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Evaluation of the Quality and Antibacterial Efficacy of Some Alcohol-Based Hand Sanitizers Sold in Benue South, Nigeria

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ABSTRACT
Hospital and community-acquired infections are a serious problem worldwide often leading to increased deaths, costs and other problems. Hand hygiene has been promoted as the most important way to prevent these infections. In this study, the alcohol content and antibacterial efficacy of some popular hand sanitizers sold in Benue South, Nigeria were determined. Gas chromatography-mass spectrophotometry was used for the qualitative and quantitative determination of alcohol while the well-variant of the agar diffusion test was used to evaluate the efficacy of the products against *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*. The result of the study showed the use of two toxic contaminants (1-propanol and ethyl methanol) in some samples. The study also found many of the alcohol-based hand sanitizers to have far less percentage alcohol compared to their label claims and acceptable standards. Though most of the hand sanitizers showed strong anti-bacterial activity, the result also demonstrate that not all alcohol-based hand sanitizers possess the same antibacterial effectiveness. Hence, it is imperative for regulatory bodies to ensure compliance from manufacturers of alcohol-based hand sanitizers.

Keywords: Alcohol-based, hand sanitizers, antibacterial

INTRODUCTION
The need for use of non-pharmaceutical intervention such as the washing of hands with soap and water or alcohol-based hand sanitizers (HS) have been re-iterated by the World Health Organization (WHO) (Agbana *et al.,* 2020). Studies have shown that good hand hygiene reduces the incidence of nosocomial infections (Erasmus *et al.,* 2010). 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. 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. 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 (Fallica *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. Alcohol is the main antimicrobial component of most hand sanitizers and WHO particularly recommends either ethanol or isopropyl alcohol for the production of hand sanitizers.
Some alcohol-based hand sanitizers have been reported to kill up to 99.9% of pathogens within 15 seconds of application. Ethanol has the record of being the oldest skin disinfectant, acting as a permeation enhancer when applied topically to human skin (Lachenmeier, 2008). The availability of quality health commodities is important in promoting good health and to ensure 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 especially with covid-19 and heavy reliance on hand sanitizers as a veritable means of preventing infection and transmission. The recommended amount of alcohol in an alcohol-based HS should be between 60 to 80%. This study aimed to quantify the alcohol content of the sanitizers and alcohol types, and also assess their overall antibacterial capacities.

Literature Review
Hospital and community-acquired infections constitute a serious public health problem all over the world (Hassan *et al.,* 2012). Hospital acquired (nosocomial) infections are infections developing in hospitalized patients and which were neither present nor in incubation at the time of their admission (Atul-Jain, 2007). Community-acquired infections on the other hand are those acquired anywhere other than in a healthcare facility, in settings such as schools, exercise facilities, or any place where people come in contact with others or with surfaces that have been contaminated (Hassan *et al.*, 2012). These infections have considerable impacts on individuals such as prolonged hospitalization, long-term disability, increased risk of antimicrobial resistance, huge financial burden, high costs for patients and their families and deaths (WHO, 2009). The Center for Disease Control and Prevention (CDC) estimates that approximately 2 million people acquire hospital-associated infections each year and that approximately 90,000 of these patients die as a result of their infections (Zerr *et al.*, 2005). The CDC, WHO and many other experts promote hand hygiene as the single most important measure in the prevention of hospital associated infections (WHO, 2009; CDC, 2002).
Health care workers in developed and developing countries comply with hand hygiene less than 50% of the times they

