International Journal of Applied Biological Research 2016

Original Article

EVALUATION OF THE ANTIBACTERIAL EFFICACIES OF HAND-WASH DISINFECTANTS ON SOME PATHOGENIC BACTERIAL HAND SWAB ISOLATES FROM HEALTH WORKERS IN ILORIN, NIGERIA

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Submitted: November, 2016; Accepted: December, 2016; Published: December, 2016

ABSTRACT

Hands are the most implicated in the transmission and spread of pathogens that causes disease. Hand hygiene has been said to be the most important way to avoid these infections. Hand washing with the use of hand wash is one of the ways to tackle the barriers to efficient hand hygiene. This study assessed the efficacy of seven popular brands of Hand washes (HW): DET, SAV, CHE, VIS, NIV, DOV and CAR (all abreviations in full for the first time) against some clinically important bacterial pathogens: *Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli* and *Proteus mirabilis* recovered from hands of health care workers. The antibacterial susceptibility and minimum inhibitory concentration of the hand washes was determined using the agar diffusion and broth dilution method, respectively. Each brand showed different activities against the isolates. VIS had the highest inhibitory effect against all organisms it was tested on, while SAV was the least effective. *S. aureus* was the most susceptible test organism, with the highest susceptibility to CAR (32.5 mm). *P. mirabilis* was most resistant, with the highest resistance to DET (9.0 mm). Antibacterial activity of the hand wash decreased with increased dilution. The minimum bactericidal concentration (MBC) was obtained for all the hand washes when not diluted. It is, therefore, recommended that the dilution of hand wash which is a commonly done in most households, hospitals, offices and eateries should be discontinued as these products are not active when diluted.

Keywords: Hand wash, Hand Hygiene, pathogens, Nosocomial infection.

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INTRODUCTION

Food and water-borne diseases as well as, hospital acquired infections claim millions of lives annually (WHO, 2003; NIH, 2006). The Centre for Disease Control and Prevention (CDC) reported that about 2 million people suffer from hospital-associated infections each year and that not less than 90,000 of these Patients die due to these infections (Zerr *et al.,* 2005). The CDC, WHO and many other experts encourage hand hygiene and has proved it to be the most important measure in the avoidance of hospital acquired infections (CDC, 2002; WHO, 2009). Any discussion on preventing nosocomial infections and food associated infections and intoxications would be deficient without emphasis on hand hygiene. Hand hygiene precautions to prevent complication of surgical procedures by hand transferred contaminants and that food is handled and consumed with the greatest probability of being free of pathogenic organisms from human and contaminants from contact surfaces. Hand hygiene has two key components: hand washing, which is the elimination of microorganisms with usual soap and water; and hand antisepsis, which is the complete removal of microorganisms using an antimicrobial soap or a hand sanitizer. Several researches have demonstrated the usefulness of different forms of hand hygiene in the reduction of both the carriage of pathogens on the hands and incidences of healthcare- associated infections (Mensah *et al.*, 2002; ASM, 2005; Oranusi *et al.*, 2013a).

Hand washing has been shown to be the easiest and cost-effective way of preventing the transmission of infection and subsequently reducing the rate of health-care associated and food related infections (Rotter *et al.*, 1998; Rotter 1999; CDC, 2002). Washing hands to rid the hands of pathogens (bacteria, fungi, protozoa, helminthes or viruses) and chemicals which can cause individual harm or disease is imperative for people who handle food or work in the medical field, but it is also a vital practice for the populace as it helps in reducing community acquired infections. Effective hand washing guards against diseases transmitted via direct physical contact and faecal-oral routes. However, despite expert opinions and proofs showing that hand hygiene is vital, there is low observance among individuals. Health care workers in developing and developed countries abide by 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 unavailability of materials needed for hand hygiene at the point of care, forgetfulness, skin irritation, insufficient time, etc.

Hand washing with contaminated soap could increase colonization of hands with microorganisms, which results in an increase in bacterial counts on the skin (Oranusi *et al.,* 2013b). In recent times the use of antiseptic hand washes and soaps has been heavily promoted to the public. There is evidence that antiseptics or disinfectants select for antibiotic-resistant organisms in nature (Hibbard, 2005; Weber and Rutala,

2006). Similarly, antibacterial soaps and washes contain antibacterial agents such as chlorhexidine gluconate, triclosan which have a wide-ranging list of resistant strains of organisms (Thomas *et al.*, 2000).

