

Comparative Analysis of the Antimicrobial Effect of Different Brands of Alcohol Based Hand Sanitizers on Some Selected Bacteria Isolates

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Abstract

Alcohol-based hand sanitizer is a topical solution designed for the purpose of disinfecting and sanitizing the hands when traditional hand washing with soap and water is not immediately feasible. It has become an integral part of personal hygiene, particularly in situations where access to water and soap is limited or impractical. The aim of this study is to comparatively analyze the antimicrobial effect of different brands of Alcohol Based Hand Sanitizers on some isolated test bacteria. A total of six (6) popular brands of alcohol-based hand sanitizers (Live it up, 2sure, Herbal blend, Best, Kaizen and Enliven) and clinical isolates *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumonia* and *Pseudomonas aeruginosa* were selected for this study. The well-variant of the agar diffusion method was modified and adopted in assessing the susceptibility of the test bacteria to the hand sanitizers. Also, the the minimum inhibitory concentrations (MIC) of the hand sanitizers were determined using a series of increasing concentrations (5% to 100%, in 5% increments) of each sanitizer in sterile nutrient broth tubes, each containing 9 mL. Data were presented as means, standard deviations, and percentages. The results showed that Herbal Blend sanitizer exhibited the highest zone of inhibition against *Klebsiella* species (40.00 ± 1.55 mm), while Kaizen sanitizer had the highest inhibition against *Pseudomonas* spp. (34.03 ± 1.29 mm), *Staphylococcus aureus* (42.00 ± 1.55 mm), and *Escherichia coli* (55.00 ± 2.78 mm). Resistance patterns were also observed: *Klebsiella* species was resistant to Best sanitizer, *Pseudomonas* spp. to Live It Up sanitizer, *Staphylococcus aureus* to 2 Sure and Enliven sanitizers, and *Escherichia coli* to Enliven sanitizer. The Minimum Inhibitory Concentration (MIC) values, expressed as volume/volume percentage (v/v%), showed substantial differences in the antimicrobial effectiveness of the tested hand sanitizers. Kaizen and Herbal Blend demonstrated the greatest potency, requiring only 5% v/v to inhibit *Klebsiella* spp., *E. coli*, and *Staphylococcus aureus*, and 10% v/v to inhibit *Pseudomonas* spp. Enliven showed moderate activity with MICs ranging from 40–50% v/v, while Live It Up required 40–60% v/v for inhibition. Best exhibited poor activity, with very high MICs (70–80% v/v) across all organisms. 2Sure showed no inhibitory effect within the tested concentration range. Overall, Kaizen and Herbal Blend were identified as the most effective sanitizers based on their consistently low MIC values across all bacterial isolates. The use of reliable and effective hand sanitizers should be promoted as a preventive measure against hand-transmissible infectious diseases. It is also important to emphasize adherence to other hand hygiene practices, such as washing hands with soap and water, in addition to using hand sanitizers.

Keywords: Antimicrobial activity, alcohol-based hand sanitizers, Bacteria isolates, resistance pattern, minimum inhibitory concentration.

1. INTRODUCTION

The gold standard for hand hygiene and prevention of the spread of non-airborne infectious diseases is regarded as washing of hands with warm water and soap, because water and soap remove oils from hands that can harbour pathogens (WHO, 2020). However, in the absence of water, hand sanitizers are recommended (Ochwoto, 2017). An alcohol-based hand sanitizer is a topical solution designed for the purpose of disinfecting and sanitizing the hands when traditional hand washing with soap and water is not immediately feasible. Hand sanitizer formulations exist in the form of liquids, gels and foams. Depending on the active ingredient used, hand sanitizers can be classified as one of two types: alcohol-based and alcohol-free. Alcohol-based hand sanitizers are recommended for general use, whereas the alcohol-free ones are not (Gold *et al.*, 2020; Golin *et al.*, 2020). For alcohol-based hand sanitizers, the

United State Centres for Disease Control and Prevention (CDC) recommends a concentration of 60–95% ethanol or 2-propanol mixed with distilled water (Jing, 2020). Hand sanitizers with less than the recommended alcohol content (60–95% alcohol) have been found not to be active against many types of pathogens, in that they may merely reduce their growth rate and hence reduce their numbers rather than kill them outrightly (Gold *et al.*, 2020).

