumoniae	0	8	0	11	0	21	0
Salmonella typhi	0	10	0	12	0	25	0
Coagulase Negative Staphylococcus	0	14	0	12	0	24	0

KEY: *+C = Positive Control, -C = Negative Control*

Table 4 shows the bactericidal activities of Brand A hand sanitizer on selected test isolates. The hand sanitizer displayed inhibitory activities against all the test isolates. However, the mean zone diameter of inhibition varied between the five batches of the same hand sanitizer. Batch 2 gave the highest zone diameter of inhibition against *P. aeruginosa* (15mm) and the least against *E. coli* (7mm). Batch 4 gave the highest zone diameter of inhibition against E. *coli, Salmonella typhi* and *Coagulase-negative Staphylococcus spp.* (12mm) and the least against *Pseudomonas aeruginosa* (10mm). Meanwhile, Batch 1, 3, and 5 did not display any inhibitory activity on the test isolates.

The bactericidal activities of Brand B hand sanitizer on selected test isolates are presented in Table 5. All the batches of Brand B hand sanitizer examined, displayed inhibitory activities against all the test isolates with varying mean zone diameter of inhibition between each batch. Batch 1 gave the highest zone diameter of inhibition against Coagulase Negative Staphylococcus (12mm) but had no inhibitory activity against *Salmonella typhi* (0mm). Batch 2 gave the highest zone diameter of inhibition against *P. aeruginosa* (15mm) and the least against *K. pneumoniae* (8mm). While Batch 3 gave the highest zone diameter of inhibition against *K. pneumoniae* (14mm) and the least against *E.coli* (10mm). Batch 4 gave the highest zone diameter of inhibition against *P. aeruginosa* (13mm) and the least against *K. pneumoniae* (11mm). Lastly, Batch 5 gave the highest zone diameter of inhibition against *E.coli, K. pneumoniae*, and *Coagulase Negative Staphylococcus* (12mm) and the least against *Salmonella typhi* (10mm). The positive control gave a range of 21-25mm zone of inhibition against all the test isolates, while the negative control did not show any zone of inhibition (0mm).

Table 5

Bactericidal activities of Brand B hand sanitizer on selected test isolates

Isolates	Mean Zone Diameter of Inhibition (mm)						
	Batch 1	Batch 2	Batch 3	Batch 4	Batch 5	+C	-C
Escherichia coli	11	12	10	12	12	25	0
Pseudomonas aeruginosa	7	15	12	13	11	25	0
Klebsiella pneumoniae	7	8	14	11	12	21	0
Salmonella typhi	0	12	11	12	10	25	0
Coagulase Negative Staphylococcus	12	14	13	12	12	24	0

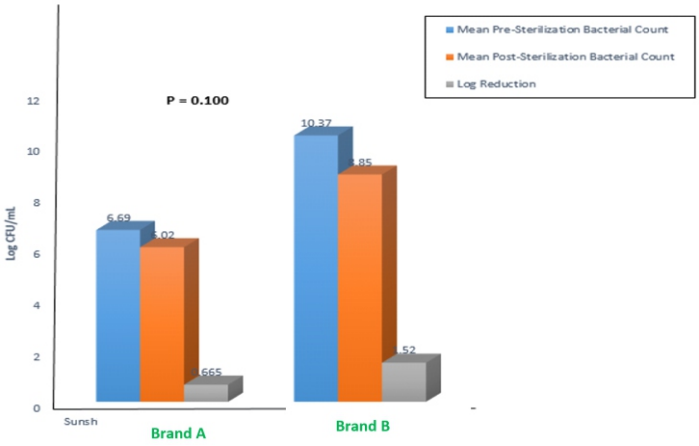
KEY: *+C = Positive Control, -C = Negative Control*

The mean bacterial count before and after sterilization with the selected hand sanitizers is presented using a histogram (Figure 1). There was a Log reduction of 0.665 and 1.52 after sterilization with Brand A and Brand B hand sanitizer, respectively. The mean bacterial count was not significantly decreased after sterilization using both brands of hand sanitizers. The hand sanitizers' bactericidal activity was considered inadequate since Log reduction was <5.