The alarming increase in brands of antimicrobial hand washes in Nigerian markets and its concomitant usage in most hospitals, offices, eateries and homes calls for effective quality monitoring at the consumer level. In most Nigerian settings, hand washes are often diluted to increase its quantity and reduce cost. However, the dilution often leads in loss of activity and subsequently could be detrimental to the consumers' health. This study, therefore, seeks to ascertain the: microbiological quality of common antibacterial hand washes sold in Ilorin, Nigeria and to assess the susceptibility of bacterial isolates from hand swabs of health care workers to different dilutions of these hand washes results will provide information on the quality of the assessed hand washes.

MATERIALS AND METHODS

Collection of hand swabs

Samples of hand swabs were collected from 20 health care workers from various hospitals in Ilorin, Kwara, Nigeria. These samples were taken in the afternoon when most health workers would have handled some patients and they were not pre infromed before sample collection.

Isolation of pathogens from swabs

Swabs were inoculated on Mannitol Salt Agar (MSA) for *Staphylococcus aureus,* Eosine ethylene blue for *E. coli,* Centrimide agar for *Pseudomonas aeruginosa,* and CLED for *Proteus mirabilis*, distinct resultant colonies were characterized by standard microbiological procedures (Speck *et al.*, 1976).

Hand Wash Disinfectants

Seven brands of hand washes obtained in triplicates were purchased from local retail outlets in Ilorin. The products were coded DET, SAV, CHE, VIS, NIV, DOV and CAR for convenience.

Assessment of bacteriological quality of hand washes by sterility test

Evaluation of hand washes was performed following the methods of Oranusi *et al.* (2013b). This was done by introducing 1ml of the hand wash into 9ml of sterile peptone water in a test tube. The mixture was homogenised and 1ml of the aliquot was introduced into a sterile plate. Sterile molten nutrient agar was added, swirled and allowed to solidify and the plate was incubated at 37°C for 24 hours. The presence or absence of growth on the plate was observed at the end of the incubation period.

Preparation of McFarland standard

Mcfarland 0.5 turbidity standard was prepared according to the method described by NCCLS (1999). The standard was prepared by adding 0.5ml of 1.175% w/v barium chloride dihydrate ($BaCl .2H_20$) solution to 99.5ml of 15% w/v sulphuric acid ($H_2 SO_4$). The accuracy of the density of the standard was confirmed using a spectrophotometer. The absorbance of the 0.5 McFarland standard at wavelength 625nm was 0.08-0.10 (Cheesbrough, 2006).

Standardization of test organisms

A loopful of inoculums was picked from a pure culture of the test organism using a sterile wire loop. This was then transferred and suspended in a tube of sterile normal saline, the tube was

compared with the MacFarland standard (Vandepitte *et al.*, 2003).

Determination of susceptibility of test organisms to hand washes

The susceptibility of the test organisms to the hand washes was done using the agar diffusion method (Oke *et al.*, 2013). With the aid of a sterile cork borer, holes were bored in the agar plate. Fifty microlitres (50µL) of the hand wash was then introduced into each of the 4 wells while the central well was filled with an equal volume of sterile distilled water to serve as control. This was done for all the test organisms and hand washes. The plates were incubated for 24 hours at 37°C in an upright position, they were then examined for zones of inhibition. Inhibition zones were measured with the aid of a ruler (mm).

Determination of minimum inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) of hand washes against test isolates

This was done by method as described by Candido *et al.* (1996). To 10ml of the undiluted and 1:2, 1:4, 1:10 and1:100 dilutions of hand washes in test tubes was added 1ml of the standardized test organisms. The tubes were incubated for 24hours at 37°C and then examined for growth evidenced by turbidity of medium. The MIC was recorded as the lowest concentration of the hand wash that inhibited the growth of the test organisms indicated by lack of turbidity. Tubes showing no growth were plated out on sterile nutrient agar and the highest dilution that yielded no growth of bacteria colonies after 24hours incubation was recorded as MBC.

