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By  
U. S. Oranusi, V. A. Akande and S. O. Dahunsi

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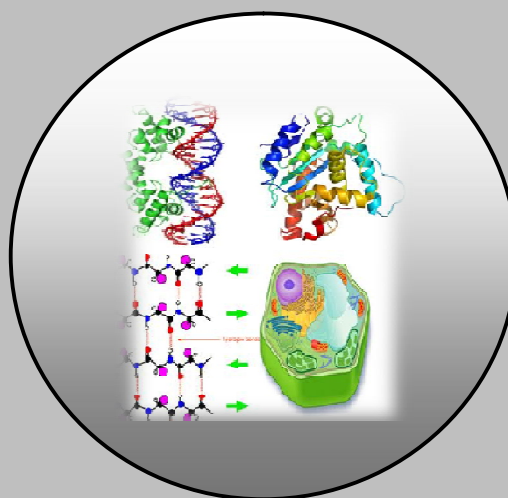
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## Assessment of Microbial Quality and Antibacterial Activity of Commonly used Hand Washes

\*U. S. Oranusi, V. A. Akande and S. O. Dahunsi

Biological Sciences Department, Covenant University, Ota, Ogun State, Nigeria

### ABSTRACT

*Hands are the highways to the transmission and spread of pathogens that causes diseases, food borne illnesses and nosocomial infections. Hand washing is the act of cleansing the hands with water or another liquid, with or without the use of soap or other detergents, to ensure proper hand hygiene. To determine the microbiological quality and the antibacterial property and dilution effects on activity of hand wash, seven brands of hand washes were evaluated using susceptibility test by agar well diffusion, minimum inhibitory dilution and time kill test. This was done by assessing different dilutions of the hand washes against standardized  $1.5 \times 10^8$  cells of Staphylococcus aureus, Escherichia coli and Pseudomonas aeruginosa. Results showed that all the hand washes were sterile and all the brands had some level of antibacterial activity. The hand washes were more active on Staphylococcus aureus and Escherichia coli than on Pseudomonas aeruginosa. Activity decreased with dilution as neat and  $2^{-1}$  dilutions gave better results compared to  $2^{-2}$ ,  $10^{-1}$  and  $10^{-2}$ . Hib hand wash killed all test organisms when exposed for 2, 5 and 10 minutes and at neat and  $2^{-1}$  dilutions but not at  $2^{-2}$ ,  $10^{-1}$  and  $10^{-2}$ . Rev and Pan hand washes though are sterile, were least active in all the tests. The minimum inhibitory dilution and minimum bactericidal dilution for most of the hand wash was at neat concentration. The time kill test showed that the effect of the hand wash was highest at 5 and 10 minutes and at neat (undiluted) for all the test organisms. It is advised that the dilution of hand washes a common practice in most eateries must stop as these products are not active when diluted, hands should be washed for five to ten minutes for maximum hand hygiene.*

**Key words:** Antibacterial Activity, Food Borne Illnesses, Hand Hygiene, Microbiological Quality, Nosocomial Infections and Pathogens.

## INTRODUCTION

Food and water borne diseases coupled with nosocomial infections claim millions of lives annually (WHO, 2001; 2003; NIH, 2006). Any discussion on controlling nosocomial infections and food related infections and intoxications would be incomplete without the inclusion of hand hygiene. These describes the means of ensuring that surgical procedures are not complicated by hand transferred contaminants and that food is handled and consumed with the greatest probability of being free from human pathogen and contaminants from contact surfaces.