should (Zerr *et al*., 2005; McGuckin *et al*.,2009). Some of the identified barriers to hand hygiene compliance include lack of easy access to hand hygiene at the point of care, insufficient time, forgetfulness, skin irritation, etc. Alcohol based hand-rub solutions (hand sanitizers) have been suggested as one of the ways to overcome some of these barriers (CDC, 2002). Hand sanitizers are alcohol-containing preparations designed for application to the hands for reducing the number of viable microorganisms on the hands (CDC, 2002). They are also used as supplements or alternatives to hand washing with soap and water (Hammond *et al*., 2000). Various preparations of hand sanitizers are available including the gel, foam and liquid solutions (Pandit, 2020). Active ingredients of hand sanitizers include isopropanol, ethanol, n-propanol or providone-iodine while the inactive ingredients usually include a thickening agent (such as polyacrylic acid for gels), humectants (such as glycerin for liquid rubs) or propylene glycol and essential oils of plants. Hand sanitizers address the barriers to hand hygiene compliance because they require a fraction of the time for effective hand washing (Mody *et al*., 2003), they are less damaging to the skin than soap and water (Boyce *et al.,* 2000) and they are more effective in killing many microorganisms. While alcohol-based hand sanitizers have been demonstrated to be effective against a wide range of Gram-positive and Gram-negative bacteria, multi-resistant pathogens, fungi and many viruses (Kampf *et al*., 1999), they have also been reported to have very poor activity against bacterial spores, protozoan oocysts and certain non-enveloped (nonlipophilic) viruses (CDC, 2002). Despite several reports stating their efficacy, consumers have been warned against false claims of efficacy by some manufacturers (FDA, 2011).

MATERIALS AND METHODS

Materials

Sample Collection Procedure

A total of 12 different brands of alcohol-based hand sanitizers were randomly purchased from different pharmacies, supermarkets, and stores, from the nine LGAs in Benue South. In the laboratory, samples were checked for their label claims which include; NAFDAC registration number, manufacturing and expiry dates, type and percentage alcohol. The details of the collected hand sanitizers are shown in Table 1 with codes representing each brand. The samples were stored in their original container under ambient conditions as per the manufacturers' recommendations until analysis. All samples were within their shelf lives during analysis.

Table 1: Label Claims of the Selected Alcohol-based Hand Sanitizers

Abhs Code	Nafdac No	Manufacturing Date	Expiry Date	Type Of Alcohol	% Alcohol
HS 1	03-3095	9/24	08/27	Alcohol denat	70
HS 2	NS	NS	NS	Ethyl alcohol	62
HS 3	A2-0646	01/24	12/25	Ethyl alcohol	NS
HS 4	O3-7342	NS	NS	NS	NS
HS 5	NS	NS	NS	Ethyl alcohol	65
HS 6	NS	3/23	3/26	Isopropanol	NS
HS 7	NS	NS	NS	ethyl alcohol	62
HS 8	O3-3275	11/23	11/25	NS	NS
HS 9	03-1640	NS	NS	Ethyl alcohol	NS
HS 10	03-7298	NS	NS	Ethyl alcohol	NS
HS 11	NS	NS	NS	NS	NS
HS 12	NS	NS	NS	NS	NS

Methodology

Determination of pH*:*

The pH of ABHSs was determined using calibrated digital pH meter (HI 2550 Hanna I made in Italy). Briefly, a 20 cm^3 quantity of alcohol-based HS was transferred into a beaker and the pH reading taken using the pH meter (Mettler Toledo) in triplicates.

Determination of the Type and Percentage alcohol of the ABHSs

For the determination of the type and percentage of alcohol, a gas chromatography equipped with mass spectrophotometer was used adopting standard procedure.

Antimicrobial Efficacy Test

The antimicrobial efficacy study for the ABHS was conducted in the microbiology laboratory of the Moses Oshio Adasu University, Makurdi, Nigeria

Test organisms

The following organisms were obtained from the culture collection of the Department of Microbiology, Moses Oshio Adasu University, Makurdi: *Staphylococcus aureus, Streptococcus pneumoniae, Escherichia coli, Pseudomonas aeruginosa and Klebsiella pneumoniae*. They were stored on nutrient agar slants and kept at 4^o C.

Preparation of McFarland Standard

Mcfarland 0.5 turbidity standard was prepared according to the method recommended by the National Committee for Clinical Laboratory Standards (NCCLS, 1999). The standard was prepared by adding 0.5 cm^3 of 1.175% w/v barium chloride dihydrate ($BaCl\ .2H_2O$) solution to 99.5 cm^3 of 15% w/v sulphuric acid (H_2SO_4).

