

Antibacterial Activities of Some Hand Sanitizers Sold in Aba North, Abia State, Nigeria

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ABSTRACT

The use of hand sanitizers has made numerous claims, notably their ability to eliminate 99.9% of microorganisms. The present study investigated the antibacterial effect of different commercial and homemade hand sanitizers on clinical isolates from human skin using standard microbiological procedures. Five different samples of hand sanitizers designated sample E, sample N, sample L, sample G, and sample P were used for the study. A total of three isolates namely, *E. coli*, *Staphylococcus* and *Pseudomonas* sp were identified. The results of the study showed that sample L had more effect compared to other hand sanitizers with zone of inhibition ranging from 5.0 - 11.5mm followed by sample G (8.0- 9.0mm), P (3.0- 6.4mm), E (2.1- 4.2mm) and N (2.0- 6.0mm) which showed varying zones of inhibition according to the test isolates. The decreasing order of the hand sanitizers tested was therefore, L > G > P > N > E. The minimum inhibitory concentration of the sanitizers on the test isolates showed that all the samples had an MIC at 100% concentration. Some of the products assayed had good efficacy and all products tested were not active against all the test organisms using the dilution method.

Keywords: Hand sanitizers, human skin, antibacterial effect, zone of inhibition, minimum inhibitory concentration (MIC).

Introduction

Traditionally, bacteria on hands can be categorized as resident and transient floras. Common resident floras such as *Staphylococcus epidermidis*, and *Enterococcus faecalis* which colonize deep layers of the skin are resistant to mechanical removal while transient floras such as *Escherichia coli*, and *Pseudomonas aeruginosa*, colonize the superficial layers of skin (Jain et al., 2016).

There are also numerous bacterial strains that can be transmitted to the host from other sources that can potentially develop into a variety of bacterial infections.

Hand washing and the use of hand sanitizers are important interventions in disease prevention.

Engaging in frequent hand washing is especially effective in preventing the spread of viruses, as this removes microbes and prevents the spread to others. Hand dermatitis, however, is a common occurrence in certain occupations, such as healthcare workers. With the onset of the SARS-CoV2 (COVID-19) pandemic, hand hygiene measures are further enforced as there is no cure or vaccine for this virus at its onset.

Alcohol based hand sanitizers (ABHS) are very effective for quickly destroying many pathogens by the action of the aqueous alcohol solution without the need for water or drying with towels. According to the Centers for Disease Control and Prevention (CDC, 2002), ABHS have excellent *in vitro*

antimicrobial activity, including multidrug-resistant pathogens, such as methicillin-resistant *S. aureus*, vancomycin-resistant *Enterococcus* (Gerberding *et al.*, 2002). Specific *in vitro* studies show that hand sanitizers containing 60%-80% ethanol produced 4 to 6 log reduction in 15-30 seconds against a range of bacterial and fungal species (Fendler *et al.*, 2002). The effectiveness of hand sanitizer depends on multiple factors, including the manner in which the product is applied (e.g., quantity used, duration of exposure, frequency of use) and whether the specific infectious agents present on the person's hands are susceptible to the active ingredient in the product (David *et al.*, 1998; Eva and Badar, 2014).

In general, alcohol-based hand sanitizers, if rubbed thoroughly over finger and hand surfaces for a period of 30 seconds, followed by complete air-drying, can effectively reduce populations of bacteria, fungi, and some enveloped viruses.

In 2010 the World Health Organization produced a guide for manufacturing hand sanitizers, which received renewed interest in 2020 because of shortages of hand sanitizers in the wake of the COVID-19 pandemic (Okafor, 2020). Dozens of liquor and perfume manufacturers switched their manufacturing facilities from their normal product to hand sanitizer. In order to keep up with the demand, local distilleries started using their alcohol to produce hand sanitizer (Kaur, 2020). Distilleries producing hand sanitizer originally existed in a legal grey area in the United States, until the Alcohol and Tobacco Tax and Trade Bureau declared that distilleries could produce their sanitizers without authorization (Levenson, 2020)..

In the beginning of the pandemic, because of hand sanitizer shortages due to panic buying, people resorted to using 60% to 99% concentrations of isopropyl or ethyl alcohol for hand sanitization, typically mixing them with glycerol or soothing moisturizers or contain aloe vera to counteract irritations with options of adding drops of lemon or lime juice or essential oils for scents, and thus making "do it yourself" (DIY) hand sanitizers. However, there are cautions against making them, such as a wrong measurement or ingredient may result in an insufficient amount of alcohol to kill the coronavirus, thus rendering the mixture ineffective or even poisonous (Mitroff, 2020).

Additionally, some commercial products are dangerous, either due to poor oversight and process control, or fraudulent motive.

The present study investigates the efficacy of different commercial and homemade hand sanitizers sold within Aba North considering the increase in demand of the product.

Materials and Methods

Sample Collection

Five (5) different brands of hand sanitizers viz:- Ema Hand Sanitizer, Neo Sanitizer, Lemo Fresh, Gello Hand Sanitizer, Pure Bliz Sanitizer were purchase from stores within Umungazi market. the hand sanitizers were designated E, N, L, G, and P respectively and immediately transported to the Microbiology laboratory, Abia State Polytechnic, Aba for analysis to determine their antimicrobial properties against clinical isolates collected from patients visiting the school medical centre.