Hand hygiene relates to hand washing , the act of cleansing the hands with water or another liquid, with or without the use of soap or other detergents, for the purpose of removing soil, dirt, and/or microorganisms and most importantly to ensure proper hand hygiene. Hands are the highways to the transmission and spread of pathogens that cause diseases, food-borne illness, and nosocomial infections. Numerous studies support the finding that hand washing reduces both the carriage of pathogens on the hands and nosocomial infections (Steere and Mallison, 1975., Mensah *et al*, 2002., ASM, 2005., Oranusi *et al*,. 2013). Hand washing is the simplest and most cost-effective way of preventing the transmission of infection and thus reducing the incidence of health-care associated and food related infections (Rotter *et al*, 1998, Rotter 1999, CDC, 2002). Washing hands to cleanse the hands of pathogens (bacteria, fungi, protozoa, helminthes or viruses) and chemicals which can cause personal harm or disease is especially important for people who handle food or work in the medical field, but it is also an important practice for the general public. Effective hand washing protects best against diseases transmitted through direct physical contact and via fecal-oral routes; gastrointestinal illnesses, diarrhea, polio, pneumonia and as many forms of stomach flu. For effective hand washing, the application of water alone is inefficient for cleaning skin because water is often unable to remove fats, oils, and proteins, which are components of organic soil. (www.hi-tm.com, 2009). Therefore, removal of microorganisms from skin requires the addition of soaps or detergents to water. Use of liquid soaps have wider acceptability because solid soap due to its reusable nature, may hold bacteria acquired from previous uses, so it is important to wash the soap itself before and after use.(www.pubmedcentral.nih.gov, 2009). Hand washing with contaminated soap could colonize the hands with Gram-negative bacteria, which results in an increase in bacterial counts on the skin (www.learnwell.org, 2009). In recent times the use of antibacterial hand washes and soaps has been heavily promoted to a health-conscious public. Though there is no evidence that using recommended antiseptics or disinfectants selects for antibiotic-resistant organisms in nature (Jones, 1999; Barry *et al*, 1999; Hibbard, 2005; Weber and Rutala, 2006). However, antibacterial soaps and washes contain common antibacterial agents such as triclosan, chlorhexidine gluconate which has an extensive list of resistant strains of organisms (Westergren and Emilson, 1980, Tattawasart *et al*, 1999., Thomas *et al*, 2000). So, even if antibacterial soaps and washes aren't selected for antibiotic resistant strains, they might not be as effective as they are marketed to be.

More so plain soaps which are cheap, mild on the skin and readily available are as effective as consumer-grade anti-bacterial soaps containing triclosan, chlorhexidine gluconate, benzalkonium chloride, isopropyl alcohol or ethanol, in preventing illness and removing bacteria from the hands. (www.physorg.com, 2007). Although Alcohol-based hand sanitizers which don't require water are an excellent alternative to hand washing, particularly when soap and water aren't available. They're actually more effective than soap and water in killing bacteria and viruses that cause disease and using these products can result in less skin dryness and irritation than hand washing (Rotter, 2001, Kampf and Kramer, 2004, Kampf and Ostermeyer, 2005). Plain soap and water hand washing is preferable when the hands are visibly dirty, soiled or contaminated with blood because alcohol-based hand rubs are ineffective in the presence of organic material. In addition, alcohols are ineffective against non-lipid-enveloped viruses e.g. Noro viruses and the spores of bacteria e.g. *Clostridium difficile* and *B. anthracis* and protozoan cysts e.g. *Giardia lamblia* (Ansari, 1989, [www.learnwell.org](http://www.learnwell.org), 2009, [www.wtxl.com](http://www.wtxl.com), 2009).

The alarming increase in brands of antimicrobial hand washes in Nigerian markets and its concomitant usage in almost every eatery and homes call for effective quality monitoring at the consumer level. Similarly, in most Nigerian eateries, the hand wash is often diluted to increase the quantity and reduce cost. However, the implication of the dilution effect in terms of the hand wash being able to deliver on the claim(s) of the manufacturer is not considered to the detriment of the consumers' health. This work therefore seeks to ascertain.

1. The microbiological quality of common antibacterial hand washes in Nigerian markets.
2. The susceptibility of microorganisms isolated from hand swabs to different dilutions of these hand washes.
3. The effective dilution for maximum activity of these hand washes.
4. Adequately inform the consuming public on products status. .