Standardization of Test Organisms, Agar Diffusion Test (Well Variant) to determine susceptibility of test organisms to hand sanitizers, determination of minimum inhibitory concentration (MIC) and determination of minimum bactericidal concentration (MBC) was done using standard methods (Nester, 2009).

RESULTS AND DISCUSSION

Results

Determination of pH:

The pH of ABHSs was determined using calibrated digital pH meter (HI 2550 Hanna I made in Italy) and the results are as shown in Table 2.

Table 2: pH values for the ABHSs

ABHS CODE	pH
HS 1	7.2
HS 2	7.6
HS 3	7.2
HS 4	7.3
HS 5	7.9
HS 6	7.6
HS 7	7.3
HS 8	7.5
HS 9	7.5
HS 10	7.2
HS 11	7.4
HS 12	7.8

Percentage Alcohol

Alcohol content of hand sanitizers was determined using GC-MS. The gas chromatograms of analyzed samples were studied and the results computed from the chromatograms are shown in Table 3.

Table 3: Percentage Alcohol per Label Claim and Result of the Analysis

T	Label claim (%)	Analysis result (%)
HS 1	70	25.48
HS 2	62	67.18
HS 3	NS	30.57
HS 4	NS	33.41
HS 5	65	21.45
HS 6	NS	70.32
HS 7	62	20.32
HS 8	NS	74.83
HS 9	NS	39.54
HS 10	NS	27.03
HS 11	NS	65.32
HS 12	NS	83.87

Determination of the Type of Alcohol

The type of alcohol was determined using a gas chromatography equipped with mass spectrophotometer and the results computed from the chromatograms are shown in table 4.

Table 4: Type of Alcohol per Label Claim and Result of the Analysis

ABHS CODE	Label claim	Analysis result
HS 1	Alcohol denat	Isopropanol and methanol
HS 2	Ethyl alcohol	Ethyl alcohol and 2-hexene-1-ol
HS 3	Ethyl alcohol and isopropanol	Ethyl alcohol, isopropanol, 1-propanol and butanol
HS 4	NS	Ethyl alcohol and 2-penten-4-yn-1-ol
HS 5	Ethyl alcohol and isopropanol	Ethyl alcohol and 7-octen-2-ol
HS 6	Isopropanol	Isopropanol, 1-Propanol and methanol
HS 7	Ethyl alcohol	Ethyl alcohol
HS 8	NS	1-propanol and methanol
HS 9	Ethyl alcohol	Ethyl alcohol
HS 10	Ethyl alcohol	Ethanol Isopropanol and 1-propanol
HS 11	NS	Isopropanol
HS 12	NS	Isopropanol and 1-propanol

Antimicrobial Evaluation

The results for antimicrobial activity were assessed by measuring the zone of inhibition against the specific test bacteria. Tables 5, 6 and 7 show the results for the susceptibility of test organisms to hand sanitizers, determination of minimum inhibitory concentration (MIC) and determination of minimum bactericidal concentration (MBC) respectively.

Table 5: Zones of Inhibition of Selected ABHS against Test Organisms

Hand sanitizer	Zones of Inhibition (mm)				
	Staphylococcus aureus	*Streptococcus pneumoniae,*	*Escherichia coli,*	*Pseudomonas aeruginosa*	*Klebsiella pneumonia*
HS 1	6	5	8	7	7
HS 2	14	14	10	11	13
HS 3	6	5	8	5	5
HS 4	6	5	7	6	4
HS 5	4	4	7	6	3
HS 6	13	9	9	10	**7**
HS 7	3	5	7	3	4
HS 8	15	13	11	13	9
HS 9	7	9	9	7	6
HS 10	5	6	8	7	9
HS 11	13	13	11	10	12
HS 12	15	13	10	15	12

