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Antibacterial Activity of Alcohol-Based Hand Sanitizers on Some Clinical Bacterial Isolates

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ABSTRACT: This study was carried out to investigate the antibacterial activities of commercially available alcohol-based handsanitizers (2Sure, Dettol and Carex) on some clinical bacterial isolates. The isolates were obtained from the laboratory stock culture of University of Benin Teaching Hospital, Benin City. Two Gram-positive bacteria (*Staphylococcus aureus* and *Bacillus subtilis*) and four Gram-negative (*Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Proteus* spp.) were used for the assay.The test organisms were subjected to different concentrations of the handsanitizers from 20 % to 100 %. The result showed that the handsanitizers used showed variable bacteriostatic and bacteriocidal activities against the test organisms. *Staphylococcus aureus* and *Pseudomonas aeruginosa* were susceptible to all the test handsanitizers with *Staphylococcus aureus* having the highest diameter of zone of inhibition (23.33± 2.03mm) using 2Sure handsanitizer. The highest inhibitory activity was exhibited by 2sure handsanitizer with highest values of diameter of zone of inhibition for all the test isolates except *Bacillus subtilis* which was resistant. The handsantizers showed inhibitory or bacteriocidal activity at a minimum concentration of 100% for the susceptible organism except 2sure which had an MIC of 80% for *Pseudomonas aeruginosa*

Keywords: Hand, Sanitizer, Antibacterial, Alcohol.

Introduction

Following the rise of infections in developed and developing countries, there has been public concern on methods to adopt to break the chain of infectious diseases and hand hygiene is an important measure in the prevention of infections (WHO, 2009; CDC, 2002). Hand hygiene is defined as any method employed to destroy microorganisms on hands. This can be achieved through hand washing with soap and water or the use of hand sanitizers which have been proven to be an affordable means to reduce morbidity and mortality due to infectious diseases (Stedman-Smith *et al.,* 2015).

Hand sanitizers could be alcohol-based or non-alcohol based, containing preparations designed for application to the hands to reduce the number of viable microorganisms on it (CDC, 2002).Various forms or preparations of hand sanitizers are available and they include; gel, foam and liquid solutions. The active components contained in hand sanitizers are: isopropanol, ethanol, n-propanol and povidine-iodine while the inactive component usually includes a thickening agent (such as polyacrylic acids for gels), humectant (such as glycerin for liquid rubs) or propylene glycol and essential oil of plants. Alcohol-based hand sanitizers have varying concentration or level of alcohol ranging from 60-95 % (Sandora *et al.,* 2008). Alcohol- based handsanitizers have been proven to be more effective (60- 80 %) owing to their rapid activity and its broad spectrum bactericidal acitivities (WHO, 2009). The use of handsanitizers have become more common as handwashing with soap and water is not practical and convenient as it may not be easily accessible, hence the use of handsanitizers but their effectivity are variable. This present study focuses on evaluating the effectiveness of three (3) alcohol-based commercially sold hand sanitizers on some clinical bacterial isolates of some selected Gram-negative and Gram-positive organisms.

