BACTERIOCIDAL ACTIVITY OF HANDWASH PRODUCTS AND SOAPS SOLD IN PORT HARCOURT, NIGERIA – AN IN VITRO STUDY.

Otokunefor K., Onebunne S.C., Onodu B.C
Department of Microbiology, Faculty of Science, University of Port Harcourt, P.M.B. 5323, Port Harcourt, Nigeria.

CORRESPONDENCE:
E-mail: kome.otokunefor@uniport.edu.ng

ABSTRACT
Background
Hand hygiene is still noted as one of the most important factors essential for control of infectious diseases particularly in hospital settings and soap and water is still of essence, especially when hands are visibly soiled. The use of medicated handwash products is becoming more widespread. These products sometimes claim a better efficacy than plain soap and water. This study set out to determine trends in antibacterial activity of medicated and non-medicated locally sold soap products and bacteriocidal activity of these products against commonly encountered clinical pathogens (Staphylococcus aureus, Klebsiella pneumoniae and Escherichia coli).

METHODS: *Antibacterial activity of 4 handwash and 3 bodywash products was determined using the Agar-well diffusion, minimum inhibitory concentration and minimum bacteriocidal concentration tests.*

RESULTS: *At 100% product concentration, both non-medicated handwash products (Pears and Cussons) gave zones of inhibition ranging from 8 – 17 mm and 9 – 12 mm respectively, while the medicated products resulted in zones of inhibition ranging from 23 – 30 mm and 17 – 29 mm. Liquid bodywash products were less inhibitory with 'no inhibition' observed in 66.7% (18/27) of cases than 11.1% (4/36) of cases of the handwash. All handwash products had bacteriocidal activity against all three test strains, while for the bodywash products, no bacteriocidal activity was observed in 4 cases.*

CONCLUSION: *This study highlights the important role soaps particular the medicated versions play in hand hygiene. Further studies involving human volunteers and time-kill assays would be necessary to provide a more complete story of the effectiveness of these products in infection control.*

INTRODUCTION

Initial guidelines for hand hygiene, recommended the use of soap and water[1]. Despite the Centers for Disease Control and Prevention (CDC) revised guidelines in 2002[2], the use of soap and water is still widespread particularly where the hands are visibly dirty[3]. Soaps act by reducing microbial load either via mechanical removal, or/and death. This variation of activity depends on the composition of the soaps. Soaps are a timeless product described as early as 1500 B.C.[4] and are generally classed as medicated or non-medicated, based on the presence or absence of a biocide at active concentrations.

The more commonly used antibacterial products in the soaps include chlorhexidine, triclosan, trichlorocarbamide and trichloroxylenol. These substances are often contained at differing concentrations, which may result in either bacteriostatic or bacteriocidal activity[4, 5]. Different studies report on different substances as the most common in use. Chlorhexidine, triclosan and triclocarbon have been mentioned as the most common antibacterial agents in use[5, 6, 7]. These antimicrobial products have been found to inhibit both Gram positive and negative bacteria, though with varying activity levels[5].

Controversial results have however been associated with the use of medicated antibacterial products as opposed to the non-medicated products. One group of studies report higher levels of bacterial reduction in soaps containing antimicrobial agents[8, 9, 10, 11]. This is in contrast to a second set of studies which seem to report no significant difference in levels of bacterial reduction when comparing

medicated and non-medicated soaps[4, 11, 12, 13]. One major reason for this variation in result is the non-standardization of methodology, with the various studies differing in concentration of substrate used and contact time[5].

Though hand hygiene practices in Nigeria improved significantly since the 2014 Ebola virus disease (EVD) outbreak, most studies exploring hand washing practices in Nigeria generally focus on the use of soaps and water in general, rather than the use of antibacterial soaps[14,15,16]. This same trend was reported by a previous study which noted a significantly higher use of ordinary soap and water (93.9%) as opposed to the use of an antiseptic soap and water (6.1%) among community health officers in Rivers State[17]. With all the variations previously reported in the antibacterial efficacy of various antimicrobial soaps, it is essential to explore the local situation as this would provide a basis to strengthen or alter current hand washing practices. This study therefore set out to determine if any variations exist in antibacterial activity between medicated and non-medicated locally sold soap products and determine the bacteriocidal activity of these products against commonly encountered clinical pathogens.