Table 6: Percent of Minimum Inhibitory Concentration (MIC) of Selected ABHS against Test Organisms

Hand sanitizer	Minimum inhibitory concentration (MIC (%))				
	Staphylococcus aureus	*Streptococcus pneumoniae,*	*Escherichia coli,*	*Pseudomonas aeruginosa*	*Klebsiella pneumonia*
HS 1	70	80	60	80	80
HS 2	40	50	30	40	30
HS 3	80	70	60	60	70
HS 4	70	50	70	60	60
HS 5	80	80	60	70	80
HS 6	30	40	50	40	40
HS 7	80	70	90	70	70
HS 8	30	20	30	40	40
HS 9	60	60	70	60	50
HS 10	70	90	80	60	70
HS 11	40	50	50	30	40
HS 12	20	20	40	30	20

Table 7: Percent of Minimum Bactericidal Concentration (MBC) of Selected ABHS against Test Organisms

Hand sanitizer	Minimum bacteria concentration (MBC (%))				
	Staphylococcus aureus	*Streptococcus pneumoniae,*	*Escherichia coli,*	*Pseudomonas aeruginosa*	*Klebsiella pneumonia*
HS 1	60	80	70	50	80
HS 2	30	40	20	50	30
HS 3	70	60	60	80	80
HS 4	60	50	80	70	60
HS 5	80	50	70	70	60
HS 6	30	20	40	30	30
HS 7	80	80	70	60	60
HS 8	20	30	30	20	40
HS 9	50	60	60	70	70
HS 10	80	80	60	60	70
HS 11	40	30	50	50	40
HS 12	20	20	20	30	20

Discussion

Determination of pH:

The pH of hand sanitizers observed in the range of 7.20 – 7.90 is slightly alkaline which is overall consistent with the pH of 100% ethanol, which is very slightly basic at 7.33 (Shahd *et al,* 2024). The fluctuation in pH of these hand sanitizers is mainly due to the additives and different percentages of ethanol present. The product with the highest pH value was HS 5 at 7.90 followed by HS 12 at 7.80 then HS 2 and HS 6 at 7.6 respectively. Given that the skin's natural pH is slightly acidic, using a product with neutral to slightly alkaline pH like HS 5 and HS 12 could potentially disrupt the skin's acid mantle. This could lead to skin dryness and irritation with frequent use (Proksch, 2028). However, the GC-MS result of these hand sanitizers also revealed the presence of glycerin as one of the ingredients which serve as a moisturizer to help mitigate these effects. The lowest pH value was observed in HS 1, HS 3 and HS 10 at 7.2. The near neutrality of these hand sanitizers is imperative to the efficacy and acceptability in the market (Selam *et al.,* 2022).

Determination of Percentage Alcohol

Though only a few of the products chosen had specific claims of the percentage alcohol, this study aimed to determine if they met this claim and followed the WHO standards. The results in Table 3, which are computed from the gas chromatograms show that, only 41.67% of the ABHSs have alcohol content within the WHO recommended range of 60% to 90%. HS 12, HS 8, HS 6, HS 2 and HS 11 have alcohol contents within the WHO recommended range at 83.87, 74.83, 70.32, 67.18 and 65.32 respectively. However, seven samples contained less alcohol content than the WHO standards. The products, HS 7, HS 5 and HS 1 had the least amount of alcohol at 20.32, 21.45 and 25.48 respectively. These figures fall far below the recommended concentration for viral or bacterial inactivation. According to Shahd *et al.,* 2024, products with the highest alcohol content have greater antibacterial activity.

According to a review conducted by Villa and Russo 2021, hand sanitizers require a mixture of water and alcohol as key active ingredients to achieve effective protein denaturation of viruses. It should be noted that if the alcohol concentration is greater than 90% and water is excluded from the sanitizer, it is not efficacious in killing pathogens since water is required for protein denaturation. None of the ABHS samples analyzed in this study contained more than 90% alcohol, which is an imperative finding as above this, would not result in adequate denaturation of the virus.