Material and methods

Sample collection: Three (03) different brands of alcohol-based hand sanitizers (*2Sure*, *Dettol* and *Carex*) were purchased from local supermarkets in Benin City, Edo State, Nigeria.
Test organisms: The clinical isolates used for this research were obtained from the laboratory stock culture of the Department of Medical Microbiology, University of Benin Teaching Hospital (UBTH), Benin City. Two Gram-positive bacteria (*Staphylococcus aureus* and *Bacillus subtilis*) and four Gram-negative bacteria (*Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae,* and *Proteus* spp.) were used. These isolates were identified following standard microbiological techniques. They were then stored on nutrient agar slants and kept at 4 °C until when needed.
Sterilization of materials/preparation of culture media: Glasswares such as test tubes, glass rod, measuring cylinder, beakers and conical flasks were washed and rinsed with distilled water then dried in an oven at 160 °C-170 °C for 45-60 min. All media were prepared according to manufacturers' instructions employing standard laboratory practices. The media used were Nutrient Agar, MacConkey Agar, Salmonella-Shigella Agar, Eosin Methylene Blue Agar and Mueller-Hinton Agar.
Identification of isolates: Cultural and morphological and biochemical characterizations of the bacterial isolates was done using standard methods.
Standardization of innoculum: The concentration of the test isolates was adjusted to 0.5 McFarland turbidity standard ($1.5x10^8$ cfu/ml). 0.5 McFarland turbidity standard was prepared by adding 0.05 ml of 1% barium chloride dihydrate ($BaCl_2.2H_2O$) to 9.95 ml of 1% sulfuric acid (H_2SO_4) (Cheesbrough, 2006).
Antibacterial activity: The efficacy of the various alcohol-based hand sanitizers against the test organisms were determined using previously described methods (Otokunefor and Dappa, 2017; Magaldi *et al.*, 2004; CLSI, 2012) which are the well-variant of the agar diffusion test. The antibacterial susceptibility was indicated by the zone diameter of inhibition measured in millimeter (mm).
Determination of Minimum inhibitory concentration (MIC): The minimum inhibitory concentration (MIC) was determined using broth dilution method (Cheesbrough 2006). MIC was done by preparing various concentrations of each hand sanitizer (20 %, 40 %, 60 %, 80 % and 100 %). A test tube containing only nutrient broth and the test organism without the hand sanitizer served as negative control while a test tube containing the sanitizer and broth without bacteria served as the positive control. The tubes were inoculated for 18-24 h and then examined for visible growth or turbidity. The concentration of the hand sanitizer at which no visible growth was observed was regarded as the MIC.
Determination of Minimum bactericidal concentration (MBC): The MBC was determined by taking a loop full of the inoculum from the minimum inhibitory concentration tubes which showed no visible growth and streaked on a fresh sterile nutrient agar plates. The plates were incubated at 37°C for 24 h and observed for growth. The streaked nutrient agar plates showing no growth indicated bactericidal effect of the hand sanitizer at that concentration.
Statistical analysis: The statistical analysis of this research was done using the analysis of variance method (ANOVA). All statistical procedures were performed using Statistical Package for Social Sciences (SPSS).

Results

The antibacterial effect of the hand sanitizers against the test bacteria isolates is presented in Table 1. *2Sure* hand sanitizer had the highest antibacterial activity against *Staphylococcus aureus* at 100 % concentration with 23.33 ± 2.03 mm zone of inhibition. This hand sanitizer had no antibacterial activity against *Bacillus subtilis*. There was significant difference between the inhibition of *2Sure* and *Carex* hand sanitizers on *Escherichia coli* and *Klebsiella pneumoniae* ($P<0.05$). *Detto*l hand sanitizer had antibacterial activity against only *P. aeruginosa* and *Staphylococcus aureus* of all the test microorganisms with zones of inhibition of 5.00 ± 2.89 mm and 13.67± 1.33 mm respectively. *Carex* hand sanitizer showed no antibacterial activity against *Proteus* spp but had the highest antibacterial activity against *Bacillus subtilis* with zone of inhibition of 11.00 ± 2.08 mm and the least antibacterial activity against *E. coli* with zone of inhibition diameter of 2.33 ± 2.33 mm. *2sure* and *Carex* hand sanitizers had inhibitory effect on *Klebsiella pneumoniae* with zones of inhibition of 15.33 ± 1.33 mm and 9.67 ± 0.33 mm respectively.

