

EVALUATION OF ANTIBACTERIAL EFFICACY OF SOME ALCOHOL-BASED HAND SANITIZERS SOLD IN ILORIN (NORTH-CENTRAL 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. The use of hand sanitizers is one of the ways to address the barriers to effective hand hygiene. In this study, we evaluated the antibacterial efficacy of some popular hand sanitizers sold in Ilorin. 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*. Hygel alone inhibited all the test organisms with zones of inhibition ranging between 14.3mm and 28.0mm while Dettol was only effective against *P. aeruginosa* (14.5mm). The other products (SKP and Samcleans) were not active against any of the test organisms. Hygel and Dettol were bacteriostatic at 100% concentration (MIC) and none was bactericidal. In vivo tests to determine the efficacy of these two in reducing bacterial counts from hands of subjects showed mean percentage cfu reduction of 89.9% for Hygel and 73.8% for Dettol. There was no significant difference in the efficacy of the two products in this regard. Despite the claims of efficacy and 99.9% bacterial count reduction by hand sanitizer manufacturers, there still exists a need for verification of these claims by regulatory bodies and for the enforcement of good quality control measures.

Keywords: Hand Sanitizer, Hand Hygiene, Bactericidal, Bacteriostatic, MIC, MBC

INTRODUCTION

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). Hand hygiene has 2 major components: hand washing, which is the removal of microorganisms with ordinary soap and water; and hand antiseptics, which is the removal or destruction of microorganisms using an antimicrobial soap or an alcohol-based hand rub. Several studies have demonstrated the effectiveness of different forms of hand hygiene in reducing incidences of healthcare-associated infections (Maki, 1989; Massanari and Hierholzer, 1984; Doebbeling *et al.*, 1992). Other studies also demonstrated increased frequency of hand hygiene and reduced frequency of hospital-associated infections with provision of alcohol hand gels in the context of institution-wide hand hygiene campaigns (Zerr *et al.*, 2005).

However, despite the evidences and expert opinions supporting hand hygiene, there is low compliance among individuals. 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. 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 (Larson *et al.*, 2001). 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 (Price, 1939; Sakuragi *et al.*, 1995, Kampf *et al.*, 1999), they have also been reported to have very poor activity against bacterial spores, protozoan oocysts and certain non-enveloped (non-lipophilic) viruses (CDC, 2002). Despite several reports stating their efficacy, consumers have been warned against false claims of efficacy by some manufacturers (FDA, 2011).

Hand sanitizers are relatively new in the Nigerian market and the government regulatory body, NAFDAC (National Agency for Food and Drugs Administration and Control), has registered a number of commercial hand sanitizers. It is therefore necessary to evaluate the efficacy of these products. In this study, we evaluated the antibacterial efficacy of 4 popular brands of hand sanitizers sold in Ilorin, a north-central Nigerian town.

MATERIALS AND METHODS

Test organisms

The following organisms obtained from the culture collection of the Department of Microbiology, University of Ilorin were used in this study: *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*. They were stored on nutrient agar slants and kept at 4°C until when needed.

Hand sanitizers

Four brands of alcohol-based hand sanitizers were purchased from local retail outlets in Ilorin. The products are Hygel, Dettol, SKP and Samclean. Table 1 shows the composition of the hand sanitizers.

Table 1: Hand Sanitizers Used in the Study and their Ingredients.

PRODUCT	INGREDIENTS
Hygel	62% ethanol, glycerin.
Dettol	Alcohol denat, aqua, PEG/PPG-17/16 copolymers, Acrylate/c10-30 Akyl Acrylate cross polymer, Tetrahydroxyl propyl Ethylenediamine, parfum, and limonene.
SKP	62% Isopropyl Alcohol, water, glycerin, propylene glycol, fragrance, triethanolamine, carbopol 940, acetate isopropyl myristate.
Samclean	62% Ethyl alcohol, water, isopropyl alcohol, carbomer, tocophenyl acetate, glycerin, propylene glycol, isopropyl myristate, fragrance.

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 0.5ml of 1.175% w/v barium

chloride dihydrate ($\text{BaCl}_2 \cdot 2\text{H}_2\text{O}$) solution to 99.5ml of 15% w/v sulphuric acid (H_2SO_4). This was mixed well and then aliquoted into test tubes identical to the ones used in preparing inoculum suspensions of the test organisms. The accuracy of the density of the standard was verified using a spectrophotometer. The absorbance of the 0.5 McFarland standard at wavelength 625nm was 0.08-0.10. The tubes were stored in a well-sealed container in the dark at room temperature until when needed (Cheesbrough, 2006).

Standardization of Test Organisms

A sterile loop was used to pick a loopful of inoculum from a pure culture of the test organism. This was then transferred and suspended in a tube of sterile normal saline (NaCl 8.5g, distilled water 1 litre). The tube was compared with the turbidity standard and the density of the organism was adjusted to that of the standard by adding more bacteria or more sterile saline (Vandepitte *et al.*, 2003).