From our results, the general trend is that, 58.33% of the samples contained less than 60% alcohol. If the alcohol content in hand sanitizers is below the aforementioned requirement, it will limit the growth of pathogens instead of killing them. Alternatively, such ABHS should be for cosmetic use only and not as a biocide (Berardi *et al,* 2020*).*

Determination of the Type of Alcohol

Of the twelve alcohol-based hand sanitizers selected for this study, four, which represent 33.33% did not have their type of alcohol stated on the container while the other eight which represent 66.67% had their types of alcohol as either ethyl alcohol or propanol. The US FDA allows ethanol and isopropanol as the key active ingredients for ABHS, and ingredients such as 1-propanol and methanol are classified as toxic. Methanol is toxic when inhaled, taken orally, or applied to the skin; and should never be incorporated into any hand hygiene product. Methanol poisoning may lead to central nervous system depression and severe metabolic acidosis, causing blindness, confusion, and eventual death (*Yip et al., 2020).*

The result of this analysis as shown in Table 4 indicates that, of the twelve samples, HS 1, HS 2, HS 3, HS 6, HS 7, HS 9 and HS 10 had their type of alcohol to be the same as what is stated on the label.

A discrepancy between the label claim and the analysis result regarding the type of alcohol was evident in sample HS 4 and HS 5 while. Alarmingly, six samples, viz. HS 2, HS 3, HS 6, HS 8, HS 10 and HS 12 were found to have 1-propanol, an alcohol that is considered toxic at high concentrations.

Another finding of this study leads to a grave concern regarding the safety of users of ABHSs as four of the samples, viz. HS 1, HS 6, and HS 8 were found to contain methanol. A study conducted in Nairobi showed about 14.9% of the tested 74 samples had methanol, instead of ethanol, as the main component of ABHS (Abuga *et al,* 2021).

Such circulation of falsified hand sanitizer products in the market compromises the control of infection transmission and may expose users to the undesired effects. Nausea, vomiting, blindness, seizures, coma, damage to the nervous system or death may be resulted from methanol exposure that seek immediate treatment for reversal of such toxic effects (FDA). However, the public should not misconstrue that all ABHS has false labelling. To avoid substandard ABHS production, higher governing authorities must implement stricter and consistent manufacturing guidelines.

Determination of the Anti-microbial Activity

Table 5 shows the zones of inhibition (mm) produced by twelve (12) different alcohol-based hand sanitizers (HS1–HS12) against five bacterial species: *Staphylococcus aureus, Streptococcus pneumoniae, Escherichia coli, Pseudomonas aeruginosa* and *Klebsiella pneumoniae.*

From the results, there is a clear difference in antibacterial effectiveness among the tested sanitizers. The zones of inhibition ranged from 3 mm to 15 mm, indicating varying levels of antimicrobial potency. Larger inhibition zones indicate stronger antibacterial activity, while smaller zones suggest weaker effectiveness.

Among the tested samples, HS8 and HS12 demonstrated the highest antimicrobial activity, producing zones of inhibition between 9 mm and 15 mm against all tested organisms. This can be attributed to their high percent alcohol content which stands at 74.83 and 83.87 respectively. In contrast, HS5 and HS7 exhibited the lowest activity, with inhibition zones as small as 3–7 mm, suggesting weaker antibacterial performance.

Based on the results, the sanitizers can generally be categorized as HS8, HS12, HS2, HS11 being highly effective, HS6, HS9, HS10 moderately effective and HS1, HS3, HS4, HS5, HS7 poorly effective. The higher effectiveness observed in some sanitizers (HS8, HS12, HS2, HS11) may be due to their higher alcohol concentration (60–80%), presence of additional antimicrobial agents such as lemone and better formulation and product quality.