Alcohol acts on microbes in the presence of water by making the organism cell membrane permeable leading to cytoplasm leakage, denaturing of proteins and eventually, cell lysis (Kampf, 2018). At higher concentrations (> 95%) alcohol is not effective since microbial denaturing of proteins only takes place in the presence of water (Greenaway *et al.*, 2018). The primary active ingredients in these sanitizers are typically ethanol (ethyl alcohol) or isopropyl alcohol (isopropanol), or a combination of both. The need for use of non-pharmaceutical intervention such as alcohol-based hand sanitizers have been re-iterated by the World Health Organization (WHO) (Agbana *et al.*, 2020; Pandit, 2020). Studies have shown that good hand hygiene reduces the incidence of nosocomial infections (Agbana *et al.*, 2020). Presently, washing of hands with soap and water and the use of hand sanitizers are the two most important hand hygiene methods in clinical practice, and they have been reported not only to reduce hand bacterial contaminations but also enhance hygiene compliance among health care workers (Erasmus *et al.*, 2010). It has also been reported that, about 80% of individuals retain some pathogens on their hands after washing, and this exercise removes the body's own fatty acids from the skin, sometimes resulting in cracked skin that provides an entry portal for pathogens (Fendler *et al.*, 2020). To overcome these limitations, hand sanitizers discovered to be effective against those pathogens while improving the skin condition due to the added emollients were introduced (McGuckin & Govednik, 2017). The correct use of hand sanitizers takes less time than hand washing, and at the same time, does not require drying of hands with potentially contaminated surfaces. Some alcohol-based hand sanitizers have been reported to kill up to 99.9% of pathogens within 15 seconds of application (Dastider *et al.*, 2020). Ethanol has the record of being the oldest skin disinfectant, it acts as a permeation enhancer when applied topically to human skin (Lachenmeier, 2018). The availability of quality health commodities is important in promoting good health and to assure this, there is the need for routine quality assessment tests of pharmaceutical products and other health commodities. Poor quality health commodities pose a significant threat to consumers and heavy reliance on hand sanitizers as a veritable means of preventing infection and transmission. This study aimed to compare the antimicrobial efficacy of different brands of alcohol-based hand sanitizers against selected bacterial isolates, thereby assessing their effectiveness and reliability in reducing bacterial contamination.

2. MATERIALS AND METHODS

2.1. Study Area

This study was conducted in the Medical Microbiology Laboratory in the Faculty of Medical Laboratory Science, Rivers State University Port Harcourt, Rivers State. Port Harcourt is the capital and largest city of Rivers State, Nigeria. It is situated in the South-South geopolitical zone of Nigeria and lies between latitude 4°15' and 5°45' North, and longitude 6°20' and 7°35' East of the equator.

2.2. Study Design

This research employed a Randomized Controlled Trial (RCT) to compare the antimicrobial effect of different brands of alcohol based hand sanitizers on some selected bacteria isolates.

2.3. Sampled Alcohol-based Hand Sanitizer

Six popular brands of alcohol-based hand sanitizers (Live it up, 2sure, Herbal blend, Best, Kaizen and Enlivenbrands) locally purchased from pharmacies and supermarkets were selected for the analysis. The brands were chosen based on market popularity, availability, and representation of various types (gel, spray, foam) and formulations (alcohol concentration only). In the laboratory, samples were checked for their label claims, NAFDAC registration numbers, batch numbers, manufacturing and expiration dates. Each of the products were stored as recommended by the respective manufacturers and analysis were carried out well before their expiration dates.