Table 1: Antibacterial effect of hand sanitizers against the test clinical bacterial isolates

Bacteria isolates	Zone of Inhibition of Hand Sanitizers		
	2Sure	***Dettol***	***Carex***
S. aureus	23.33±2.03[b]	13.67±1.33[a]	6.67±3.33[a]
B. subtilis	0.00±0.00[a]	0.00±0.00[a]	11.00±2.08[b]
E. coli	19.00±1.53[b]	0.00±0.00[a]	2.33±2.33[a]
P. aeruginosa	14.33±1.45[b]	5.00±2.89[a]	8.67±1.76[ab]
K. pneumoniae	15.33±1.33[c]	0.00±0.00[a]	9.67±0.33[b]
Proteus spp.	12.00±1.53[b]	0.00±0.00[a]	0.00±0.00[a]

*Values are the mean and standard error of triplicate
a-b: different characters in the same row indicate values with significant difference ($p<0.05$)

The Minimum Inhibitory Concentration (MIC) of *2Sure*, *Dettol* and *Carex* hand sanitizers on the test isolates are presented in Table 2. The Minimum Inhibitory Concentration for *2Sure* hand sanitizer against *S. aureus*, *Escherichia coli*, *Klebsiella pneumoniae* and *Proteus* spp was at 100 % concentration except for *Pseudomonas aeruginosa* (80 %). The MIC of *Dettol* hand sanitizer against *Pseudomonas aeruginosa* was 100 %. *Carex* showed the same MIC of 100% against *S. aureus, Pseudomonas aeruginosa* and *Klebsiella pneumoniae.*

Table 2: Minimum Inhibitory concentration (MIC) of the hand sanitizers on the clinical test isolate

		Test isolate					
Hand sanitizer	**Conc (%)**	***Staphylococcus aureus***	***Bacillus subtilis***	***Escherichia coli***	***Pseudomonas aeruginosa***	***Klebsiella pneumoniae***	***Proteus* spp**
2 sure	20	+		+	+	+	+
	40	+		+	+	+	+
	60	+	N/A	+	+	+	+
	80	+		+	-	+	+
	100	-		-	-	-	-
MIC%		100		100	80	100	100
Dettol	20	+			+		
	40	+	N/A	N/A	+	N/A	N/A
	60	+			+		
	80	+			+		
	100	-			-		
MIC%		100			100		
Carex	20	+	+	+	+	+	
	40	+	+	+	+	+	
	60	+	+	+	+	+	N/A
	80	+	-	+	+	+	
	100	-	-	-	-	-	
MIC%		100	80	100	100	100	

Key: +: Growth, -: No growth, N/A: Not applicable

Table 3 shows the Minimum Bactericidal Concentration (MBC) of *2Sure*, *Dettol* and *Carex* hand sanitizers on the test isolates. *2Sure* hand sanitizer had a bactericidal effect on *Escherichia coli* and *Proteus* spp at 100 % but 80 % for *Pseudomonas aeruginosa*. *Dettol* hand sanitizer showed bactericidal activity on *Pseudomonas aeruginosa* at 100 % concentration. *Carex* hand sanitizer had a bactericidal effect on *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* at 100 % concentration also.

Table 3: Minimum Bactericidal concentration (MBC) of hand sanitizers on the test isolates

Test organisms	**MBC of Handsanitizer (%)**		
	2Sure	***Dettol***	***Carex***
S. aureus	ND	ND	ND
Bacillus subtilis	N/A	N/A	ND
E. coli	100	N/A	ND
P. aeruginosa	80	100	100
K. pneumoniae	ND	N/A	100
Proteus spp.	100	N/A	N/A

Key: ND: No dilution indicating growth of organism, N/A: Not applicable

Discussion

This study revealed that the hand sanitizers used in this research had antibacterial activity, though with varying strength from the zone of inhibition of the test organisms. This study corroborates with the findings of Oke *et al.* (2013) that observed *Dettol* hand sanitizer was effective against *P. aeruginosa* and did not have inhibitory effect on *Escherichia coli,* but is in contrast with the findings of Ichor *et al*., (2018) who showed that *Dettol* hand sanitizer was effective against *Escherichia coli, Proteus* spp. and *Salmonella typhi* with zones of inhibition of 10.00 mm, 1.00 mm and 5.00 mm respectively. *Carex* hand sanitizer showed antibacterial activity on *E. coli, P. aeruginosa* and *K. pneumoniae*. This result agrees with the findings of Otokunefor and Princewill (2017) which showed that *Carex* hand sanitizer was effective against *E. coli, P. aeruginosa* and *K. pneumoniae* but with zones of inhibition of 15.00 mm, 28.00 mm and 48.00 mm.