MATERIALS AND METHODS

Test Isolates

The test organisms used in this study were of clinical origin and included *Staphylococcus aureus, Klebsiella pneumoniae* and *Escherichia coli*.

Test Products

Test products to be analysed were purchased from various stores around the Port Harcourt metropolis and represented both medicated and non-medicated liquid hand and body wash.

Antibacterial Activity of Products

The efficacy of the various products against select clinical isolates was determined using previously described[18, 19, 20] diffusion and dilution methods; the agar well diffusion test and the minimum inhibitory concentration (MIC) dilution technique. To do this, stock solutions were first prepared by diluting each test substance twofold serially, using sterile distilled water to give stocks of 50%, 25% and 12%.

Agar well diffusion test

The agar well diffusion test was carried out as a preliminary screen to assess the antimicrobial activities of the various products. This involved the use of an inoculum corresponding to 0.5 McFarland. The test inoculum was swab inoculated on a Mueller Hinton agar plate and allowed to stand at room temperature for 15 minutes. Following this, 4 wells were created on the plates using a 6 mm cork borer and 0.2 ml of differing concentrations (100%, 50% and 25%) of the test substance added to individual wells. After a 24 hour incubation at 37˚C, the zones of inhibition were then measured.

Minimum Inhibitory Concentration (MIC) Determination

MIC testing was carried out to quantitatively determine the lowest 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. Following a 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.

Minimum Bacteriocidal Concentration (MBC) Determination

To determine the MBC of each test substrate, against each test isolate, the three lowest concentrations which resulted in an inhibition of the test organism were subcultured unto nutrient agar plates, incubated at 37C for 24 hours and observed for growth. The MBC was taken as the least concentration which did not result in growth of the organism.

RESULTS

Test Products

Seven products were chosen to represent various brands, which varied in their antibacterial content (Table 1). Of these seven products, four were liquid handwash products (2 indicated as medicated and 2 non-medicated). The other 3 products were liquid bodywash products, 2 of which were non-medicated.

Table 1: Test Products used and their active ingredients

Products Tested	Product Class	Active Antibacterial Ingredient(s)
Pears	Handwash	None
Cusson	Handwash	None
Astonish	Handwash	Triclosan
Enliven	Handwash	Triclosan
Lemon Fresh	Bodywash	Not Indicated
Extract	Bodywash	None
Idole	Bodywash	None

Preliminary Screen for Antibacterial Activity Using Agar well diffusion

A preliminary analysis of the antibacterial activity of each product against test isolates using the agar well diffusion technique, revealed a range of zones of inhibition (Table 2). The non-medicated products generally resulted in lower zones of inhibition than the medicated products. At 100% product concentration, both non-medicated handwash products (Pears and Cussons) gave zones of inhibition ranging from 8 – 17 mm and 9 – 12 mm respectively, while the medicated products resulted in zones of inhibition ranging from 23 – 30 mm and 17 – 29 mm. In comparison with the handwash products, the liquid bodywash products were less inhibitory with 'no inhibition' observed in 66.7% (18/27) of cases of the bodywash as opposed to 11.1% (4/36) of cases of the handwash.

The degree of inhibition of these products was generally concentration dependent but with varying activity against the clinical organisms (Figure 1), and a significant difference in inhibitory activity exhibited by the medicated handwash products as opposed to the non-medicated ones.