## **MATERIAL AND METHODS**

### **Sampling**

Twenty one hand washes comprising of triplicate samples of seven different brands of antimicrobial hand washes were purchased from supermarkets, shopping malls and vendors around Lagos, Ogun, Ondo and Edo states of Nigeria. All the samples are within their expiry date from date of manufacture. The batch numbers, manufacture date, expiry date, product composition and address of manufacturing company were recorded before analyses of samples for microbiological quality and antimicrobial activities. The samples were coded for convenience as Coc, Hib, Lib, Pao, Ren, Rev, and Pan.

### **Microbiological Quality Assessment**

All the media used Nutrient agar, Mueller-Hinton agar, Peptone water and Nutrient broth (all Oxoid, England) were prepared based on the manufacturer's instructions.

Microbial isolates *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli* were isolated from hands swabs of students and food vendors. Characterization of isolates was by standard microbiological procedures (Speck, 1976; Jolt *et al*, 1994). Microbial cultures for antimicrobial susceptibility tests were cultured in nutrient broth for 24h incubation and standardized to  $1.5 \times 10^8$  cells using 0.5 McFarland standard and distilled water as diluents.

#### **Assessment of Microbial Quality of Hand Washes**

**Sterility test:** Evaluation of microbiological quality of hand washes was performed following the methods as described by Ogunledun *et al.*, (2008) and Okpalugo *et al.*, (2009). Aliquot 1ml of the undiluted hand wash was added to 1ml and 9ml of peptone water respectively and serial dilutions were made to  $2^1$ ;  $2^2$ ;  $10^{-1}$  and  $10^{-2}$ . These dilutions were chosen based on questionnaire interview on hand wash dilutions for customers use in restaurants. The sample homogenates were agitated manually for about one minute for thorough mixing. Approximate 1ml of each dilution was inoculated onto Nutrient agar via the spread plate method. Incubation of plates was at  $37^\circ\text{C}$  for 24h to 48h. Plates were examined at the expiration of incubation period for colony formation and enumeration using digital colony counter (SC6-Baloworld Scientific, United Kingdom), counts were expressed as Cfu/ml.

**Susceptibility test by agar well diffusion method:** Agar well diffusion method as described by Adeniyi and Ayepola (2008) with slight modification was adopted. Mueller-Hinton agar was seeded uniformly by spread plate method with 1ml of standardized culture of each microbial isolate. The inoculated plates were allowed to set. A sterile cork borer was used to cut uniform wells of 5mm diameter on the surface of the agar and the wells were filled with neat and each dilution ( $2^1$ ;  $2^2$ ;  $10^{-1}$  and  $10^{-2}$ ) of the hand washes using sterile Pasteur pipette. Sterile distilled water and Oxoid Gentamicin ( $10\mu\text{g}$ ) was used as negative and positive controls. The plates were allowed to stand for diffusion of the hand washes and were incubated at  $37^\circ\text{C}$  for 24h. The antibacterial susceptibility was indicated by the zone diameter of inhibition and was measured using a transparent ruler.

**Time kill test for microbial isolates:** This was carried out using the method of Bouchara *et al* (2004) with slight modification. Two, Five and Ten minutes was chosen for this test based on questionnaire interview on length of time people spend in hand washing before eating/after using the toilet and also to accommodate for sustenance (persistent) effect (Boyce and Pittet, 2002., Kampf and Ostermeyer, 2005). To 1ml of the undiluted hand washes was added 1ml and 9ml of standardized cultures in distilled water as diluents. Dilutions were made to  $2^1$ ;  $2^2$ ;  $10^{-1}$  and  $10^{-2}$ . The tubes were swirled to mix and agitated constantly at  $37^\circ\text{C}$  in incubator with shaker (Guangzhon healthy ling- HZQ-X300) for two minutes. Aliquot 0.1ml was plated out on Nutrient agar and Mueller-Hinton agar respectively. At five and ten minutes, the same procedure was repeated. Plates were incubated at  $37^\circ\text{C}$  for 24h, after which colonies observed on plates were counted using digital colony counter (SC6-Baloworld Scientific, United Kingdom).

