Evaluation of Antibacterial Efficacy of Randomly Selected Alcohol Based Hand Sanitizers Sourced from Grocery Shops within Lagos Metropolis on Some Local Bacterial Strains in COVID-19 Era

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ABSTRACT

Background: Health experts promote the use of alcohol based hand rubs to contain the spread of microbes. The emergence of novel SARS-CoV-2 virus has brought a worsening public health challenge that re-enacted the importance of hand hygiene globally.

Objective: This study evaluated the antimicrobial efficacy of locally made alcohol-based hand rubs sourced from grocery shops within Lagos metropolis, Nigeria.

Methods: We conducted a laboratory based in vitro experiment, using 20 randomly sourced hand sanitizers against standard Escherichia coli (ATCC 25922) and three locally characterized Multi-drug-Resistant bacterial strains (Staphylococcus aureus (NIMR/NTCC/GP056), Klebsiella pneumonia (NIMR/NTCC/GN065) and Proteus stutzin - (NIMR/NTCC/ GN029). Reference standard, 60 % isopropanol was used as positive control. Test suspension method as per European standard PrEN12054 was employed. The Colony Forming Unit/mL (CFU/mL) at base line and after each contact time (15, 30 & 45 seconds) with samples was recorded. The Logarithmic reduction factor (RF) and percent reduction were computed and expressed using descriptive statistics.

Results: Out of the 20 solutions tested (10 sprays and 10 gels), 11 (55%) had standard efficacy of 5-Log₁₀ reduction factor (6.7- 6.8) recommended at 15 seconds exposure time on the 4 bacteria. Out of such 7 (64%) were spray solution (L1, L3, L5, L10, L11, L12 & L16), while 4 (36%) were gel solutions (L9, L15, L18 & L20). Another 2 (10%) had relative time based efficacy at between 30 to 45 seconds exposure (L2 spray and L14 gel). Seven (35%) (L7 & L17 spray; L4, L6, L8, L13 & L19 gel solutions) failed the test. Escherichia coli and Proteus stutzin were more susceptible to the samples tested and produced higher RF.

Conclusion: About 45% of the hand sanitizers had poor efficacy and this is quite high, especially in this era of pandemic. This report underscores the need for production policy review by the regulatory body. It is imperative to enforce quality management regime, particularly, internal and external production quality control. Periodic batch efficacy validation is necessary to ensure precision. Poor quality products must be actionable. We recommend this experiment be scaled up to national level and to cover major microbial pathogens.
Introduction
Community and nosocomial acquired infections are on the rise globally [1,2]. Human hands are considered to be the major route of transmitting microbes from man-to-man, man-to-environment and man-to-animals and vice versa [3]. For instance, contaminated ‘hand-to-nose’ is one of the primary routes of corona virus (COVID-19) transmission [4]. The emergence of novel SARS-CoV-2 virus has brought a worsening public health challenge that re-enacted the importance of hand hygiene globally. As at present, most of activities in handling the case of the virus are mainly supportive and the effectiveness of the hand rubs on the virus is extrapolative [5].

Health experts promote hand hygiene practices that involve washing hands with running water and soap at regular intervals or the use of alcohol based hand rub (spray or gel) as a panacea to contain the spread of microbes [6]. It has been reported that bacterial fingertips contamination among health workers ranged from 0 to 300 colony forming units (CFU), when sampled by agar contact methods [7].

Essentially, hand rub is to reduce or inhibit the growth of microorganisms without the need for an exogenous source of water and requiring no rinsing or drying with towels or other devices [8]. According to World Health Organization [7], hand hygiene products include alcohol-based hand rub (liquid, gel or foamy) designed for application to the hands. Such preparations may contain one or more types of alcohol (ethanol or isopropyl) with excipients (bulking & stabilizing agents), other active ingredients (chlorhexidine gluconate, chlorine derivatives, iodine, chloroxylenol (PCMX), quaternary ammonium compounds, triclosan, H₂O₂, NaOH, etc.) and humectants (moisturizers) at a concentration which is sufficient to reduce or inhibit the growth of microorganisms on living tissues [7].

Many studies reported that hand hygiene practice significantly reduced the incidence of community and hospital acquired infections [7,9,10]. Furthermore, Pittet et al [9], demonstrated that use of an alcohol-based hand rub led to reduction of the prevalence of bacterial transmission from human to human and surfaces and recommended hand hygiene practices in hospitals and at other points where the hand is soiled and there is no readily available water and soap for proper hand washing.

Essentially, some factors may influence the outcome of the use of such hand sanitizers (i) the quality of the active ingredient and composition of the agent (ii) volume of hand hygiene solution to be used and, (iii) the ability of the user to observe the proper procedure [7].

Since the outbreak of Ebola and SARS-CoV-2, there has been emphasis on the use of hand sanitizers in public and private places. The WHO therefore provided a guideline on production of alcohol based hand rubs; recommending ethanol (96%), or isopropyl alcohol (99.8%), hydrogen peroxide (3%), Glycerol (90%) and sterile distilled water as composition of sanitizer and finally recommended final acceptable limits of alcohol content 75-85% (ethanol) or 60% isopropanol [11]. The Organization equally prohibited the use of any substance that may promote skin allergy or toxicity to human as component of the hand sanitizer [11].