Antimicrobial Assay

The agar well diffusion method as described by Otokunefor and Dappa, (2017) was used as a preliminary screening to assess the antimicrobial activities of the various products. The inoculums used was prepared using the isolates from a 24-hour culture on nutrient agar, a suspension was made in a sterile saline solution (0.85%). The turbidity of the suspension was then adjusted with a spectrophotometer at 530 m to obtain a final concentration to match that of a 0.5 McFarland standard ($0.5—2.5 \times 10^3$). The test isolate was spread on Mueller Hinton agar plate and allowed to stand at room temperature for 15 minutes.

Following this, three wells were created on the plates using a 6 mm cork borer and 0.2 ml of differing concentrations (100%, 50%, 25% and 12.5%) of the test hand sanitizers added to individual wells. After 24 hours of incubation at 37°C, the zones of inhibition were then measured and reported in millimeter (mm) and the results interpreted as sensitive, intermediate and resistant according to National Health Insurance Scheme (CLSI, 2017). All antimicrobial assays for each isolate were made in duplicate.

Minimum Inhibitory Concentration (MIC)

MIC testing was carried out to determine the minimum concentration of test substance which could cause an inhibition of the growth of the test isolates. This involved the inoculation of 5×10^8 CFU of organisms to doubling dilutions of the test substances. After 24 hour incubation at 37°C, the MIC was determined as the lowest concentration of test substance which caused an inhibition of the growth of the test organisms.

Results

The result of the mean values of the zone of inhibition (mm) of the different hand sanitizers against the test clinical isolates is as shown in the Table 1 below. While the minimum inhibitory concentration (MIC) (%) of different hand sanitizers on clinical isolates is presented in Table 2.

Table 1: Mean values of zone of inhibition (mm) of different concentrations of different hand sanitizers on clinical isolates

Clinical Isolate	100%			50%			25%			12.5%		
	<i>E. coli</i>	<i>Staphylococcus</i>	<i>Pseudomonas</i>	<i>E. coli</i>	<i>Staphylococcus</i>	<i>Pseudomonas</i>	<i>E. coli</i>	<i>Staphylococcus</i>	<i>Pseudomonas</i>	<i>E. coli</i>	<i>Staphylococcus</i>	<i>Pseudomonas</i>
Concentration												
Sanitizer Sample	Zone of Inhibition (mm)											
E	4.2	-	2.1	-	-	-	-	-	-	-	-	-
N	3.0	6.0	2.0	-	-	-	-	-	-	-	-	-
L	5.0	11.5	5.3	-	-	-	-	-	-	-	-	-
G	9.0	-	8.0	-	-	-	-	-	-	-	-	-
P	3.4	4.0	6.2	-	-	-	-	-	-	-	-	-

Key: E= Ema Hand Sanitizer, N= Neo Sanitizer, L= Lemo Fresh, G= Gello Hand Sanitizer, P= Pure Bliz Sanitizer

Table 2: Minimum inhibitory concentration (%) of different hand sanitizers on clinical isolates

Samples	Isolate / MIC (%)		
	<i>E. coli</i>	<i>Staphylococcus</i>	<i>Pseudomonas</i>
E	100	-	100
N	100	100	100
L	100	100	100
G	100	-	100
P	100	100	100

Discussion

The human hands are the parts of the human body that are mostly in contact with the outside environments, people use their hands for different activities almost for the whole day and often come in contact with different microbial contamination which they equally transfer to object while carrying out other activities even while eating. Hand hygiene is one effective way to prevent contact spread of disease. Therefore, there is high demand for hand sanitizers. The present study investigated the antibacterial effect of different hand sanitizer used within Aba metropolis. The result of the study showed that the hand sanitizer had promising effect on the test isolates. These observed effect can be attested to that reported by Otokunefor and Princewill (2017) and Ochwoto *et al.* (2017) who reported similar finding in their study.

However, sample E and G had no effect on *Staphylococcus* sp showing no zones of inhibition on the test isolate. This supports the findings of Tambekar *et al.* (2007) who stated that some hand sanitizers had no effect on Gram positive bacteria isolates. Furthermore, the zone of inhibition reported in the present study is less than those reported by previous authors the poor activities can be attributed to the differences, in the test isolate used in the studies, formulation or types of sanitizers used and possibly due to the adulteration of the products as a result of higher demand of sanitizers in the country and the pandemic. The study also noted that the hand sanitizer were only effective at 100% concentration.

The impact of hand hygiene in disease control and prevention has been well documented (Kampt and Kramer (2004) and Nathu, (2011). Hand Sanitizer has more recently been the proscribed method of hygiene, possibly due to the compliance rates associated with it and its usefulness in areas lacking adequate water supply.

With increase in compliance in use of hand sanitizers, there is a need to assess the efficacy of these products available in the market. Variable levels of activity of hand sanitizers in the market have previously been reported. Sharif and Ansari, (2015) reported that some sanitizer were not effective in preventing the growth of tested bacteria isolates used in their study.

In conclusion, hands greatly serve as a means by of transmission of pathogens, thus effective hand hygiene can reduce infection and prevent diseases. The use of hand sanitizers has gained popularity in Nigeria recently. This has led to the development, production and importation of several hand sanitizers by various companies with the aim of commercialization as well as supporting the health care system in preventing transmission of pathogens. While the results of this study showed that some of the products assayed have a good efficacy than others, all products tested were not active against all the test organisms using the dilution method. More stringent checks of products introduced into the Nigerian market may therefore be necessary to ensure that they meet set international standards both in composition of inhibitory substance and texture to ensure uniformity in activity against pathogens.

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