**Determination of minimum inhibitory dilution (MID) and minimal bactericidal dilution (MBD) of hand washes against test isolates:** This was done by a modification of methods as described by Candido *et al* (1996). To 10ml of the neat and dilutions of hand washes in test tubes was added 1ml of standardized test organisms. The tubes were incubated for 24h at 37°C and then examined for growth evidenced by turbidity of medium. The MID was recorded as the lowest dilution of the hand wash that inhibited the growth of the test organisms evidenced by lack of turbidity. Tubes showing no growth were plated out on Nutrient agar, the highest dilution that yielded no growth of bacteria colonies after 24h incubation was recorded as MBD

## RESULTS

All the hand washes were sterile as none had growth of microbial colonies after 24 to 48h incubation at 37°C. Table1 presents the antimicrobial susceptibility profile for the test organisms. Hib hand wash has the greatest activity against all the organisms and at all dilutions except at  $10^1$  and  $10^2$  dilutions for *Pseudomonas aeruginosa*. All the hand washes had no activity beyond  $2^2$  dilution except however, for Hib. The effect of the hand washes on all the test organisms waned out with increase in dilution. All the hand washes were active on *S. aureus* and *E. coli* than on *Pseudomonas aeruginosa*. Rev and Pan Hand washes had no effect on *Pseudomonas aeruginosa*.

**Table1. Susceptibility test by agar well diffusion for test isolates.**

Dilutions	Hand wash Zone diameter of Inhibition (mm)						
	Coc.	Hib.	Lib.	Pao.	Ren.	Rev.	Pan.
<b><i>Staphylococcus aureus</i></b>							
Neat	20	30	22	19	10	10	5
$2^{-1}$	-	17	13	10	3	7	4
$2^{-2}$	-	15	8	5	5	-	4
$10^{-1}$	-	8	-	-	-	-	-
$10^{-2}$	-	5	-	-	-	-	-
<b><i>Escherichia coli</i></b>							
Neat	15	18	15	16	10	15	5
$2^{-1}$	10	8	14	10	5	10	-
$2^{-2}$	7	5	3	4	-	8	-
$10^{-1}$	-	4	-	1	-	2	-
$10^{-2}$	-	1	-	-	-	-	-
<b><i>Pseudomonas aeruginosa</i></b>							
Neat	10	12	15	14	4	-	-
$2^{-1}$	5	6	-	9	-	-	-
$2^{-2}$	2	4	-	-	-	-	-
$10^{-1}$	-	1	-	-	-	-	-
$10^{-2}$	-	-	-	-	-	-	-

= No inhibitory effect

Table 2 shows the time kill test for the test organisms. Activity was recorded only at neat,  $2^1$  and  $2^2$  dilutions thus  $10^1$  and  $10^2$  dilutions were not presented. Hib hand wash killed the test organisms at neat,  $2^1$ ,  $2^2$  dilutions and in 2, 5 and 10 minutes respectively. Lib at neat and  $2^1$  dilutions killed *S. aureus*, *E. coli* and *Pseudomonas*. Pao was effective on *S. aureus* and *E. coli* as undiluted (neat) for all the time ranges but was active against *Pseudomonas* only in 10min as undiluted sample. Coc and Pan were not able to kill completely any of the test organisms at all time and dilutions. Rev and Pan had no activity on *Pseudomonas* at all concentrations and time. Reduction in microbial load was more at 5 and 10minutes for all the hand wash and at neat and  $2^1$  dilutions. Table 3 shows the inhibitory dilutions for the hand washes. Hib inhibited *S. aureus* at neat,  $2^1$  and  $2^2$  dilutions but it inhibited *Pseudomonas* only at neat and  $2^1$  dilution. Lib at neat and  $2^1$  inhibited *S. aureus* and *E. coli*. All the hand washes except Pan inhibited *S. aureus* and *E. coli* at neat (undiluted). Hib had MBD at  $2^1$  for all the isolates. All the hand washes except for Pan had MBD as neat (undiluted) for *S. aureus* and *E. coli*. Lib and Pao had MBD as neat for *Pseudomonas*.