There are concerns however; that the need to mass produce hand rubs due to pressure of demand may have led to quality compromise. Many of the available sanitizers are not necessarily validated, weather registered by the regulatory body or not, but each brand claims 99.9% efficacy on their various brand labels. Meanwhile, according to EN 13697, for any hand rub to be effective it must meet a minimum of 5-log reduction standards (99.999%) i.e. ability to lower the number of microorganisms by 100,000-fold [12]. Many of such claims may be frivolous and there by calls for evaluation.

Therefore, in other to assist generate quality tools for setting/evaluating standards of hand sanitizer quality in Nigeria and for the re-enforcement of good-quality measures, this study evaluated the efficacy of newly manufactured hand sanitizers in Lagos metropolis in SARS-COVID-19 era.

Materials and Methods
Study Design
The study was a laboratory experimental study. The study took place at the Center for Infectious Disease Research (CIDR) laboratory, Microbiology Department, Nigerian Institute of Medical Research (NIMR), Yaba, Lagos, Nigeria.

Study Site
The study toke place in Lagos, Southwest Nigeria, harboring over 14.8 million people in a small land mass of 3,577Km², situated on the coast of the Bight of Benin, located approximately along longitude 2°42’E and 3°22’E and between latitude 6°22’N and 6°42’ N. It is bounded in the North and East by Ogun State of Nigeria, in the West by the Republic of Benin, and in the South by the Atlantic Ocean. The state is delimitated into three senatorial districts. It has many urban and peri-urban cities and many shantytowns and is the most densely populated state in Nigeria.

Sample Size
Twenty purposively sampled hand sanitizers (10 gels and 10 sprays), 6 randomly purchased from grocery shops across the three senatorial zones (Lagos east, Lagos west and Lagos central) and two (one gel and one spray) commonly in use at NIMR, Yaba, Lagos were studied.

Test Bacterial Organisms
Four bacteria species one non-pathogenic Escherichia coli (ATCC 25922) standard and three locally characterized Multi-drug-Resistant strains: Staphylococcus aureus (NIMR/NTCC/GP056) -Typical, Klebsiella pneumonia (NIMR/NTCC/GN065) -Typical.
and *Proteus stutzin* - (NIMR/NTCC/ GN029) – opportunistic pathogen.

**Test materials**

Isopropyl alcohol 60% (Standard), media, neutralizer (10 % tween 80 in 3 % asolectin plus 0.3 % Sodium thiosulphate) and McFarland standard (0.5), sterile distilled water, normal saline, test tubes, pipette tips (100 -1000µl), timer, vortex mixer and colony counter.

**Testing method Suspension Method (PrEN12054) [13]**

Actively growing (18 – 24 hours) bacterial cells on Muller Hinton Agar (MHA) was suspended on sterile physiological saline and standardized with McFarland no 5 turbidity standard [14]. The turbidity was adjusted to constitute each bacterium stock to contain between 1.0 -1.5 x 10⁷ Colony Forming Unit (CFU/mL). Serial dilutions of the test organisms were conducted from 10⁻⁴ to 10⁻⁶.

**Efficacy Analysis**

The antibacterial efficacy was conducted on three of the dilutions: 10⁻⁴, 10⁻⁵ and 10⁻⁶. One thousand microliter (1000 µl) of each well-mixed bacterial cells was put into a sterile text tube and 1000µl of test sample added and simultaneously set time for 15s, 30s and 45s respectively and vortexed for 10 seconds. At the end of each set time, 1000µl of the neutralizer was added and vortexed to stop the activity of the hand sanitizer. One hundred microliter (100µl) of each sample was plated in duplicate onto two plates (MHA and Blood agar [BA]). Meanwhile, 100µl of each dilution (10⁻¹, 10⁻² and 10⁻³) was plated out to enumerate the baseline CFU/mL (contained neither the hand sanitizer nor the neutralizer). The plates were incubated at 35 ± 2°C for 18 -24 hours. At the end of each incubation period, the baseline and the viable cells on the test samples were counted in duplicate and the average recorded. The Colony Forming Units (CFU/mL) were calculated using the dilution factors [15]. Thus:

\[
\text{Logarithmic Reduction Factor (RF)} = \log_{10}(A) - \log_{10}(B) \quad [13, 16].
\]

Where A = CFU/mL of baseline bacterial cells

B = CFU/mL of bacteria after exposure to test hand sanitizer.

The \( \log_{10} \) RF was converted to Percent Reduction using the formula:

\[
P = (1 - 10^{-L}) \times 100 \quad [13,16].
\]

Where P is the percent (%) reduction and L is the Logarithmic reduction factor calculated.

**Quality Control**

Standard positive control for the study was 60% isopropanol alcohol as recommended [11].

**Batch Positive Control**

For positive control, 100µl from a mixture of 1000 µl of physiological saline and 1000 µl test hand sanitizer was plated alongside each test procedure.

**Negative Control**

The negative control had 100µl of mixture of 1000µl physiological saline and 1000µl bacterial cells at various dilutions cultured, without test hand sanitizer.