2.4. Test Microorganisms

Pure cultures of *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumonia* and *Pseudomonas aeruginosa* were obtained from the Microbiology Laboratory of the Rivers State University Teaching

Hospital, Port Harcourt. These isolates were sub-cultured and maintained on nutrient agar slants at 4°C prior to use.

2.5. Antimicrobial Susceptibility Test

The agar well diffusion method was employed. Sterile nutrient agar plates were inoculated with 0.1 mL of standardized bacterial suspension (0.5 McFarland standard). Wells of 6 mm diameter were made using a sterile cork borer and filled with 0.1 mL of each sanitizer sample. Plates were incubated at 37°C for 24 hours, and zones of inhibition were measured in millimeters.

2.6. Minimum inhibitory concentration volume/volume percentage (MIC v/v%) determination

The minimum inhibitory concentration (MIC) refers to the lowest concentration of an antimicrobial agent that completely halts the growth of a test organism, detectable without the need for magnification. The Minimum Inhibitory Concentration (MIC) was expressed as **volume/volume percentage (v/v%)** because the test agents were commercial hand sanitizer solutions requiring dilution by volume. Serial two-fold dilutions of each sanitizer (5%–100% v/v) were prepared in nutrient broth. Each tube received 100 µL of standardized bacterial inoculum. A growth control (broth + organism) and sterility control (broth + sanitizer) were included. After incubation at 37°C for 18 hours, the MIC was defined as the lowest sanitizer concentration (v/v%) showing no visible growth compared to the controls.

2.7. Data Analysis

Statistical analysis was performed on the obtained data. Data were presented as means, standard deviations, and percentages.

3. RESULTS

Table 1 shows the zone of inhibition (mm) of various Alcohol Based hand sanitizers on the isolated test organism. Herbal blend sanitizer had the highest zone of inhibition on *Klebsiella* species while Kaizen sanitizer had the highest zone of inhibition on *Pseudomonas* spp, *Staphylococcus aureus*, and *Escherichia coli*.

Table 1. Mean Zone of Inhibition (mm) of Alcohol-Based Hand Sanitizers Against Test Organisms

Sanitizer Test Organism	<i>Klebsiella</i> species	<i>Pseudomonas</i> spp	<i>Staphylococcus aureus</i>	<i>E. coli</i>
2 Sure	33.4±2.18	13.90±1.29	-	29.70±2.32
Kaizen	25.00±2.16	34.03±1.29	42.00±1.55	55.00±2.78
Live it Up	10.00±1.75	-	20.06±1.34	10.00±2.28
Enliven	20.07±1.34	15.07±1.65	-	-
Herbal Blend	40.00±1.55	19.00±1.55	15.13±1.93	29.7±2.32
Best	-	117.27±2.77	20.07±1.34	21.73±1.98

Values represent Mean ± Standard Deviation (mm). “—” indicates no measurable zone of inhibition.

The Minimum Inhibitory Concentration (MIC) values, expressed as volume/volume percentage (v/v%), showed substantial differences in the antimicrobial effectiveness of the tested hand sanitizers (Table 2). Kaizen and Herbal Blend demonstrated the greatest potency, requiring only **5% v/v** to inhibit *Klebsiella* spp., *E. coli*, and *Staphylococcus aureus*, and **10% v/v** to inhibit *Pseudomonas* spp. Enliven showed moderate activity with MICs ranging from **40–50% v/v**, while Live It Up required **40–60% v/v** for inhibition. Best exhibited poor activity, with very high MICs (**70–80% v/v**) across all organisms. 2Sure showed no inhibitory effect within the tested concentration range. Overall, Kaizen and Herbal Blend were identified as the most effective sanitizers based on their consistently low MIC values across all bacterial isolate

Table 2. Minimum Inhibitory Concentration (v/v%) of various Alcohol Based hand sanitizers on test organism