Mean bacterial count was not significantly decreased after sterilization using both selected hand sanitizers ($P>0.05$ is considered statistically not significant). The bactericidal activity of the hand sanitizers was considered not adequate since Log reduction was <5.

Fig. 1

A histogram showing means bacterial counts before and after sterilization with selected hand sanitizers



The killing rate of Brand A and Brand B hand sanitizer for each bacterial isolate is presented in Figures 2 and 3, respectively, using line charts. The log of living cells remained almost constant for the control (*i.e.*, organism + neutralizer only) throughout the 10 minutes of contact time; whereas for the test (*i.e.*, organism + hand sanitizer + neutralizer), it differed with different contact times. There was a log reduction of 1 for *E. coli* in the first minute of contact with both Brand A and Brand B hand sanitizer. However, at the 5th minute, Brand A hand sanitizer gave a log reduction of 4 for E. coli, while a zero bacterial population was recorded for Brand B hand sanitizer. There was no log reduction for *Pseudomonas aeruginosa* in the first minute of exposure to Brand A. However, a log reduction of 2 was observed at the 3rd minute. On the other hand, a log reduction of 1 was observed in the first minute, while a log reduction of 3 was observed at the 3rd minute of contact with Brand B hand sanitizer. At the 5th minute, a log reduction of 4 was observed for Brand A hand sanitizer, while a zero bacterial population was observed for Brand B hand sanitizer. For K. pneumoniae, Brand A and Brand B gave a log reduction of 1 in the first minute, but a log reduction of 2 and 3, respectively, in the 3rd minute. And while the bacterial load was reduced to 1log CFU/ml at the 5th minute by Brand A, it had reduced to zero for Brand B hand sanitizer. On the other hand, no log reduction of S. typhi was noted when exposed to Brand A hand sanitizer for the first minute; however, there was a log reduction of 2 when exposed to Brand B hand sanitizer. By the 5th minute, Brand A gave a log reduction of 3, while a zero bacterial load was observed for Brand B's hand sanitizer.

In conclusion, Brand A hand sanitizer and Brand B hand sanitizer gave a log reduction of 2 and 3, respectively, for *Coagulase Negative Staphylococcus* in the first minute of exposure. And a log reduction of 3 and 4 at the 3rd minute for Brand A and Brand B hand sanitizer, respectively. At the 5th minute, the bacterial load was decreased to 1 log CFU/ml by Brand A, and a zero bacterial population was recorded for Brand B hand sanitizer.

Fig. 2

A line chart showing the killing rate of bacterial Isolates when exposed to Brand A hand sanitizer for 10 minutes