Effective hand sanitizers play a critical role in controlling the spread of infectious microorganisms, especially in healthcare and community settings. Products that demonstrated larger inhibition zones (such as HS8 and HS12) may provide better protection against pathogenic bacteria including *Staphylococcus aureus* and *Escherichia coli*, which are common causes of infections. However, sanitizers with smaller zones of inhibition may not provide adequate antimicrobial protection and may therefore be less suitable for infection control purposes.

The Minimum Inhibitory Concentration (MIC) values represent the lowest concentration (%) of each hand sanitizer required to inhibit visible growth of specific microorganisms. Thus, lower MIC values indicate higher antimicrobial effectiveness, while higher MIC values suggest weaker activity.

Results from Table 6 indicates that, HS 12, HS 8, HS 6, HS 2 and HS 11 have low MIC values and overall, were the most effective against the tested organism. While HS 9, HS 4 were moderately effective, HS 1, HS 3, HS 5, HS 7, HS 10 were generally shown to have high MIC values and hence, the least effective against the tested organisms. The implications are that, not all commercially available hand sanitizers are equally effective as clearly shown by this study and high MIC values in some products may pose a risk of inadequate disinfection, especially against resistant Gram-negative organisms.

The MIC analysis reveals significant variability in antimicrobial efficacy among the tested hand sanitizers. HS 12 stands out as the most effective broad-spectrum sanitizer, while several others demonstrate insufficient inhibitory activity at practical concentrations.

The Minimum Bactericidal Concentration (MBC) represents the lowest concentration of a hand sanitizer required to kill bacteria. Thus, lower MBC values indicate higher bactericidal effectiveness, while higher values suggest weaker activity. The result from Table 7 shows a considerable variation in bactericidal activity among the twelve hand sanitizers (HS 1–HS 12) against the tested organisms. HS 12, HS 8, and HS 6 exhibit the lowest MBC values overall between 20–40% for all tested organisms, indicating excellent broad-spectrum bactericidal activity. While HS 12 shows a better activity across the tested organism, HS 8 was particularly strong against *Staphylococcus aureus* and *Pseudomonas aeruginosa* and HS 6 shows good activity especially against *Streptococcus pneumoniae* and *Pseudomonas aeruginosa*. In line with this, a study conducted by Selam et al (2022) revealed that 25% was the minimum concentration of bacterial inhibition. The effectiveness of these sanitizers can be attributed to their optimal concentrations of active antimicrobial agents.

According to the results, HS 2, HS 11 and HS 9 were moderately effective against the tested organisms and require moderate concentrations (40–60%) to achieve bactericidal effects. HS 1, HS 3, HS 5, HS 7, and HS 10 which constitute about 41.7 % of the samples, shows high MBC values, indicating poor bactericidal efficiency. Minimum bactericidal activities could be due to the relatively decreased concentration of alcohol in hand sanitizer as the efficacy of alcohol-based hand sanitizer is affected mainly by the type and concentration of alcohol used.

The antimicrobial results demonstrate that not all hand sanitizers are equally effective. HS 12, HS 8, and HS 6 exhibit strong bactericidal activity and are suitable for broad-spectrum antimicrobial use. In contrast, sanitizers such as HS 7 and HS 10 may be less reliable, requiring higher concentrations to achieve bacterial killing. This highlights the importance of quality formulation and adequate active ingredient concentration in ensuring sanitizer efficacy.

CONCLUSION

GC–MS-based method was used for the quality, safety, and efficacy assessment of ABHS products. The method's applicability was demonstrated using 12 ABHS samples obtained from stores across Benue south and the study discovered the use of two toxic contaminants (1-propanol and ethyl methanol) in some samples. The study also found many of the ABHSs to have far less percentage alcohol compared to their label claims and acceptable standards.

The results demonstrate that not all alcohol-based hand sanitizers possess the same antibacterial effectiveness. While some products exhibited strong inhibitory effects against both Gram-positive and Gram-negative bacteria, others showed limited antimicrobial activity.

Therefore, it is critical for regulatory bodies like the NAFDAC to ensure that ABHSs contain the required percentage and type of alcohol and are free of any potentially hazardous impurities like methanol.

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