Agar Diffusion Test (Well Variant) to Determine Susceptibility of Test Organisms to Hand Sanitizers

The susceptibility of the test organisms to the hand sanitizers was investigated using the well variant of the agar diffusion method described by Valgas *et al.* (2007). Sterile Mueller Hinton agar plates were inoculated with standardized test organisms. A sterile cotton swab was dipped into a tube containing the inoculum and was rotated properly to allow maximum contact. Excess inoculum was removed by pressing and rotating the swab firmly against the inside of the tube above the liquid level. The swab was then streaked over the surface of the medium three times while rotating the plate through an angle of 60° after each application. The swab was also passed round the edge of the agar surface. The inoculum was left to dry for a few minutes at room temperature with the lid closed.

With the aid of a sterile 6mm cork borer, 4 equally spaced holes were bored in the agar plate with a fifth hole in the centre of the plate. The agar plugs were discarded using a sterile needle. Fifty microlitres (50 μL) of the hand sanitizer was then introduced into each of the 4 wells while the central well was filled with an equal volume of sterile water to serve as control. This was done for all the test

organisms and hand sanitizers. The plates were incubated for 24 hours at 37°C in an upright position. They were then examined for zones of inhibition which indicate the degree of susceptibility or resistance of the test organism to the antibacterial agent. The test was carried out in duplicates and the average of 2 readings was taken as the zone of inhibition in each case. Inhibition zones were measured with the aid of a ruler (mm).

Determination of Minimum Inhibitory Concentration (MIC)

The hand sanitizers which showed activity against test organisms in the agar diffusion test were subjected to further test to determine their MIC values using the broth dilution method. MIC is the lowest concentration of a specific antimicrobial needed to prevent the growth of a given antimicrobial substance in vitro (Nester *et al.*, 2009).

Various concentrations of the sanitizers were prepared in increasing order (20%, 40%, 60%, 80% and 100%). One milliliter of each sanitizer was introduced into tubes containing equal volume (1 ml) of standardized test organisms. Each of the concentrations of the sanitizers was used in each case. A tube containing only nutrient broth and bacteria without sanitizer served as negative control while a tube containing just the sanitizer and broth without bacteria served as positive control. The tubes were incubated for 18-24 hours and examined for visible growth or turbidity. The concentration of the sanitizer at which no visible growth was observed when compared with the controls was regarded as the MIC.

Determination of Minimum Bactericidal Concentration (MBC)

MBC is the lowest concentration of a specific antimicrobial substance that kills 99.9% of cells of a given bacterial strain (Nester *et al.*, 2009). MBC was determined by assaying for live organisms in the tubes from the MIC tests which showed no visible growth. A loopful of inoculum from the MIC tubes was streaked on fresh nutrient agar plates without the hand sanitizer incorporated into them. The plates were incubated at 37°C for 24 hours after which they were observed for growth. Absence of growth indicated a bactericidal effect of the sanitizer at that concentration which is the MBC.

Determination of Efficacy of Hand Sanitizers in Reducing Viable Counts of Bacteria on the Hands of Subjects

The two products (Hygel and Dettol) which showed activity against test organisms in the agar diffusion test were further evaluated for their efficacy in reducing baseline bacterial counts of resident flora on the hands of subjects. Ten individuals were randomly selected for the study and verbal informed consent was obtained from all participating subjects prior to the conduct of the experiment.

The hands of 5 of the randomly selected subjects were examined for baseline bacterial count reduction with Hygel while the other 5 subjects' hands were examined with Dettol. Sterile nutrient agar plates were serially numbered and each was

divided into 2 equal halves A and B. The test was carried out with unwashed hands of the subjects. Subjects' left hands were gently used to make a finger impression on the agar by pressing and rolling the finger on the agar in the section A of the plate. Three milliliters of the sanitizer was then applied to the hand and then rubbed thoroughly on the palm, hands and fingernails until the hands became dry. The finger impression was then repeated on the B section of the plates. This was done for all subjects. The plates were incubated at 37°C for 24 hours and the number of colonies were counted.

RESULTS AND DISCUSSION

Table 2 below shows the susceptibility pattern of the test organisms to the hand sanitizers in the agar diffusion test.

Table 2: Susceptibility Pattern of Test Organisms to Hand Sanitizers.

Test Organisms	Zones of inhibition (mm) of hand sanitizers against test organisms			
	Hygel	Dettol	Samclean	SKP
<i>E. coli</i>	26.0	-	-	-
<i>S. aureus</i>	25.5	-	-	-
<i>P. aeruginosa</i>	28.0	14.5	-	-
<i>K. pneumoniae</i>	19.0	-	-	-
<i>S. pneumoniae</i>	14.3	-	-	-

- No inhibition.