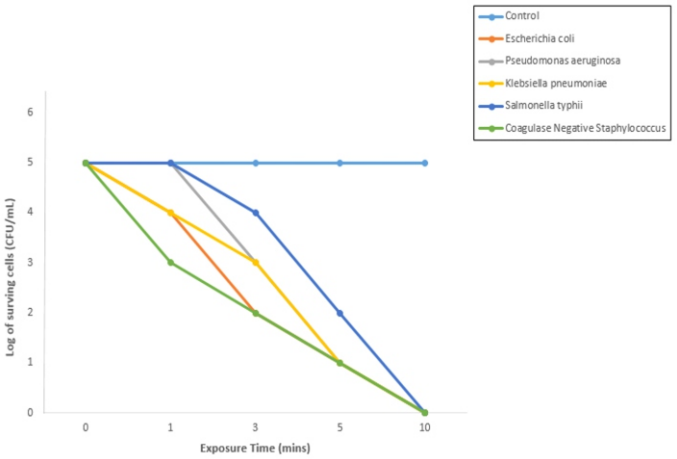


Fig. 3

A. Line chart showing the killing rate of bacterial isolates when in contact with Brand B hand sanitizer for 10 minutes

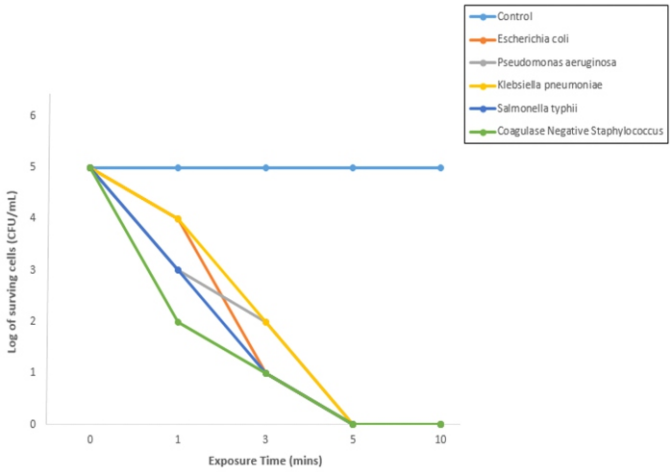
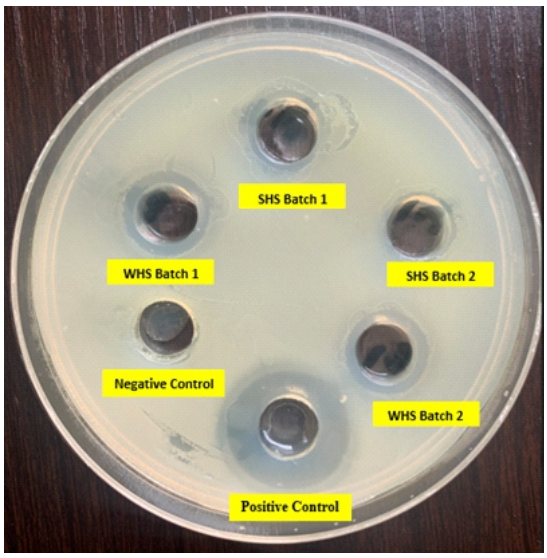


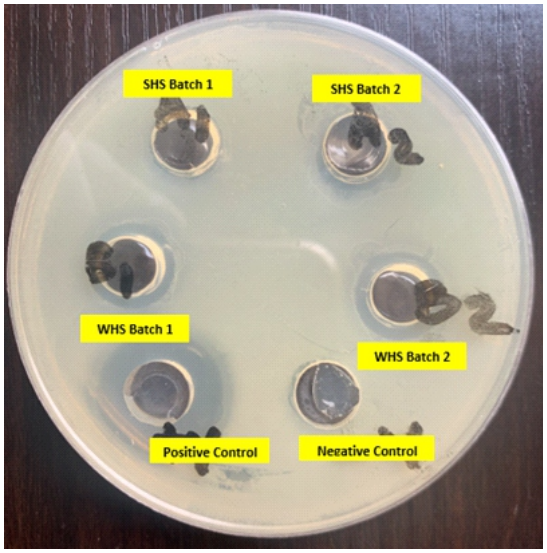
Fig. 4

Picture showing inhibitory activity of Brand A and Brand B Hand Sanitizer against Klebsiella pneumoniae



Key: *SHS = Brand A Hand Sanitizer, WHS = Brand B hand Sanitizer*

Fig. 5
Picture showing inhibitory activity of Brand A and Brand B Hand Sanitizer against Escherichia coli



Key: *SHS = Brand A Hand Sanitizer, WHS = Brand B hand Sanitizer*

Discussion

This current study was designed to assess the bacteriological quality and efficacy of two hand sanitizers (Brand A and Brand B) sold within the Ilishan-Remo community of Ogun State, Nigeria. The zero bacterial counts recorded for the two test hand sanitizers (Brand A and Brand B) show that the five batches of the two hand sanitizers tested were of great bacteriological quality and can therefore be considered safe for use. The outcome of this work is consistent with the study conducted by Enitan *et al.* (2018), who reported a zero bacterial count for the two disinfectants tested (Jik and Lysol). Although, the current study differs from their work, which went further to assess the mycological quality of the disinfectants which 1 to 2 colonies of either *Microsporum spp. Trichophyton spp.* or Aspergillus spp. were recovered from Jik disinfectant in particular.