RESULTS

All the hand washes were sterile as none had bacterial colonies after 24 to 48hours incubation at 37°C.The antibacterial susceptibility profile for the test organisms to the different hand washes is as presented in Table 1. VIS hand wash was most active on *Staphylococcus aureus* with the highest zone of inhibition (32.5 $\pm$ 1.00 mm) at undiluted concentration where as DOV had the least activity at 1:4 dilution with diameter zone of inhibition of 6.50$\pm$1.00mm. *E. coli* was most susceptible to CHE hand wash at undiluted concentration with diameter zone of inhibition of 31.5$\pm$1.00mm while the VIS had the least activity at 1:10 dilution with diameter zone of inhibition of 11.5$\pm$1.00mm. For all the hand washes, their undiluted concentrations had the highest degree of inhibition. The effectiveness of the hand washes on all the test organisms waned with increase in dilution. The dilution of 1:100 was unable to cause inhibition on any of the test isolates for all the hand washes. All the hand washes were active on *S. aureus*, *E. coli, Pseudomonas aeruginosa* than on *Proteus mirabilis*. SAV Hand wash had no effect on *Proteus mirabilis.*

The Minimum Inhibitory Concentration (MIC) of DET and SAV on *P. mirabilis* was at undiluted concentration while CAR and CHE hand washes had MIC of 1:4 on *P. mirabilis.* Similarly, the MIC OF DOV and VIS was 1:10 on this same organism. NIV had the least MIC of 1:100 on *P. mirabilis*. The MIC of other hand washes on other isolates is as shown in Table 2. Table 3 showed the Minimum Bactericidal concentration (MBC) of the tested hand washes. Bactericidal effect was only recorded at undiluted concentration for all the tested hand washes.

Table1. Susceptibility pattern of test organisms to hand wash.

Dilutions	Zones of inhibition (mm) of hand washes against test organisms						
	DET	CAR	NIV	SAV	CHE	DOV	VIS
Staphylococcus aureus							
Undiluted	26.5 ± 1.00^{e}	24.0 ± 1.00^{d}	24.5 ± 1.00^{d}	24.5 ± 1.00^{d}	21.0 ± 1.00^{e}	24.0 ± 1.00^{d}	32.5 ± 1.00^{e}
1:2	23.5 ± 1.00^{d}	20.5 ± 1.00^{c}	21.5 ± 1.00^{c}	20.0 ± 1.00^{c}	14.0 ± 1.00^{d}	17.5 ± 1.00^{c}	24.5 ± 1.00^{d}
1:4	20.5 ± 1.00^{c}	18.5 ± 1.00^{b}	11.5 ± 1.00^{b}	19.0 ± 1.00^{e}	12.0 ± 1.00^{c}	6.50 ±1.00^{b}	21.5 ± 1.00^{c}
1:10	18.0 ± 1.00^{b}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	11.5 ± 1.00^{b}	9.00 ± 1.00^{b}	0.00 ± 1.00^{a}	11.5 ± 1.00^{b}
1:100	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 1.00^{a}
Escherichia coli							
Undiluted	21.0 ± 1.00^{d}	23.5 ± 1.00e	20.0 ± 1.00^{d}	18.0 ± 1.00^{b}	31.5 ± 1.00^{e}	29.0 ± 1.00^{d}	27.5 ± 1.00^{d}
1:2	20.0 ± 1.00^{d}	21.5 ± 1.00^{d}	17.5 ± 1.00^{c}	0.00 ± 0.00^{a}	20.0 ± 1.00^{d}	21.5 ± 1.00^{c}	25.0 ± 1.00^{c}
1:4	18.0 ± 1.00^{c}	19.5 ± 1.00^{c}	15.5 ± 1.00^{b}	0.00 ± 0.00^{a}	15.0 ± 1.05^{c}	20.5 ± 1.00^{c}	24.0 ± 1.00^{c}
1:10	13.0 ± 1.00^{b}	15.0 ± 1.00^{b}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	12.0 ± 1.00^{b}	17.5 ± 1.00^{b}	11.5 ± 1.00^{b}
1:100	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
Pseudomonas aeruginosa							
Undiluted	16.5 ± 1.00^{d}	25.5 ± 1.00^{d}	31.5 ± 1.00^{d}	19.5 ± 1.00^{b}	24.0 ± 1.00^{d}	24.0 ± 1.00^{d}	24.0 ± 1.00^{d}
1:2	9.00 ± 1.00^{c}	18.5 ± 1.00^{c}	23.0 ± 1.00^{c}	0.00 ± 0.00^{a}	20.0 ± 1.00^{c}	21.5 ± 1.00^{c}	15.5 ± 1.00^{c}
1:4	7.00 ± 1.00^{b}	15.5 ± 1.00^{b}	16.5 ± 1.00^{b}	0.00 ± 0.00^{a}	18.5 ± 1.00^{c}	14.0 ± 1.00^{b}	11.40 ± 0.00^{a}
1:10	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	12.5 ± 1.00^{b}	0.00 ± 0.00^{a}	16.5 ± 1.00^{b}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
1:100	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
Proteus mirabilis							
Undiluted	9.00 ± 0.00^{b}	31.5 ± 1.00^{d}	21.5 ± 1.00^{c}	0.00 ±0.00^{a}	28.0 ± 1.00^{d}	33.0 ± 1.00^{e}	29.0 ± 1.00^{d}
1:2	0.00 ± 0.00^{a}	24.0 ± 1.00^{c}	15.5 ± 1.00^{b}	0.00 ± 0.00^{a}	8.50 ± 1.00^{c}	24.0 ± 1.00^{d}	19.0 ± 1.00^{c}
1:4	0.00 ± 0.00^{a}	9.00 ± 1.00^{b}	14.5 ± 1.00^{b}	0.00 ± 0.00^{a}	6.50 ± 1.00^{b}	21.5 ± 1.00^{c}	14.0 ± 1.00^{b}
1:10	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	10.0 ± 1.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	19.0 ± 1.00^{b}	11.0 ± 1.00^{b}
1:100	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	9.00 ± 1.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 1.00^{a}