Hygel was the only product that showed inhibitory activity against all the test organisms with the highest activity against *P. aeruginosa* (28.0 mm) and the lowest against *S. pneumoniae* (14.3mm). Dettol was only active against *P. aeruginosa* (14.5mm). SKP and Samclean showed no activity against all the test organisms.

Hygel and Dettol were further tested to determine their MIC and MBC values. For Hygel, inhibition of all the test organisms was only observed at the 100% concentration thus indicating that the MIC

value of the product against all test organisms was 100% (Table 3). The contents of the 100% concentration tubes were further plated out on sterile nutrient agar plates (which had no antibacterial incorporated into them) in order to determine the bactericidal concentration. All the plates showed growth of the organisms thus indicating that the product was only bacteriostatic against the organisms and not bactericidal. Similarly, Dettol had an MIC value of 100% concentration against *P. aeruginosa* and was not bactericidal (Table 3).

Table 3: Minimum Inhibitory Concentration (MIC) of Test Organisms to Hygel and Dettol Hand Sanitizers.

Hand sanitizer	Hand sanitizer concentration (%)	Test organisms					MIC
		<i>E. coli</i>	<i>S. aureus</i>	<i>P. aeruginosa</i>	<i>K. pneumoniae</i>	<i>S. pneumoniae</i>	
Hygel	20	+	+	+	+	+	100%
	40	+	+	+	+	+	
	60	+	+	+	+	+	
	80	+	+	+	+	+	
	100	-	-	-	-	-	
Dettol	20	N/A	N/A	+	N/A	N/A	100%
	40			+			
	60			+			
	80			+			
	100			-			

Key: + growth, - no growth, N/A - not applicable

These two hand sanitizers displayed bacteriostatic activity against at least one of the test organisms. This is attributable to the presence of alcohols as the main active ingredients in the products. Alcohols are known to exert disinfectant activity in bacteria by causing protein denaturation, disruption of tissue membranes and dissolution of several lipids (Kar, 2008). Hygel which had the highest activity against the organisms in this study contained 62% ethanol as the main active ingredient while Dettol which was active against *P. aeruginosa* contained alcohol denat as the active ingredient. Isopropyl alcohol was the main active ingredient in SKP and Samclean (in addition to ethyl alcohol) (Table 1).

Although isopropanol has been reported as being superior to ethanol as an antiseptic, the poor activity of SKP, Samclean and Dettol observed in this study is probably due to the negative interactive effects of the additional ingredients

such fragrance, emollients, humectants and thickening agents added to them and which are not present in Hygel. These could probably limit the cidal effect of the alcohol from reaching the bacterial cells. However, in vitro tests need to be carried out to rule out or confirm this possibility. The efficacy of alcohol-based hand sanitizers is affected by several factors such as the type, concentration and volume of alcohol used, the contact time (CDC, 2002), the test method (in vitro and in vivo), target organism and matrix (Liu *et al.*, 2010). Generally, the lack of bactericidal activity observed among all the products could be due to poor or prolonged storage of the products which could lead to increased temperature causing evaporation of the active ingredient.

Table 4 shows the percentage reduction of baseline bacterial counts on hands of subjects after applying Hygel and Dettol sanitizers.

Table 4: Percentage cfu Reduction of Viable Bacterial Count Reduction on Hands of Subjects by Hygel and Dettol.

Subjects	cfu reduction (%)	
	Hygel	Dettol
A	91.10	60.00
B	100.00	70.60
C	100.00	60.00
D	75.00	100.00
E	83.30	78.60
Mean reduction	89.90	73.80

Statistical comparison showed that there was no significant difference in the mean percentage reduction of viable bacterial counts by the two products (**two-sample t test**, $t(8) = 2.31$, $p < 0.05$) thus indicating that there is no significant difference in the efficacy of the two products in

this regard. The highest mean reduction of bacteria by these products was 89.9% which is lower than the 99.9% reduction usually put on the labels of these products. Reynolds *et al.* (2006) found that a number of products with alcohol concentrations as low as 33% and 40% were

available in American stores despite label claims of “reducing germs and harmful bacteria by 99.9%”. They found that the products with 40% ethanol yielded no significant reductions in cfu while those with 62% ethanol reduced mean cfu by up to 90%.

CONCLUSION

We have evaluated the antibacterial efficacy of the most popular brands of hand sanitizers sold in Ilorin. Only one of the products inhibited growth of all the test organisms in vitro and none of the products was bactericidal. Also, the most efficacious product was only able to effect 89.9% bacterial count reductions in vivo.

From these findings, we identify the need to confirm the concentration of alcohol in the hand sanitizers sold in consumer outlets in order to verify the claims of the manufacturers and thus protect consumers from buying substandard products. Regulatory authorities and manufacturers should enforce stringent quality control measures during production and routine inspections to ensure the efficacy of these products. Lastly, consumers should be alerted on the existence of substandard sanitizers on the shelves of some retail outlets.

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