With reference to the efficacy of the test hand sanitizer, a greater Log reduction of the bacterial load was achieved with Brand B hand sanitizer than Brand A hand sanitizer. However, the bactericidal activity of both sanitizers was considered not satisfactory since the Log reduction obtained was less than 5. The outcome of this work agreed with that of Enitan *et al.*(23), in which the Log reduction achieved using 30% dilution of Jik was less than five and therefore thought not to be microbiologically satisfactory. Although, the 2.5% Lysol dilution tested was considered to be microbiologically satisfactory since the log reduction obtained was more significant than or equal to 5. It is, however, important to note that the efficacy of alcohol-based hand sanitizers may be affected by numerous factors, such as; the type of alcohol used, its concentration, the technique of application, as well as whether the hands are visibly soiled or greasy before the application of the hand sanitizer.

Furthermore, at 10 minutes of contact time, all of the test isolates exposed to Brand A hand sanitizer were killed entirely. Meanwhile, for the isolates exposed to Brand B hand sanitizer, it was observed that 5 minutes of contact time was sufficient for the killing of all the isolates.

The outcome of this present study differs from the work of Enitan *et al.*(23), who reported growth for all the isolates exposed to 30% Jik, except for *E. aerogenes, S. epidermidis*, and *P. mirabilis* at 10 minutes contact time. Although, 5 minutes of contact time was sufficient for the destruction of the same, except for *P. mirabilis* which was killed at 10 minutes when 2.5% Lysol was used.

In this study, the zone diameter of inhibition obtained for the following organisms: *E. coli* (7-12mm), *P. aeruginosa* (7-15mm), and *K. pneumoniae* (7-14mm) were found to be lower for the same organisms as reported by Oke *et al.*(24), 26mm, 28mm and 19mm, respectively, when tested using Hegel sanitizer. However, our result is comparable to the work of Oke *et al.*(24), who recorded 14.5mm for P. aeruginosa when tested against Dettol but disagrees with their report on Samclean and SKP which showed no activity against all the test organisms.

The outcome of this study also agrees with Jain et al.(25), in which all the hand sanitizers examined in their study (Sterillium, Pure hands, Dettol and Lifebuoy) were effective against all the test organisms (*S. aureus, S. epidermidis, P. aeruginosa, E. coli* and *E. faecalis*). The maximum inhibition was given by Sterilium against S. aureus (2±71.414mm). Meanwhile, the minimum inhibition was given by Pure hands against S. aureus (3.5±4.95mm). The varying zones diameter of inhibition confirms the existence of ineffective products sold in the market, which may be due to inconsistency in preparation protocol, low potency of the hand sanitizers used, or it could be a result of the intrinsic resistance of the bacterial isolates to the test hand sanitizers.

Bacterial contaminants of the hands consist of transitory flora. Transient bacterial floras are recurrently acquired and may be conveyed by direct hand interaction between human skin and the inanimate environment such as work surfaces or food. They are acknowledged to colonize the superficial layers of the skin and can be easily removed by performing appropriate hand hygiene practices. Some examples of transient flora that survive well in the hospital environment include; *Staphylococcus aureus*, enterococci, and Gram-negative bacilli such as *Pseudomonas spp, Klebsiella spp*, and *Acinetobacter spp*.

The organisms tested in this study are known contaminants and colonizers of the hand surfaces. *Escherichia coli* are usually harmless and normally constitute the normal flora of a healthy human intestinal tract. However, they are also known to be pathogenic outside the intestinal tract and cause illness in humans, including diarrhea, abdominal pain, fever, and sometimes vomiting, transmitted through contaminated water or food or contact with animals or persons. *Pseudomonas aeruginosa* can also be found in the intestinal tract, water, soil, and sewage, and it is frequently found in moist environments in hospitals, and as a result, P. aeruginosa is often implicated in hospital-acquired infections.

Klebsiella pneumoniae, in healthcare settings, can be spread through person-to-person contact (for example, from patient to patient via the contaminated hands of healthcare personnel or other persons). Salmonella typhi is known to cause enteric fever (Typhoid). The infection is frequently passed on through contaminated drinking water and food, and it is more prevalent in dwellings where hand washing is less common(26). Furthermore, *Coagulase-negative staphylococci* are normal flora of the skin. They are not regarded as pathogens on intact skin but are capable of causing infections when the skin has been broken(27).