Proteus spp was inhibited by only *2sure* hand sanitizer but was resistant to *Carex* and *Dettol*. This finding agrees with that of Ahmed (2018) which revealed that *Proteus* showed resistivity to most hand sanitizers since it was resistant to all of the five (5) hand sanitizers used. This resistance could be as a result of the swarming motility and ability of *Proteus* spp to self-elongate and secrete a polysaccharide which allows it to attach and move along surfaces. *Dettol* showed inhibitory effect on *S. aureus* which is similar to the study conducted by Enwa *et al.* (2015) but is in contrast with the findings of Ichor *et al.* (2018) and Oke *et al.* (2013) in which *Dettol* did not show any inhibitory effect. *Detto*l hand sanitizer did not show any inhibitory effect on *K. pneumoniae. 2Sure* hand sanitizer was the most effective having the highest diameter of zone of inhibition against most of the isolates among all the hand sanitizers used for this study inhibiting the growth of *S. aureus, E. coli, P. aeruginosa* and *K. pneumoniae*. This might be attributed to the presence of high alcohol content (70.00 %) in comparison with *Dettol* and *Carex* hand sanitizers having 63.00 %. Reports have shown that alcohol denature proteins of microorganisms hence inhibiting their growth and metabolism (WHO, 2009). The three hand sanitizers used had the same MIC for all the test microorganisms (100 %) except *2sure* on *P. aeruginosa* at 80 %.

Carex hand sanitizer showed bactericidal effect on *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* at 100% concentration. This agrees with the work of Otokunefor and Princewill (2017) with similar results. The presence of capsules in *Klebsiella pneumoniae* aid in conferring resistance to many antibacterial agents. Although all the hand sanitizers inhibited the growth of *Staphylococcus aureus,* they were not bactericidal against it. *Pseudomonas aureuginosa* was most sensitive to all the hand sanitizers used.

These 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 ingredient in the products. Alcohols are known to exert disinfectant activity on bacteria by causing protein denaturation, disruption of tissue membranes and dissolution of several lipids (Kar, 2008). Hand sanitizers should therefore, exhibit significant levels of inhibitory activity against these isolates as the active ingredient is alcohol (Otokunefor and Princewill, 2017). Ochwoto *et al*. (2017) reported a possible link of efficacy to composition and noted that the ethanol based products resulted in a higher efficacy than the isopropyl based products. Depending on the type of alcohol present, the difference in efficacy of the various hand sanitizers could also arise from the actual composition of alcohol present in the product. For most alcohol-based hand sanitizers, the alcohol components are the major active ingredients. Although isopropanol has been reported as being superior to ethanol as an antiseptic, the poor activity of *Dettol* and *Carex* hand sanitizers observed in this study is probably due to the negative interactive effects of the additional ingredients such as fragrance, emollients,

humectants and thickening agents added to them which may be absent in *2Sure* hand sanitizer. These could probably limit the bactericidal effect of the alcohol from reaching the bacterial cells. 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). The lack of bactericidal activity observed among some of the products could be due to poor or prolonged storage, increased temperature of storage causing evaporation of the active ingredient and finally, the structure the organisms possesses enable them to evade bactericidal effect.

Conclusion

Not all hand sanitizers are efficient in killing microorganisms. Therefore, awareness of choosing effective alcohol-based hand sanitizers is important in reducing infections and its transmission especially when dealing with patients in hospitals, clinical laboratories and among school children. Of the test hand sanitizers, *2Sure* possessed maximum antimicrobial effect against all the test organisms used in the study. Although people should use hand sanitizers with caution to avoid misuse, regulatory authorities/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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