**Neutralizer toxicity effect test**

The efficacy and non-toxicity of the neutralizers were tested by making serial dilution with the test organisms. Using 10⁻², 1:10 dilution was made by adding 100µl of bacterial suspension to 900 µl of neutralizer and vortex for 10 seconds. One hundred (100 µl) of the suspension was plated onto MHN & blood agar. Viable counts were performed. The difference between the baseline CFU/mL and that of sanitizer and bacterial suspension CFU/mL determined the efficacy of the neutralizer. It is expected that effective and non-toxic neutralization/recovery ratio/ percent rate must be ≥ 0.75 (75%) [17].

**Data Analysis**

Statistically, the data generated were expressed using descriptive instrument and Percent expression after computation of RF applying the approved formula. Data were double entered into excel spread sheet (Excel version 5.0, Microsoft Redmond, WA, USA) and expressed as a graph.

**Results**

Out of the 20 (10 spray solutions and 10 gel solutions), 11 (55%) had near perfect efficacy of 5-Log10 reduction factor recommended at 15 seconds exposure time, out of such 7 (64%) were spray solution (L1, L3, L5, L10, L11, L12 & L16), while 4 (36%) were gel solution (L9, L15, L18 & L20) as shown in table 6. Another 2 (10%) had relative time based efficacy at between 30 to 45 seconds exposure (L2 spray and L14 gel). Seven (35%) (L7 & L17 spray; L4, L6, L8, L13 & L19 gel solutions) failed to measure up with WHO approved 5-Log10 reduction factor for hand rubs/sanitizers, table 6.

The key components almost all the hand rubs studied include between 60 to 75 % alcohol (ethyl/ethanol or isopropyl) and some contained glycol/glycerine, triclosan, chlorohexidine, hydrogen peroxide, essential oil and flagrance. Most of them contained excipients and humectants, mainly the gels (Table 6).

Table 1 shows the base line CFU/mL that formed the bases for the computation of reduction factors (RF) of the organisms studied.

**Table 1**: Average base line colony count of bacterial organisms after plating each 100µl of three dilutions (10⁻⁴ – 10⁻⁶) and incubating for 24 hours at 35 ± 2°C on MHA and BA culture plate.

<table>
<thead>
<tr>
<th>Organism</th>
<th>10⁻⁴ dilution</th>
<th>10⁻⁵ dilution</th>
<th>10⁻⁶ dilution</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CFU/mL</td>
<td>CFU/mL</td>
<td>CFU/mL</td>
</tr>
<tr>
<td></td>
<td>MHA  BA</td>
<td>MHA  BA</td>
<td>MHA  BA</td>
</tr>
<tr>
<td><em>E. coli</em></td>
<td>223*</td>
<td>&gt;300</td>
<td>50</td>
</tr>
<tr>
<td><em>S. aureus</em></td>
<td>213</td>
<td>254</td>
<td>70</td>
</tr>
<tr>
<td><em>K. pneumonia</em></td>
<td>200</td>
<td>&gt;300</td>
<td>62</td>
</tr>
<tr>
<td><em>Proteus stutzin</em></td>
<td>115</td>
<td>214</td>
<td>57</td>
</tr>
</tbody>
</table>

*Note: Computational, the colony forming unit per mL (CFU/mL) could be demonstrated using *E. coli* at 10⁻⁴ dilution concentration and inoculation of 100µl vol on MHA with bacterial cell count of 223. The CFU/mL is 10⁻⁴ x 223 x 1/10 = 2.23 x10⁻⁶/mL.*
Table 2 presents the outcome of the 60% isopropanol standard control test for the study. The outcome showed that at a higher bacterial concentration of 10⁶, the isopropanol standard could not achieve the recommended efficacy of 5-Log₁₀ reduction factor on the local Staphylococcus aureus (multi-antibacterial resistant) strain tested, when exposed for 5 seconds. However, at 30 and 45 seconds exposure, the standard became efficacious.

Efficacy Analysis
Below is the arithmetical computation of the standard Log₁₀ reduction factor and percent (%) reduction using 10⁴ or 10⁵ CFU/mL and the control standard (60% isopropanol) employed for the study of the 4 organisms studied.

RF = Log₁₀(A) - Log₁₀(B)
Where A = baseline count, B = count after exposure to the 60% isopropanol for 15, 30 & 45 seconds.

(i) E. coli (ATCC 25922)
RF = Log₁₀(6.8 x 10⁶) – Log₁₀(0) = 6.8 x 10⁶ = 6.83250891271

Percent Reduction
Using P = (1 - 10⁻ⁿ) x 100 [13].
P = (1 - 10⁻⁶.83250891271) x 100 = 99.9999%

(ii) Staphylococcus aureus
(a) At 15 seconds exposure of dilution 10⁻⁴, RF = Log₁₀(5.2 x 10⁶) – Log₁₀(10⁻⁴) = 6.71600334363 – 5.0 = 1.71600334363
P = (1-10⁻¹.71600334363) x 100 = 98.0769082 %
(b) At 30 & 45 seconds of the same baseline dilution 10⁻⁴ Log₁₀(5.2 x 10⁶) – Log₁₀(0) = 6.71600334363
P = (1-10⁻¹.71600334363) x 100 = 99.9999%
(C) A lower dilution of 10⁻⁵ standard achieved RF of 6.7 and percent reduction of 99.9999 at 15, 30 and 45 seconds of exposure/