Conclusions

The bacteriological quality of the two hand sanitizers tested was considered satisfactory as no bacterial contaminants were recovered from them following the sterility test. The bactericidal activity of the hand sanitizers was considered not satisfactory since the Log decrease was <5. Brand B hand sanitizer appeared to be more potent than Brand A at the contact time tested. All of the selected bacterial isolates were completely destroyed by the two hand sanitizers within 10 minutes of contact time. The test hand sanitizers displayed a batch-dependent antibacterial activity against the bacterial isolates. The outcome of this study underscores the need to periodically assess the bacteriological value and efficacy of hand sanitizers to ensure their effectiveness in reducing bacteria in the hands of end users, as well as to ensure proper control of infections and to further eliminate the prevalence of ineffective products sold in the market.

Recommendation

We recommend that the manufacturers should increase the concentration of the anti-bacterial agents in the sanitizers in order to boost their potency.

Limitation of the study

Molecular characterization of the test isolates used for the study was not done due to cost.

Consent

All authors declared that 'written' informed consent was obtained from the participants with an assurance of anonymity and confidentiality before the commencement of the study.

Ethical Approval

The Babcock University Health Research Ethics Committee (BUHREC) gave ethical approval for the study with ethical approval registration number: BUHREC 469/21.

Competing Interests

No competing interests exist, as declared by the authors

References

1. Ford C, Park LJ. Hand hygiene and handwashing: key to preventing the transfer of pathogens. British J of Nurs. 2018; 27(20):1164-166.
2. Singh D, Joshi K, Samuel A, Patra J, Mahindroo N. Alcohol-based hand sanitizers as first line of defense against SARS-CoV-2: a review of biology, chemistry and formulations. Epid J and infect. 2020;148. Available from: e229. Doi:10.1017/S0950268820002319.
3. Sharma RP, Dutta S, Kumar T, Singh S, Sharma A. Role of Alcohol-Based Hand Rubs (ABHR) in the COVID-19 Era: A Concise Review. Intl J of Pharma sc. Review and Research. 2020; 64(1): 179-182. Available from: Doi: 10.47583/ijpsrr.2020.v64i01.032.
4. Berardi A, Perinelli DR, Merchant HA, Bisharat L, Basheti IA, Bonacucina G, Cespi M, Palmieri GF. Hand sanitizers amid COVID-19: A critical review of alcohol-based products on the market and formulation approaches to respond to increasing demand. Intl j of pharma. 2020; Available from: DOI:10.1016/j.ijpharm.2020.119431.
5. Foddai AC, Grant IR, Dean M, Efficacy of Instant Hand Sanitizers Against Foodborne Pathogens Compared with Handwashing with Soap and Water in Food Preparation Settings: A Systematic Review. food protect J. 2016; 76(6):1040-054. Available from: Doi:10.4315/0362-028x.jfp-15-492.
6. Prajapati P, Desai H, Chandarana C. Hand sanitizers as a preventive measure in COVID-19 pandemic, its characteristics, and harmful effects: a review. J. Egypt. Public. Health. Assoc. 2022; Available from https://doi.org/10.1186/s42506-021-00094-x
7. Otokunefor K, Princewill I. Evaluation of antibacterial activity of hand sanitizers – an in vitro study. 2017; Doi: 10.4314/jasem.v21i7.9.
8. Golin AP, Choi D, Ghahary A. Hand sanitizers: a review of ingredients, mechanism of action, modes of delivery and efficacy against coronaviruses. Amer J of infect contrl. 2020; 48(9) 1062-067. Available from Doi: 10.1016/j.ajic.2020.06.182.
9. Oluwatuyi SV, Agbele AT, Ogunrinde ME, Ayo AT, Fayoke AB, Funmilayo OM, Deborah AA. Alcohol-Based Hand Sanitizers: Review of Efficacy and Adverse Effect. J of Health, Med and Nurs. 2020; Available from: Doi: 10.7176/JHMN/81-01.
10. Femi A, Benedicta CN, Anariochi OC. Hand Hygiene Practices and the Effectiveness of Hand Sanitizers at Controlling Enteropathogens among the Residents of a University Community in Osun State Nigeria. Microb Research J Intl. 2019; 27(3): 1-9. Available from: doi:10.9734/mrji/2019/v27i330100.
11. Centres for Disease Control and Prevention (CDC). Hand Sanitizer Use Out and About, 2021. https://www.cdc.gov/handwashing/hand-sanitizer-use.html [Last accessed on August 22, 2022].
12. World Health Organization (WHO). Guide to local production: WHO-recommended handrub formulations. 2010, https://www.who.int/publications-detail-redirect/WHO-IER-PSP-2010.5. [Last accessed on August 23, 2022].