Values are mean ± standard deviation. Means with the same superscript along the same row for the same Hand wash are not significantly different at $\alpha=0.05$.

Table 2.Minimum inhibitory concentrations of the hand washes against the test isolates.

Dilutions	Hand washes						
	DET.	CAR.	NIV.	SAV.	CHE.	DOV.	VIS.
Staphylococcus aureus							
Undiluted	NG	NG	NG	NG	NG	NG	NG
1:2	NG	NG	NG	NG	NG	NG	NG
1:4	NG	NG	NG	NG	NG	NG	NG
1:10	NG	G	G	NG	NG	G	NG
1:100	G	G	G	G	G	G	G
Escherichia coli							
Undiluted	NG	NG	NG	NG	NG	NG	NG
1:2	NG	NG	NG	G	NG	NG	NG
1:4	NG	NG	NG	G	NG	NG	NG
1:10	NG	NG	G	G	NG	NG	NG
1:100	G	G	G	G	G	G	G
Pseudomonas aeruginosa							
Undiluted	NG	NG	NG	NG	NG	NG	NG
1:2	NG	NG	NG	G	NG	NG	NG
1:4	NG	NG	NG	G	NG	NG	NG
1:10	G	G	NG	G	NG	G	G
1:100	G	G	G	G	G	G	G
Proteus mirabilis							
Undiluted	NG	NG	NG	NG	NG	NG	NG
1:2	G	NG	NG	G	NG	NG	NG
1:4	G	NG	NG	G	NG	NG	NG
1:10	G	G	NG	G	G	NG	NG
1:100	G	G	NG	G	G	G	G

NG = No growth G = Growth

Table 3.Minimum bactericidal concentrations of hand wash against the test isolates.