(iii) Klebsiella pneumonia
RF = Log₁₀(6.6 x 10⁶) – Log₁₀(0) = 6.6 x 10⁶ = 6.81954393554

Percent Reduction
P = (1 - 10⁻⁶.81954393554) x 100 = 99.9999%

Table 3 presents the outcome of the 60% isopropanol standard tested against the standard E. coli and 3 other local bacterial isolates namely S. aureus, K. pneumonia and P. stutzin. At bacterial load of 5.2 x 10⁶/mL of Staphylococcus aureus, the efficacy was only about 98 % is unaccepted by World Health Organization standard.

The study demonstrates on Table 4 hand sanitizer solution with standard efficacy with the local bacterial organisms studied. The solution achieved 99.9999% reduction of all the bacteria studied within 5 seconds exposure time.

On table 5a, the study shows a typical hand rub with poor efficacy, considering both exposure time and bacterial low and high CFU/mL and the local bacterial strains.

The possible effect of bacteria strain load on efficacy failure of some test hand sanitizers is demonstrated by computing the CFU/mL of bacterial cells recovered after exposure at 15, 30 & 45 seconds using 10⁻⁶ dilution concentration of E. coli bacterium as shown in table 5b.

Table 2: Computation of Colony Forming Unit per milliliter (CFU/mL) efficacy of 60% isopropanol control standard on MHA medium after 15 seconds time exposure.

<table>
<thead>
<tr>
<th>DF</th>
<th>E. coli</th>
<th>S. aureus</th>
<th>K. pneumonia</th>
<th>Proteus stutzin</th>
</tr>
</thead>
<tbody>
<tr>
<td>CFU/mL</td>
<td>CFU/mL</td>
<td>CFU/mL</td>
<td>CFU/mL</td>
<td>CFU/mL</td>
</tr>
<tr>
<td>Test</td>
<td>Test</td>
<td>Test</td>
<td>Test</td>
<td>Test</td>
</tr>
<tr>
<td>10⁻⁴</td>
<td>89</td>
<td>8.9 x 10⁴</td>
<td>0</td>
<td>52</td>
</tr>
<tr>
<td>10⁻³</td>
<td>27</td>
<td>2.7 x 10⁴</td>
<td>0</td>
<td>21</td>
</tr>
<tr>
<td>10⁻²</td>
<td>3</td>
<td>3.0 x 10⁴</td>
<td>0</td>
<td>1</td>
</tr>
</tbody>
</table>

Key: DF= Dilution Factor, BC= Baseline Count, CFU= Colony Forming Unit

Table 3: Summary of the efficacy of 60% isopropanol standard tested against dilutions 10⁻⁴ and 10⁻⁵ of four species of organisms after 15, 30 & 45 seconds of exposure.

<table>
<thead>
<tr>
<th>Organism</th>
<th>Reduction Factor (RF)</th>
<th>Percent Reduction (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Escherichia coli</td>
<td>6.8</td>
<td>99.9999</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>(a) 1.7 (at higher bacterial load 10⁻⁴ dilation and 15 seconds contact time)</td>
<td>98.0769</td>
</tr>
<tr>
<td></td>
<td>(b) 6.7 at 30 &amp; 45 seconds</td>
<td>99.9999</td>
</tr>
<tr>
<td></td>
<td>(c) 6.7 at lower bacterial load of 10⁻⁶ dilation and at 15, 30 and 45 seconds exposure</td>
<td>99.9999</td>
</tr>
<tr>
<td>Klebsiella pneumonia</td>
<td>6.7</td>
<td>99.9999</td>
</tr>
<tr>
<td>Proteus stutzin</td>
<td>6.8</td>
<td>99.9999</td>
</tr>
</tbody>
</table>

Note that S. aureus attained 5-Log reduction of 6.7 and 99.9999 percent efficacy only after 30 - 45 seconds exposure time.
**Table 4**: Computation of CFU/mL of 100µl of bacterial cell suspension and alcohol based hand spray culture test at 10^4, 10^5 and 10^6 dilution concentrations, plated on MHA for 18-24 hours after 5 seconds exposures.

<table>
<thead>
<tr>
<th>DF</th>
<th>Organism</th>
<th>E. coli</th>
<th>S. aureus</th>
<th>K. pneumonia</th>
<th>Proteus stutzin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>BC CFU/mL</td>
<td>CFU/mL</td>
<td>BC CFU/mL</td>
<td>CFU/mL</td>
<td>BC CFU/mL</td>
</tr>
<tr>
<td>10^4</td>
<td>223</td>
<td>2.23 x 10^7</td>
<td>213</td>
<td>2.13 x 10^7</td>
<td>200</td>
</tr>
<tr>
<td>10^5</td>
<td>50</td>
<td>5.0 x 10^7</td>
<td>70</td>
<td>7.0 x 10^7</td>
<td>62</td>
</tr>
<tr>
<td>10^6</td>
<td>5</td>
<td>5.0 x 10^7</td>
<td>9</td>
<td>9.0 x 10^7</td>
<td>0</td>
</tr>
</tbody>
</table>

*Considering the above and applying the formula RF = Log_{10} (A) - Log_{10} (B) for each bacterial dilution concentration exemplified by dilution 10^4, the RF factor and percent reduction is demonstrated by the CFU/mL of S. aureus species studied.