13. Maurer, I. M. Hospital Hygiene; 3rd Edition, Richard Clay (The Chaucer Pren) Ltd., Great Britain, United Kingdom. 1985.
14. Mokhtari M, Zandi H, Jasmi Z, Sahl AF, Montazari A. The evaluation of efficacy of common disinfectants on microorganisms isolated from different parts of Shahid Sadoughi Accidents burns hospital in Yazd in 2011, J of Toloo-e-Beshadt. 2011; 14(3): 1-11.
15. Miles AA, Misra SS. Bacterial counts. Journal of Hygiene (London). 1938;38: 732-36.
16. Ochei JO, Kolhatkar AA. Plate Culture Methods In: Ochei, J. O., Kolhatkar, A. A. (eds.). Medical Laboratory Science: Theory and Practice, Tata McGrew-Hill, New Delhi, India. 2007b; pp. 591-592.
17. Cheesbrough M. "Biochemical tests to identify bacteria" In: Cheesbrough, M. (ed.). District Laboratory Practice in Tropical Countries, Part 2. Cambridge University Press, Cape Town, South Africa. 2006a; pp. 63-70.
18. Merap O, Melike E, Ekremkili C. Evaluation of bactericidal activity of certain Gluteraldehyde free disinfectants used in the disinfection of endoscopes and surgical devices by a quantitative suspension test. Hacettepe Uni J of the Faculty of Pharm. 2007; 27: 131-38.
19. Abban S, Jakobsen M, Jespersen L. A practical evaluation of detergent and disinfectant solutions on cargo container surfaces for bacteria
20. inactivation efficacy and effect on material corrosion. African J of Biotech. 2013; 12(23):3689-698, Available from: DOI: 10.5897/AJB2013.11994.
21. Kelsey JC, Maurer IM. An improved Kelsey-Sykes test for hand sanitizers. Pharma Microb J. 2001; 6:607-609.
22. Slack MP. "Practical aspects of antimicrobial chemotherapy" In: Weatherall, D.J., Warrel, D. A. and Ledingham, J. G. G. (eds.). Oxford Textbook of Medicine. 1985; pp. 530-543.
23. Shott S. "Statistics for health professionals". Saunders W B, Co. Philadelphia. 1990; pp. 313-336.
24. Enitan SS, Ochei JO, Digban KA, Akele YR, Nwankwo KJ, Arisi CP. Assessment of the Microbiological Quality and Efficacy of Two Common Disinfectants Used in Hospital Laboratory. Advances in Biomedical Sciences. 2018; 2(6): 31-43.
25. Oke MA, Bello AB, Odebisi MB, Ahmed El-Imam AM, Kazeem MO. Evaluation of antibacterial efficacy of some alcohol-based Hand sanitizers sold in Ilorin (North-Central Nigeria). Ife J of Sc. 2013; 15(1):111-117.
26. Jain VM, Karibasappa GN, Dodamani AS, Prashanth VK, Mali GV. Comparative assessment of antimicrobial efficacy of different hand sanitizers: an in-vitro study. Dent research J. 2016; 13(5): 424-431. Available from: DOI: 10.4103/1735-3327.192283.
27. Cheesbrough M. "Bacterial pathogens" In: Cheesbrough, M. (ed.). District Laboratory Practice in Tropical Countries, Part 2. Cambridge University Press, Cape Town, South Africa. 2006b pp. 157-205.
28. Jumaa PA. Hand hygiene: Simple and complex. International J of Infect Diseases. 2005; 9(1): 3-14. Available from: doi:10.1016/j.ijid.2004.05.005.