## DISCUSSION

The microbiological analysis of all the hand wash samples recorded absence of growth meaning they are sterile and thus conform to the sterility standard required of such sanitary personal care products. All the samples showed antibacterial activity and efficacy in conformity with the submission of Randon, (2009) who observed that hand washes can be bactericidal or bacteriostatic. Hib showed an impeccable activity as compared to other hand washes, it was very effective in all the quality assessment methods used as a determinant of antibacterial activity it inhibited the growth of all the test organisms at different dilutions. The active ingredient in Hib is Chlorhexidine gluconate and this could have distinguished it from other hand washes in terms of antibacterial activity. This finding is in tandem with the reports of Aly and Maibach, (1979; 1980), Rotter and Koller, (1991), Paulson, (1996). However, persistent exposure of microorganisms to Chlorhexidine gluconate has been reported to yield resistant strains (Westergren and Emilson, 1980., Tattawasart *et al*, 1999., Thomas *et al*, 2000).The activity of the hand washes were reduced through dilution, this is evident in reduction in zone of inhibition diameter and increase in microbial load with increase in dilution. The lower activity of the hand washes against *Pseudomonas* could be explained by the hardy nature of *Pseudomonas*. It has been reported to survive in disinfectants and resistant to a wide variety of antibiotics (Becks and Lorenzoni, 1995; *Pseudomonas* genome data base) and it is known to have prolific ability to degrade a wide variety of substances due to its natural endowment with degradative enzymes and plasmids and high protein repair and regeneration mechanisms (*Pseudomonas* genome database, Winsor *et al*, 2011)

The Time kill test showed the time taken for the organism to be reduced or killed completely. Hib had a good effect recording no growth at all dilutions during the time intervals and for the three test organisms. Lib and Pao also had an appreciable effect on the organisms as there was great reduction in the organisms at 5 and 10 minutes compared to the initial 2 minutes.

At  $10^{-1}$  and  $10^{-2}$  dilutions, the activity of the hand washes waned off due to dilution effect. That Rev and Pan failed in all the tests calls for concern because manufacturers claimed they are antimicrobial hand washes. However, Sheena and Stiles (1982), in a study of efficacy of germicidal hand wash agents in hygienic hand disinfection reported that some antibacterial hand washes were no better than non-germicidal soap. Although this work was done without estimating the chemical composition of the hand washes, those with triclosan (Lib and Pao) recorded appreciable antibacterial activity in agreement with Collins *et al*, (1981), Faoagali *et al*, (1995) Paulson, (1996), who reported that hand washes with triclosan as the major chemical ingredient pose significant antibacterial activity. The hand washes had bacteriostatic and bactericidal effect as neat and  $2^{-1}$  dilutions and at 5 and 10 minutes. It is advised that hands be washed for 5 to 10 minutes for maximum result of good hand hygiene. Hand washes must not be diluted except were stated by the manufacturer, they are active as neat concentration and loses activity with dilution. Diluting hand wash makes them mere fragrance fluid and not hand wash.