Table 2: Zones of Inhibition of test substances against select Clinical Isolates as determined by Agar well diffusion technique

		Pears (NM) Conc. (%)			Cussons (NM) Conc. (%)			Astonish Conc. (%)			Enliven Conc. (%)			Extract (NM) Conc. (%)			Idole (NM) Conc. (%)			Lemon Fresh Conc. (%)		
		50	25	NC	100	50	25	NC	100	50	25	NC	100	50	25	NC	100	50	25	NC	100	50	25	NC	100	50	25	NC
Zone of Inhibition (mm)	SA	8	7	NI	NI	11	8	NI	NI	30	23	22	NI	17	11	8	NI	29	28	23	NI	NI	NI	NI	NI	8	NI	NI	NI
	KP	17	15	14	NI	12	8	7	NI	23	21	20	NI	25	18	19	NI	13	NI	NI	NI	13	NI	NI	NI	8	NI	NI	NI
	EC	NI	NI	NI	NI	9	8	7	NI	24	22	15	NI	29	30	20	NI	11	NI	NI	NI	NI	NI	NI	NI	10	NI	NI	NI

Key: SA: *S. aureus;* KP: *K. pneumoniae*; EC: *E. coli*; NI: No Inhibition

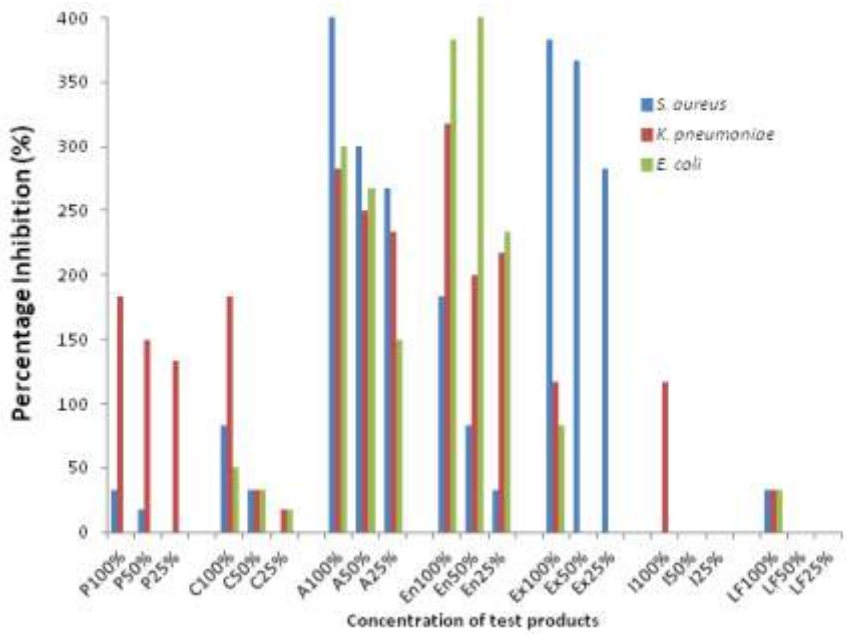

Figure 1: Variable Inhibitory levels of test substances against clinical isolates (P: Pears; C: Cussons; A: Astonish; En: Enliven; Ex: Extract; I: Idole; LF: Lemon Fresh).

MIC/MBC Determination

Further susceptibility testing using the dilution methods showed similarities to that of the agar well diffusion technique (Table 3). Dilution testing discounts the effect of product diffusion and enables a more accurate and quantitative comparison of antibacterial activity between test products. This test revealed a 100% inhibition of test isolates by handwash products, while no inhibition was noted only in one instance (Idole, *K. pneumoniae*) of the bodywash products. Of the handwash products, Pears showed the least inhibitory activity with MIC values ranging from 50% to 100%.

Similar to the results of the MIC testing, all handwash products had bacteriocidal activity against all three test strains. For the bodywash products, no bacteriocidal activity was observed in 4 cases. Lemonfresh which claims to be medicated was bacteriocidal only against *E. coli*.