RF = Log_{10} 2.23 x 10^7 - Log_{10} 0 = -6.651695137

Applying P = (1 - 10^ RF) x 100 = (1 - 10^{-6.651695137}) x 100 = 99.9999 %

**Note**: The composition of this seemingly good hand spray include: Glycerin, ethanol, IPA, BKC and fragrance; as contained on the label.

**Table 5a**: Computation of CFU/mL of 100µl of bacterial cell suspension and failed alcohol based gel test at 10^4, 10^5 and 10^6 bacteria dilution concentrations plated on MHA for 18-24 hours after 15 seconds contact time.

<table>
<thead>
<tr>
<th>DF</th>
<th>Organism</th>
<th>E. coli</th>
<th>S. aureus</th>
<th>K. pneumonia</th>
<th>Proteus stutzin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>BC CFU/mL</td>
<td>CFU/mL</td>
<td>BC CFU/mL</td>
<td>CFU/mL</td>
<td>BC CFU/mL</td>
</tr>
<tr>
<td>10^4</td>
<td>127</td>
<td>1.27 x 10^7</td>
<td>89</td>
<td>1.15 x 10^7</td>
<td>100</td>
</tr>
<tr>
<td>10^5</td>
<td>70</td>
<td>7.0 x 10^7</td>
<td>4</td>
<td>7.5 x 10^7</td>
<td>10</td>
</tr>
<tr>
<td>10^6</td>
<td>5</td>
<td>5.0 x 10^7</td>
<td>2</td>
<td>7.0 x 10^7</td>
<td>5</td>
</tr>
</tbody>
</table>

Key: BC = Baseline Count, ATC = After Test Count

**Table 5b**: Efficacy of a hand sanitizer gel at 30 & 45 seconds contact time, using the lower concentration of 10^6 dilution on the 4 bacteria tested.

<table>
<thead>
<tr>
<th>Organism</th>
<th>ATC 30 seconds CFU/mL</th>
<th>ATC 45 seconds CFU/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. coli</td>
<td>1 x 10^7</td>
<td>1.0 x 10^7</td>
</tr>
<tr>
<td>S. aureus</td>
<td>5 x 10^7</td>
<td>4.0 x 10^7</td>
</tr>
<tr>
<td>K. pneumonia</td>
<td>1 x 10^7</td>
<td>1.0 x 10^7</td>
</tr>
<tr>
<td>Proteus stutzin</td>
<td>1 x 10^7</td>
<td>0.0 x 10^7</td>
</tr>
</tbody>
</table>

Computing the RF of E.coli after 45 seconds exposure using baseline CFU/mL of 5.0 x 10^7 (A) and after 45 second exposure time CFU/mL of 1.0 x 10^7 (B)

RF = Log_{10} (A) – Log_{10} (B) = = Log_{10} (5.0 x 10^7) – Log_{10} (1.0 x 10^7) = -6.301029996 \cdot (-7) = 0.698970004; RF = 0.7

Percent reduction therefore is P = (1 – 10^{-0.7}) x 100 = 0.80 = 80.0 % reduction after 45 seconds of exposure.

That is to say that after 45 seconds exposure of bacteria to this product, it failed to achieve even 1-Log_{10} reduction i.e. ability to achieve 90% reduction of bacterial content (for instance if there was 100 bacterial cells within the hand, 1-log_{10} reduction is expected to reduce the bacteria number to 10 cells). The least recommended efficacy reduction is 5-log_{10} reduction; i.e. 10^5 smaller [16].

The composition of this failed sample included: 70 % ethyl alcohol, NaOH, glycerin, carbomer, Triethanolamin and fragrance, as contained on the container label.

Table 6, presents the summary of the test samples’ composition, the organisms and the reduction factor recorded. E. coli and P. stutzin showed efficacy of RF 6.8 at minimal time of 15 seconds for both spray and gel solutions, K. pneumonia showed RF of 6.7 across the time set for spray and gel as well. However, S aureus had lower RF of 6.4 at 15 seconds and attained 6.7 RF at 30 and 45 seconds for spray and had RF 6.6 at 15 seconds of exposure and attained 6.7 at 30 -45 seconds. Some hand rub had poor efficacy with RF as poor as 1.5 for S. aureus (L14).

**The Neutralizer Toxicity Test**

Each of the four-test bacterial suspension at 10^6 CFU/mL was exposed with the neutralizer employed in the study, vortexed for 10 seconds contact time and 100µl plated on MH agar; with concomitant plating of the validation suspension without the neutralizer and incubated for 18 to 24 hours at 35 ± 2°C, bacterial recovery rate of > 80 % was recorded on each. The outcome is a positive validation of non-toxic effect of the neutralizer used in the study.