Sanitizer Test Organisms	<i>Klebsiella</i> species	<i>Pseudomonas</i> spp	<i>E. coli</i>	<i>Staphylococcus aureus</i>
Live it up	50	60	50	40
Best	70	80	80	80
2 Sure	-	-	-	-
Kaizen	5	10	5	5
Enliven	40	40	50	40
Herbal blend	5	10	5	5

Lower MIC indicates higher antimicrobial potency

4. DISCUSSION

Result from Table 1 showed the zone of inhibition (mm) of various Alcohol Based hand sanitizers on the isolated test organism. Zone of inhibition represents a qualitative method to investigate the antimicrobial potency of an antimicrobial compound. The presence of a distinct clear zone of inhibition is usually indicative of potent antimicrobial functionality. Herbal blend sanitizer had the highest zone of inhibition on *Klebsiella* species while Kaizen sanitizer had the highest zone of inhibition on *Pseudomonas spp*, *Staphylococcus aureus*, and *Escherichia coli*. The result also showed *Klebsiella* species was resistant to Best sanitizer, *Pseudomonas spp* was resistant to Live it up sanitizer, *Staphylococcus aureus* was resistant to 2 sure and Enliven sanitizer and *Escherichia coli* is resistant to Enliven sanitizer. This indicates resistance imposed by many microorganisms as a result of their enzymes and other virulence factors that inhibit antibiotics and alcohol. While the different alcohol-based hand sanitizers all demonstrated reasonable antibacterial effects against the various gram-positive and gram-negative bacteria, propanol-based sanitizers in contrast to this study have been reported to be more effective compared to ethanol with the greatest zone of inhibition (Jain *et al.*, 2016)

A similar study on antibacterial activity of alcohol-based hand sanitizers on some clinical bacterial isolates by Saheed & Igbineweka, 2021 showed that Hygel, Dettol, SKP and Samclean sanitizers demonstrated varying degrees of antibacterial activity. Ethanol-based sanitizers generally showed higher activity than those with isopropanol or lower alcohol content. *S. aureus* and *E. coli* were **more** susceptible, while *Klebsiella spp.* and *Pseudomonas aeruginosa* showed higher resistance, similar to the findings in this study. Differences in performance were linked to alcohol concentration, formulation quality, and presence of impurities, variations in alcohol concentration and the presence of additional active components. WHO (2020) recommends that effective hand sanitizers contain at least 60% alcohol. Brands with lower efficacy likely contained substandard alcohol levels or inactive ingredients that reduced antimicrobial potency.

With the Minimum Inhibitory Concentration (v/v%) of various alcoholic-based hand sanitizers on isolated test organism (Table 2), Kaizen sanitizer and Herbal blend sanitizer both had a minimum inhibitory concentration of 5% on *Klebsiella species*, *Staphylococcus aureus* and *Escherichia coli*. The study conducted by Larson *et al.*, 2012 found that the use of alcohol-based hand sanitizers significantly reduced the colonization of methicillin-resistant *Staphylococcus aureus* (MRSA) in healthcare settings. The emergence of methicillin-resistant *Staphylococcus aureus* (MRSA), a strain resistant to multiple antibiotics, including methicillin, oxacillin, and penicillin is of grave concern. MRSA infections are associated with higher morbidity and mortality rates, making treatment challenging.