Table 3: Minimum Inhibitory and Bacteriocidal Concentrations of test substances against Select Clinical isolates

	S. aureus		*Klebsiella pneumoniae*		*Escherichia coli*	
	MIC	MBC	MIC	MBC	MIC	MBC
Pears	100%	100%	50%	100%	100%	100%
Cusson	25%	50%	25%	25%	50%	50%
Astonish	25%	50%	25%	25%	50%	100%
Enliven	25%	25%	50%	25%	50%	50%
Lemon Fresh	100%	NB	100%	NB	50%	25%
Extract	50%	50%	25%	25%	100%	NB
Idol	100%	100%	NI	NB	100%	100%

DISCUSSION

Soaps are well known to play a very important role in hand hygiene. Despite the lack of conclusive evidence of an increased effectiveness of medicated soaps in reducing bacterial load, the non-medicated soaps generally by their nature would result in removal rather than inactivation of pathogens. In line with this, a recent study[21] reported significant level differences in rinse water generated by hand cleansing with substances containing antimicrobial agents. This was in stark contrast to the little or no difference observed when non-medicated soap was used. Results of this current study present data which both appear to support the case for and against the use of medicated soaps.

Similar to previous reports[11, 22], in this study the medicated handwash products consistently resulted in higher zones of inhibition than the non-medicated ones when tested against the clinical pathogens (Table 3). Both medicated handwash products used in this study contained triclosan as the antibacterial agent (Table 1). This had previously been reported as the most common antibacterial agent in liquid handwash[5], though in more recent times this trend was noted to be changing due to the evolution of triclosan resistant bacteria[7]. Despite both products containing the same antimicrobial agent, the levels of antibacterial activity of the products varied slightly (Fig 1). This variation could be a function of the concentration of triclosan in the products. It has previously been reported that the maximum triclosan concentration allowed by law is 0.3%[12], though actual active concentrations may range up to 2%[5]. Variable levels of inhibition are associated with the different concentrations. At triclosan concentrations of 0.3% and less, reports were made of a mean Log_{10} reduction factor ranging from 2.05 to 2.8[5,12]. This was in contrast to the mean Log_{10} reduction factor of 3.23 and 3.46 at triclosan concentrations of 0.45% and 0.46%[22, 23] and the mean Log_{10} reduction factor of between 4 and 5 at 0.5% triclosan[24]. Both medicated products tested in this study did not indicate the level of triclosan in the products, but the results appear to indicate that the concentration of triclosan in both products is adequate as they both resulted in significant levels of inhibition.

The bodywash products tested in this study were consistently less inhibitory than the handwash products. This is most likely a reflection of the difference in function between handwash and bodywash. Unlike soaps which are designed as a personal hygiene product for both the body and hands, or handwash products designed specifically for hands, bodywash products are designed specifically for body care. The skin doesn't generally have the same ability as the hands to transmit infection, plus wide alterations of the normal skin flora could actually have detrimental effects, it follows therefore that skin products could be designed more to maintain skin equilibrium and hence any medicated substance present, in minimal concentrations.

The different products had differing levels of activities against the clinical isolates. In some cases the Gram positive bacteria in general had a higher level of susceptibility to the tested products. This trend has been previously noted. A recent study analysing the antibacterial activities of a soap[12] noted higher levels of log cfu/ml reduction in growth of Gram positive bacteria (1.44 – 1.80), than in Gram negative bacteria (0.63 – 0.67). Similar reports have been made by other studies over the years[25, 26, 27, 28, 29]. But this trend was not universally observed for all cases. In addition to differences in cell wall composition, prior contact with the test substance could affect the inhibitory effect on the organism. It might therefore follow that an organism would be more resistant to a substance it is encountering for the first time, rather than one it has been in contact with previously.

CONCLUSION

Results of this study show good inhibitory activity of the medicated handwash products tested against the limited subset of clinical bacteria, highlighting the usefulness of these products in infection control. The methods used in this study however only provide a preliminary screen and determine inhibitory activity after a prolonged period of time (24 hours). Further studies both involving human

volunteers and time-kill assays would be necessary to provide a more complete story of the effectiveness of these products in infection control.