Figure 1 presents the graph of the RF generated against the bacterial strains and hand sanitizers tested. Staphylococcus aureus and K. pneumonia appear to present lower reduction factors compared with E.coli standard and P. stutzin tested.
Table 6: The compositions of the 20 samples tested and the reduction factor recorded against the bacterial organisms tested.

<table>
<thead>
<tr>
<th>Sample Number</th>
<th>Constituents</th>
<th>E. coli RF 15s 30s 45s</th>
<th>S. aureus RF 15s 30s 45s</th>
<th>K. pneumonia RF 15s 30s 45s</th>
<th>P. stutzin RF 15s 30s 45s</th>
</tr>
</thead>
<tbody>
<tr>
<td>L1 spray</td>
<td>70% ethyl alcohol and fragrance</td>
<td>6.8 6.8 6.8</td>
<td>6.7 6.7 6.7</td>
<td>6.7 6.7 6.7</td>
<td>6.8 6.8 6.8</td>
</tr>
<tr>
<td>L2 spray</td>
<td>Alcohol 62%, Trolamine, Glycerin, Propylene Glycol, Aloe Vera Leaf, Carbomer, Homopolymer Type C, FD&amp;C Blue No. 1 and FD&amp;C Yellow NO. 5</td>
<td>4.8 6.5 6.5</td>
<td>1.5 5.6 5.6</td>
<td>1.6 2.3 4.2</td>
<td>5.0 5.1 5.4</td>
</tr>
<tr>
<td>L3 spray</td>
<td>Alcohol, vitamins and essential oil</td>
<td>6.7 6.8 6.8</td>
<td>6.5 6.6 6.8</td>
<td>6.7 6.7 6.7</td>
<td>6.8 6.8 6.8</td>
</tr>
<tr>
<td>L4 gel</td>
<td>Glycerin, ethanol, IPA, BKC and fragrance</td>
<td>1.7 3.2 3.8</td>
<td>1.3 2.1 3.2</td>
<td>1.3 3.6 3.7</td>
<td>1.6 2.0 2.2</td>
</tr>
<tr>
<td>L5 spray</td>
<td>Ethyl alcohol 70% Antiseptic, Glycine, Di-sodium EDTA Carbopol, Flagrance and NaOH</td>
<td>6.7 6.7 6.7</td>
<td>6.6 6.6 6.7</td>
<td>6.7 6.7 6.7</td>
<td>6.8 6.8 6.8</td>
</tr>
<tr>
<td>L6 gel</td>
<td>70% ethyl alcohol, NaOH, glycerin, carbomer, Triethanolamin and fragrance</td>
<td>1.8 3.2 3.8</td>
<td>1.3 2.1 3.2</td>
<td>1.3 3.6 3.7</td>
<td>1.9 2.0 2.2</td>
</tr>
<tr>
<td>L7 spray</td>
<td>60% isopropyl alcohol, essential oil and fragrance</td>
<td>1.7 2.5 3.7</td>
<td>0.9 1.3 2.9</td>
<td>1.2 1.7 3.1</td>
<td>2.7 2.8 3.4</td>
</tr>
<tr>
<td>L8 gel</td>
<td>Unbranded with no record of ingredients</td>
<td>1.7 2.9 3.8</td>
<td>1.2 2.5 3.0</td>
<td>1.3 3.6 3.7</td>
<td>1.8 2.0 2.1</td>
</tr>
<tr>
<td>L9 gel</td>
<td>Ethyl alcohol 70%, Antiseptic, glycerin, Disodium EDTA, Carbopol &amp; fragrance</td>
<td>6.8 6.8 6.8</td>
<td>6.5 6.7 6.7</td>
<td>6.7 6.8 6.8</td>
<td>6.8 6.8 6.8</td>
</tr>
<tr>
<td>L10 spray</td>
<td>Isopropyl alcohol and triclosan</td>
<td>6.8 6.8 6.8</td>
<td>6.7 6.8 6.8</td>
<td>6.8 6.8 6.8</td>
<td>6.8 6.8 6.8</td>
</tr>
<tr>
<td>L11 spray</td>
<td>Alcohol and Chlorhexidine</td>
<td>6.8 6.7 6.7</td>
<td>6.6 6.6 6.7</td>
<td>6.7 6.7 6.7</td>
<td>6.8 6.8 6.8</td>
</tr>
<tr>
<td>L12 spray</td>
<td>75% Isopropyl alcohol</td>
<td>6.8 6.8 6.8</td>
<td>6.6 6.7 6.7</td>
<td>6.7 6.8 6.8</td>
<td>6.8 6.8 6.8</td>
</tr>
<tr>
<td>L13 gel</td>
<td>Unbranded</td>
<td>1.7 1.9 2.8</td>
<td>1.3 1.5 2.9</td>
<td>1.4 2.6 3.2</td>
<td>1.8 1.8 2.2</td>
</tr>
<tr>
<td>L14 gel</td>
<td>Ethyl Alcohol and H₂O₂</td>
<td>4.5 6.5 6.5</td>
<td>1.5 5.5 5.5</td>
<td>1.6 2.3 4.2</td>
<td>5.0 5.2 5.4</td>
</tr>
<tr>
<td>L15 gel</td>
<td>Alcohol and glycerin</td>
<td>6.8 6.8 6.8</td>
<td>6.6 6.6 6.7</td>
<td>6.7 6.7 6.7</td>
<td>6.8 6.8 6.8</td>
</tr>
<tr>
<td>L16 spray</td>
<td>60% IPA</td>
<td>6.8 6.8 6.8</td>
<td>5.1 6.7 6.7</td>
<td>6.4 6.7 6.7</td>
<td>6.8 6.8 6.8</td>
</tr>
<tr>
<td>L17 spray</td>
<td>Alcohol and Chlorhexidine</td>
<td>1.7 1.2 2.8</td>
<td>1.3 2.1 3.2</td>
<td>1.3 3.4 3.7</td>
<td>1.6 2.1 2.3</td>
</tr>
<tr>
<td>L18 gel</td>
<td>Alcohol and Triclosan</td>
<td>6.7 6.8 6.8</td>
<td>6.6 6.6 6.7</td>
<td>6.7 6.7 6.7</td>
<td>6.8 6.8 6.8</td>
</tr>
<tr>
<td>L19 gel</td>
<td>Ethyl Alcohol</td>
<td>1.7 1.7 2.7</td>
<td>1.3 1.3 1.3</td>
<td>1.8 1.8 1.8</td>
<td>2.3 2.4 2.4</td>
</tr>
<tr>
<td>L20 gel</td>
<td>75% Isopropyl alcohol</td>
<td>6.7 6.7 6.8</td>
<td>6.6 6.7 6.7</td>
<td>6.7 6.7 6.7</td>
<td>6.8 6.8 6.8</td>
</tr>
</tbody>
</table>