**Table 2. Time kill test for test isolates.**

Time (min)	Hand wash dilutions								
	Neat	$2^{-1}$	$2^{-2}$	Neat	$2^{-1}$	$2^{-2}$	Neat	$2^{-1}$	$2^{-2}$
	Coc. <i>S. aureus</i>			Hib. <i>S. aureus</i>			Lib. <i>S. aureus</i>		
2	$1.1 \times 10^4$	$2.1 \times 10^5$	$1.5 \times 10^4$	NG	NG	NG	$2.7 \times 10^4$	$2.0 \times 10^4$	$2.8 \times 10^4$
5	$8.5 \times 10^1$	$3.2 \times 10^2$	$9.2 \times 10^1$	NG	NG	NG	$6.1 \times 10^1$	$6.8 \times 10^1$	$2.0 \times 10^1$
10	$7.1 \times 10^1$	$2.4 \times 10^2$	$8.6 \times 10^2$	NG	NG	NG	NG	NG	$1.9 \times 10^2$
	Pao. <i>S. aureus</i>			Ren. <i>S. aureus</i>			Rev. <i>S. aureus</i>		
2	NG	$5.9 \times 10^1$	$8.6 \times 10^1$	$3.6 \times 10^2$	$1.9 \times 10^2$	$1.7 \times 10^2$	$1.1 \times 10^4$	$3.2 \times 10^5$	$3.5 \times 10^5$
5	NG	$1.6 \times 10^1$	$7.2 \times 10^3$	$2.8 \times 10^1$	$9.0 \times 10^2$	$7.6 \times 10^2$	$3.2 \times 10^3$	$2.3 \times 10^3$	$1.2 \times 10^3$
10	NG	$1.1 \times 10^1$	$3.8 \times 10^2$	NG	$9.5 \times 10^1$	$7.2 \times 10^2$	$2.1 \times 10^1$	$2.2 \times 10^2$	$1.0 \times 10^2$
	Pan. <i>S. aureus</i>			Coc. <i>E. coli</i>			Hib. <i>E. coli</i>		
2	$2.0 \times 10^4$	$2.4 \times 10^5$	$3.3 \times 10^5$	$2.2 \times 10^2$	$1.8 \times 10^2$	$8.9 \times 10^3$	NG	NG	NG
5	$1.1 \times 10^3$	$2.0 \times 10^3$	$2.5 \times 10^1$	$1.8 \times 10^2$	$1.6 \times 10^2$	$7.4 \times 10^2$	NG	NG	NG
10	$1.0 \times 10^2$	$1.4 \times 10^2$	$6.0 \times 10^2$	$1.8 \times 10^1$	$1.2 \times 10^2$	$6.3 \times 10^2$	NG	NG	NG
	Lib. <i>E. coli</i>			Pao. <i>E. coli</i>			Ren. <i>E. coli</i>		
2	$1.9 \times 10^2$	$2.1 \times 10^2$	$2.8 \times 10^2$	NG	$5.9 \times 10^1$	$9.6 \times 10^3$	$9.3 \times 10^2$	$7.9 \times 10^2$	$1.2 \times 10^2$
5	$1.7 \times 10^1$	$1.2 \times 10^1$	$2.0 \times 10^2$	NG	$7.6 \times 10^1$	$2.7 \times 10^2$	$7.2 \times 10^1$	$9.0 \times 10^1$	$6.7 \times 10^1$
10	NG	< 10	$1.9 \times 10^1$	NG	$1.7 \times 10^1$	$6.8 \times 10^1$	$1.6 \times 10^1$	$8.4 \times 10^1$	$2.7 \times 10^1$
	Rev. <i>E. coli</i>			Pan. <i>E. coli</i>			Coc. <i>P. aeruginosa</i>		
2	$2.3 \times 10^3$	$3.1 \times 10^3$	$4.2 \times 10^2$	$9.4 \times 10^3$	$4.4 \times 10^3$	$5.5 \times 10^6$	$1.1 \times 10^4$	$2.8 \times 10^4$	$4.7 \times 10^3$
5	$3.1 \times 10^1$	$3.2 \times 10^2$	$3.4 \times 10^2$	$4.6 \times 10^3$	$6.3 \times 10^3$	$6.2 \times 10^5$	$8.5 \times 10^3$	$3.6 \times 10^3$	$2.9 \times 10^2$
10	NG	$3.2 \times 10^1$	$2.5 \times 10^1$	$6.2 \times 10^2$	$6.3 \times 10^2$	$4.8 \times 10^4$	$7.1 \times 10^2$	$2.4 \times 10^3$	$6.8 \times 10^2$
	Hib. <i>P. aeruginosa</i>			Lib. <i>P. aeruginosa</i>			Pao. <i>P. aeruginosa</i>		
2	NG	NG	NG	$5.7 \times 10^2$	$2.5 \times 10^3$	$2.8 \times 10^3$	$2.1 \times 10^2$	$9.5 \times 10^2$	$7.5 \times 10^2$
5	NG	NG	NG	$2.0 \times 10^2$	$2.1 \times 10^3$	$2.2 \times 10^2$	$3.1 \times 10^1$	$8.1 \times 10^2$	$6.1 \times 10^1$
10	NG	NG	NG	NG	$1.3 \times 10^2$	$8.1 \times 10^2$	NG	$1.9 \times 10^1$	$6.4 \times 10^2$
	Ren. <i>P. aeruginosa</i>			Rev. <i>P. aeruginosa</i>			Pan. <i>P. aeruginosa</i>		
2	$4.3 \times 10^4$	$2.7 \times 10^3$	$1.7 \times 10^6$	$1.5 \times 10^8$	$1.5 \times 10^3$	$1.5 \times 10^8$	$1.5 \times 10^8$	$1.7 \times 10^8$	$1.5 \times 10^8$
5	$8.2 \times 10^3$	$6.0 \times 10^2$	$7.6 \times 10^5$	$1.4 \times 10^8$	$1.5 \times 10^3$	$1.6 \times 10^8$	$1.5 \times 10^8$	$1.5 \times 10^8$	$1.6 \times 10^8$
10	$1.2 \times 10^2$	$9.8 \times 10^2$	$7.2 \times 10^5$	$1.6 \times 10^8$	$1.6 \times 10^8$	$1.3 \times 10^8$	$1.7 \times 10^8$	$1.3 \times 10^8$	$1.4 \times 10^8$