Dilutions	Hand washes						
	DET.	CAR.	NIV.	SAV.	CHE.	DOV.	VIS.
Staphylococcus aureus							
Undiluted	-	-	-	-	-	-	-
1:2	+	+	+	+	+	+	+
1:4	+	+	+	+	+	+	+
1:10	+	+	+	+	+	+	+
1:100	+	+	+	+	+	+	+
Escherichia coli							
Undiluted	-	-	-	-	-	-	-
1:2	+	+	+	+	+	+	+
1:4	+	+	+	+	+	+	+
1:10	+	+	+	+	+	+	+
1:100	+	+	+	+	+	+	+
Pseudomonas aeruginosa							
Undiluted	-	-	-	-	-	-	-
1:2	+	+	+	+	+	+	+
1:4	+	+	+	+	+	+	+
1:10	+	+	+	+	+	+	+
1:100	+	+	+	+	+	+	+
Proteus mirabilis							
Undiluted	-	-	-	-	-	-	-
1:2	+	+	+	+	+	+	+
1:4	+	+	+	+	+	+	+
1:10	+	+	+	+	+	+	+
1:100	+	+	+	+	+	+	+

-= No growth + = Growth

DISCUSSION

The bacteriological analysis of all the hand wash samples showed absence of growth indicating that all samples were sterile and thus conform to the sterility standard required of such sanitary personal care products. This is in agreement with the work of Oranusi *et al.* (2013b). All the samples showed antibacterial activity and efficacy in agreement with the submission of Randon (2009) and Oranusi *et al.* (2013b) who observed that hand washes can be bacteriostatic. DOV showed highest activity against tested isolates as compared to other hand washes. It inhibited the growth of all the test isolates at different dilutions. The active ingredient in DOV is Isopropyl Palmitate and this could have distinguished it from other hand washes in terms of antibacterial activity. This finding is in agreement with the reports of Aly and Maibach (1979, 1980); Paulson (1996) who also reported a high antibacterial activity of hand washes with Isopropyl Palmitate as the active ingredient. However, persistent exposure of microorganisms to Isopropyl Palmitate 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 diameter of zone of inhibition and increase in microbial load with increment in dilution. The reduced activity of the hand washes against *Proteus mirabilis* could be explained by the hardy nature of *Proteus mirabilis*. It has been reported to survive in disinfectants and resistant to a wide variety of antibiotics (Becks and Lorenzoni, 1995) and it is known to have prolific ability to degrade a wide variety of substances due to its natural endowment with degradative enzymes, plasmids and high protein repair and regeneration mechanisms (Winsor *et al*., 2011).

Generally, it is evident that Gram positive bacteria may be more sensitive to disinfectants than Gram negative bacteria; cocci are readily killed by halogens, phenols, especially bisphenol and quaternary ammonium compound (Sabath, 1970). The most resistant among the tested Gram negative was *Proteus mirabilis.* This could be attributed to the lipid layer present in the cell wall of Gram negative bacteria which makes contact and penetration of disinfectants difficult (Prince and Ayliffe, 1972).

The activity of various hand washes is distorted by many factors like concentration, absence of adequate active ingredient and storage condition. This was also reported by Aiello *et al*. (2008) who emphasized the fact that hand wash contains less active ingredient and more ingredients that only improves its texture. This explains the low antibacterial activity of SAV hand wash since it contains more non-active ingredients like (Perfume, Limonene, Linalool and Geranol) which are ingredients to improve texture and fragrance of the product and has no antibacterial effect.

Hand washes should not be diluted except where stated by the manufacturer. They are active when undiluted and loses activity with dilution, as it was observed in this study. Diluting hand wash makes them mere fragrance fluid and not hand wash.

CONCLUSION

The potency of hand washes is very important to enhance the antibacterial activity of these hand washes towards

controlling microbial population which includes prevention of diseases transmission and infection. Determination of antibacterial effectiveness of hand washes is essential to achieve total destruction of pathogens. This research assessed the antibacterial effectiveness of popular brands of hand washes sold in Nigeria. The products showed varying level of inhibition against the test organisms. *S. aureus* was the most susceptible to all the products while *P. mirabilis* was the most resistant. Undiluted concentration had the highest inhibitory concentration against all the test organisms. Hand washes appear to be promising for use in controlling the increase of staphylococcal infections but this would require an improvement on its consistency and efficacy in its activity with respect to the composition of its active ingredients.

Our result is in consonance with previous findings which have pointed to the poor effectiveness of many brands of hand washes against common bacteria upon dilution, which makes performance of such products quite far from the claim of the manufacturers. There is the need to educate users on the need to refrain from dilution of hand washes before use as diluting hand wash makes them lose their potency Sellers of such products should on their part store the products properly following to the manufacturers' recommendations in order to avoid loss of efficacy.

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