Key: L1 = Lagos sample 1

L1 & L10 and L9 & L15 are samples of sprays and gels with high profile of RF reflecting standard reduction of 99.9999% respectively.

Figure 1: Bacterial reduction factor graph of the hand sanitizers samples studied.

Note: E. coli and P. stutzin had the highest reduction factor; the implication of such development is that the hand rubs tested have more activities against such species in this study.
Discussion
In this study, efficacy of 55% is reported of the twenty samples studied in Lagos, Nigeria. The 60% isopropyl standard control was efficacious, plate 1; however, its activity on higher bacterial load of S. aureus local strain at 10⁻⁴ dilution concentration appears to be challenging; having achieved only 1.7 RF and 98% reduction, table 3. This is well below the WHO recommended 5- Log₁₀ reduction factor and of 99.999% bacterial reduction [11]. Some hand sanitizers tested performed better, producing RF of 6.7 – 6.8 at 15 to 30 seconds and percent bacterial reduction of 99.9999, tables 3, 4 & 6. Similar study in Ilorin (North central) Nigeria had per cent reduction of two most potent hand sanitizer as 89.9% and 73.8% respectively in 2013 [15]. These represented failed efficacy. Ochwoto et al [18] reported that 50% of 14 hand sanitizers sampled in Kenyan market had efficacy below WHO recommended efficacy, these reports are comparable. Fred et al. [19] reported 100% efficacy of locally produced hand sanitizers in Uganda. However, only two locally produced samples were evaluated. Both reports are from variable socio demographic settings, though all in Africa – resource poor setting. The report from Uganda is further marred by poor study sample size and therefore, cannot be compared with this report. Escherichia coli was identified as most susceptible and same was observed by Ochwoto and colleagues [18], this may be because the strain tested was standard non-pathogenic type. Many of the efficacious samples had RF 6.7 – 6.8 across all the organisms studied, indicative of higher efficacy profile when compared with the 60% isopropanol standard employed in the control of this study (Tables 2 & 3). The reliability of this validation is supported by the standard base line CFU/mL exemplified by 2.23 x10⁷/mL realized from E. coli 10⁻⁴ dilution concentration, and further demonstrated that blood agar medium produced more viable bacterial growth compared with growth on MHA, incubated at 25 ± 2°C, for 18 to 24 hours table 1. However, the outcome of this test will be the same when either (MHA or BA) of the viable counts is employed in the computations.

From the guidelines of hand sanitizers’ production, factors of variability include quality of alcohol (60 – 80%) active ingredient, time of exposure and bacterial load among other factors [11,20]. For instance, at 10⁻⁴ dilution concentration containing higher bacterial load, 60% isopropanol test standard attained RF 6.7 only after 30 – 45 seconds exposure and when the bacterial load was reduced to 10⁻³ the test standard attained RF 5.7 in 15 seconds, table 3.

Meanwhile, WHO [20] reported up to 4.6 x 10⁶ CFU/mL on the hands of some health care workers and has recommended ‘palmmful’ of alcohol-based hand sanitizer that could cover all surfaces of the hands; users are expected to continue to rub till dry and that may last from 15 to 60 seconds.

Alcohol based hand rubs principally kills organisms by denaturing their proteins and dissolving their lipids and is effective against most bacteria, fungi and many viruses, but may be ineffective against bacterial spores and certain bacterial species e.g. enterococcus species (WHO, 2006). As a result of limited resources and convenience, this study focused on bacteria organisms alone.