Result obtained from this study also showed that Kaizen sanitizer and Herbal blend sanitizer both had a minimum inhibitory concentration of 10% on *Pseudomonas spp*. A lower MIC (v/v%) is better because it shows higher antimicrobial potency. These result indicates that Kaizen sanitizer and Herbal blend sanitizer are very effective in the inhibition of bacterial growth. Enliven sanitizer on the other hand showed moderate activity with MICs ranging from 40–50% v/v, while Live It Up required 40–60% v/v for inhibition. Best exhibited poor activity, with very high MICs (70–80% v/v) across all organisms. 2Sure showed no inhibitory effect within the tested concentration range. It is possible that increasing the concentration of the less-effective sanitizers could produce measurable zones of inhibition, as higher alcohol content generally enhances antimicrobial activity. Therefore, these products may demonstrate improved effectiveness at concentrations above those tested in this study. In the study of the efficacy of concentrated alcohol-based hand sanitizer in preventing transmission of pathogen by Ekawati *et al.*, (2024), alcohol concentrations as the basic ingredient for hand sanitizer were adjusted to 40%, 50%, 60%, 70%, and 80% and the inhibition test of alcohol-based hand sanitizer at 80% concentration against *Escherichia coli*, Enterotoxigenic *Escherichia coli*, *Salmonella typhimurium*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Staphylococcus aureus* produced zones of inhibition. This present study is in contrast to a recent study by Lim *et al.* (2023) who assessed and evaluated the differences in antibacterial efficacy and functionalities of five different commercial alcohol-based sanitizers with different formulations. All sanitizers in their study were able to provide instant sanitization functionality, effectively killing 5×10^5 CFU/mL of inoculated bacteria while some sanitizers in this study was not effective. Lim *et al.* (2023) comparing pure alcohol-based sanitizers against alcohol-based sanitizers with a secondary active ingredient demonstrated that the addition of a secondary active ingredient enhanced the effectiveness and functionalities of the sanitizers. They

observed that alcohol-based sanitizers with secondary active ingredients demonstrated a more rapid antimicrobial mode of action, eradicating all 106 CFU/mL of bacteria within 15 seconds of contact, in contrast to the 30 min for purely alcohol-based sanitizers. The secondary active ingredient also provided additional anti-biofilm functionality to prevent opportunistic microbes from attaching and proliferating on the treated surface, leading to serious biofilm formation. Furthermore, treatment of surfaces with alcohol-based sanitizers with secondary active ingredients also imparted prolonged antimicrobial protection to the surface lasting up to 24 hours. Contrarily, purely alcohol-based sanitizers do not seem to possess such quality with the treated surface being vulnerable to microbial contamination within minutes after application. This highlight the benefits of adding a secondary active ingredient in sanitizer formulation and care needs to be taken to evaluate the type and concentration of antimicrobial agents chosen as the secondary active ingredient.

Presently, washing of hands with soap and water and the use of hand sanitizers are the two most important hand hygiene methods in clinical practice, and they have been reported not only to reduce hand bacterial contaminations but also enhance hygiene compliance among health care workers (Erasmus *et al.*, 2010). It has also been reported that, about 80% of individuals retain some pathogens on their hands after washing, and this exercise removes the body's own fatty acids from the skin, sometimes resulting in cracked skin that provides an entry portal for pathogens (Fendler *et al.*, 2020). To overcome these limitations, the use of hand sanitizers is an effective way to eliminate pathogens while improving the skin condition due to the added emollients were introduced (Asseta *et al.*, 2021; Fellica *et al.*, 2021). The correct use of hand sanitizers takes less time than hand washing, and at the same time, does not require drying of hands with potentially contaminated surfaces. Also, beyond the application on hands, alcohol-based sanitizers are also being constantly applied to different materials such as door handles, to clean the respective surfaces.

5. CONCLUSION

This study which was carried out to comparatively analyze different brands of alcoholic-based hand sanitizers sold in Port Harcourt has been able to establish that not all alcohol-based hand sanitizers on the market possess equal antimicrobial effectiveness. Among the tested brands, Kaizen sanitizer and Herbal blend sanitizer are the most effective hand sanitizers for the inhibition of bacterial growth. Therefore, this study has established that the use of alcohol-based sanitizer is an effective alternative to cleaning the hands for the killing of harmful microorganisms, especially in the case when hand washing is not doable. Continuous regulatory monitoring and public awareness are crucial to ensuring the quality of sanitizers available to consumers.

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