Table 3. Minimum inhibitory dilution of hand washes against test isolates.

Dilutions	Hand washes						
	Coc.	Hib.	Lib.	Pao.	Ren.	Rev.	Pan.
<b><i>Staphylococcus aureus</i></b>							
Neat	NG	NG	NG	NG	NG	NG	G
2 <sup>-1</sup>	G	NG	NG	G	G	G	G
2 <sup>-2</sup>	G	NG	G	G	G	G	G
10 <sup>-1</sup>	G	NG	G	G	G	G	G
10 <sup>-2</sup>	G	G	G	G	G	G	G
<b><i>Escherichia coli</i></b>							
Neat	NG	NG	NG	NG	NG	NG	G
2 <sup>-1</sup>	NG	NG	NG	G	G	G	G
2 <sup>-2</sup>	G	G	G	G	G	G	G
10 <sup>-1</sup>	G	G	G	G	G	G	G
10 <sup>-2</sup>	G	G	G	G	G	G	G
<b><i>Pseudomonas aeruginosa</i></b>							
Neat	NG	NG	NG	NG	G	G	G
2 <sup>-1</sup>	G	NG	G	G	G	G	G
2 <sup>-2</sup>	G	G	G	G	G	G	G
10 <sup>-1</sup>	G	G	G	G	G	G	G
10 <sup>-2</sup>	G	G	G	G	G	G	G

NG = No growth    G = Growth

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**Corresponding author: Dr. U. S. Oranusi**, Biological Sciences Department, Covenant University, Ota, Ogun State, Nigeria.

**Email:** [orasol2002@yahoo.com.au](mailto:orasol2002@yahoo.com.au) **Phone:** +2348152215183