From this study, the hand sprays L1 (composition: 70% ethyl alcohol & fragrance), L12 (isopropyl alcohol), L10 (isopropyl alcohol & triclosan) and L3 (alcohol, essential oil & vitamin); and gel L9 (composition: ethyl alcohol 70%, antiseptic, glycine, Disodium EDTA, cartopol and fragrance) demonstrated typical standard hand rubs by their efficacy profile, table 4. In contrast, spray L17 (alcohol & chlorhexidine) and gel L4 (glycerin, ethanol, IPA, BKC & fragrance) demonstrated poor efficacy profile, table 5a. Again, some bacteria strain showed efficacy profile of relative susceptibility against certain hand rubs after exposure for 15, 30 & 45 seconds; having produced higher RF with K. pneumonia and P. stutzin, at lower bacteria concentration of 10⁶ CFU/mL only after 45 seconds exposure time to attain 5 log₁₀ reduction level (Table 5b). The composition of such samples included 70% ethyl alcohol NaOH, glycerin, carbomer, triethanolamin and fragrance. The compositions of the products may be the source of the variability and the same opinion was expressed by previous researchers [18,19,21]. In this study also, only such samples that demonstrated efficacy against all the 4 bacteria were considered to be good enough for local use considering the RF and WHO guidelines. Majority of the hand rubs with very poor efficacy are the gel formulations and this is in agreement with other reports [18,20,21].

There is empirical evidence of reduction of transmission of pathogenic micro-organisms by good hand hygiene practices [20], especially with alcohol based hand rub liquid or gel. The era of SARS- CoV-2 pandemic has stimulated behavioral changes among the resource poor population following strategic advocacy compelling regular use of hand sanitizers and this has resulted in increase in the demand for this formulations. However, the demand pressure and the need to maximize profit have seemingly made
some manufacturers compromise the quality of some products and subvert the base line (alcohol content of 75-85% ethanol or 60% isopropanol) specification recommended by WHO [11]. For instance, many of the products studied failed to specify the volume of alcohol used and no recommended user instruction statement “apply a palm full of hand rub and rub over all surfaces of the hand … rub hand until dry” as prescribed by WHO [20]. Again, the poor quality hand rubs reported in this study may not be unconnected with poor quality control application by the manufacturers; either lower or higher alcohol content or non-application of titrimetric method of control or gas chromatography as recommended. The failed gels producers probably added excess bulking agent to save cost. This may raise issue about expertise and good manufacturing practices; including scaling and accurate measurement. This is further buttressed, since from this study at least three of the efficacious samples (L1, L5 & L9) were ethyl alcohol based and another three (L10, L12 & L20) isopropyl based), the argument as per which has more efficacy does not arise. The fact that some samples in this study had low efficacy irrespective of the claimed compliant to approved content compositions underscore the failure of the regulatory body, whose responsibility is to ensure good products quality validation and sanction defaulters. Every alcohol based hand rub is expected to pass EN 12791 test according to Kramer et al., [21] considering in particular the contact time, the volume of the hand rubs and the bacteria in question.

Some previous experiments tested the efficacy of the hand sanitizers against these organisms, *S. aureus* and *E. coli* [23,24], *K. pneumonia* [19] and *Proteus* spp. [25], and variously reported them as common bacterial organisms found within the community and hospital settings; although strains may vary.

It is warranted that absence of the name of the active ingredient or specific alcohol percent employed in some of the products pose serious limitations to our inferences and conclusions. In addition, it was not possible to sample all the products in Lagos metropolis and all manufactured in Nigeria. The reliability and reproducibility of the study is further demonstrated by the validation test of the neutralizer used, the neutralizer used had no detectable bactericidal activities or toxicity on the organisms studied and was able to neutralize the actions of the active ingredients such that time bound effects on the bacterial cells were effectively measured. Our inference is predicated upon the fact that after exposure of each of the 10^2 bacterial cell dilution concentration studied with the neutralizer for 10 seconds, recovery of CFU/ml of >80% were recorded. According to Sutton et al., [17], for a positive validation of any neutralizer, recovery rate of ≥75% must be attained. This is consistent with the report of Sheikh [26]. Sherabah and colleagues [27] recommended 10% tween 80 in 1% physiological peptone. Nonetheless, the main neutralizing ingredient remains the tween 80.

**Conclusion**

In conclusion, only about 55% of the hand rubs found in Lagos metropolis meets global efficacy reference standard of 5-Log_{10} reduction factor and percent bacterial reduction of 99.999 within 30 seconds of contact time. Failure rate of 45% is quite high, especially in this era of pandemic. This report underscores the need for production policy review by the regulatory body. It is imperative to enforce quality management regime, particularly, internal and external production quality control. Periodic batch efficacy validation is necessary to ensure precision. Poor quality products must be actionable. Furthermore, we recommend this experiment be scaled up to national level and to cover important microbial pathogens (mainly bacteria, viruses and fungi), all alcohol based hand rubs produced in Nigeria and a study on their organoleptic properties. This is apt considering global experience on Ebola outbreak in Africa and SARS-CoV-2 pandemic currently ravaging every nation and making hand hygiene an imperative.

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