REFERENCES

1. Garner JS, Favero MS. Guideline for handwashing and hospital environmental control, 1985 supersedes guideline for hospital environmental control published in 1981. *American Journal of Infection Control*. 1986; 14(3): 110 – 126.

2. Centers for Disease Control and Prevention. Guideline for hand hygiene in health-care settings. Recommendation of the Healthcare Infection Control Practices Advisory Committee and the HICPAC/SHEA/APIC/IDSA Hand Hygiene Task Force. 2002.

3. Kampf G, Löffler H, Gastmeier P. Hand hygiene for the prevention of nosocomial infections. *Deutsches Ärzteblatt International*. 2009; 106(40): 649 – 655.

4. Mwambete KD, Lyombe F. Antimicrobial Activity of Medicated Soaps Commonly Used By Dar es Salaam Residents in Tanzania. *Indian Journal of Pharmaceutical Science*. 2011. 73(1): 92 – 98.

5. Kampf G, Kramer A. Epidemiologic background of hand hygiene and evaluation of the most important agents for scrubs and rubs. *Clinical Microbiology Reviews*. 2004; 17(4): 863 – 893.

6. Perencevich EN, Wong MT, Harris AD. National and regional assessment of the antibacterial soap market: a step toward determining the impact of prevalent antibacterial soaps. *American Journal of Infection Control*. 2001. 29: 281 – 283.

7. Kaliyadan F, Aboulmagd E, Amin TT. Antimicrobial activity of commercial "antibacterial" handwashes and soaps. *Indian Dermatology Online Journal*. 2014. 5(3): 344 – 346.

8. Paulson D. A comparative evaluation of five surgical hand scrub preparations. AORN J. 1994. 60(2): 249 – 256.

9. Fuls JL, Rodgers ND, Fischler GE, Howard JM, Patel M, Weidner PL, Duran MH. Alternative hand contamination technique to compare the activities of antimicrobial and nonantimicrobial soaps under different test conditions. *Applied and Environmental Microbiology*. 2008; 74(12): 3739 – 3744.

10. Montville R, Schaffner DW. A meta-analysis of the published literature on the effectiveness of antimicrobial soaps. *Journal of Food Protection*. 2011; 74(11): 187 – 1882.

11. Pérez-Garza J, García S, Heredia N. Removal of *Escherichia coli* and *Enterococcus faecalis* after Hand Washing with Antimicrobial and Nonantimicrobial Soap and Persistence of These Bacteria in Rinsates. *Journal of Food Protection*. 2017; 80(10): 1670 – 1675.

12. Kim SA, Moon H, Lee K, Rhee MS. Bactericidal effects of triclosan in soap both *in vitro* and *in vivo*. *Journal of Antimicrobial Chemotherapy*. 2015; 70(12): 3345 – 3352.

13. Kim SA, Rhee MS. Microbicidal effects of plain soap vs triclocarban-based antibacterial soap. *Journal of Hospital Infection*. 2016; 94(3): 276 – 280.

14. Olalekan AW, Adeola E. How prepared are Nigerian schools for ebola virus disease prevention and control? *Annals of Global Health*. 2014; 80(6): 452 – 457.

15. Ilesanmi OS, Alele FO. The effect of Ebola Virus Disease outbreak on hand washing among secondary school students in Ondo State Nigeria, October, 2014. *The Pan African Medical Journal*. 2015; 22(Suppl 1).

16. Iliyasu G, Ogoina D, Otu AA, Dayyab FM,

Ebenso B, Otokpa D, Rotifa S, Olomo WT, Habib AG. A multi-site knowledge attitude and practice survey of Ebola virus disease in Nigeria. *PloS one*. 2015; 10(8): e0135955.

17. Braimoh OB, Udeabor SE. Hand hygiene practices among community health officers in Rivers State, Nigeria. *African Health Sciences*. 2013; 13(2): 507 – 511.

18. Otokunefor K, Dappa B. Antibacterial Evaluation of Nigerian *Ocimum Sanctum* Leaf Extracts against Bacterial Isolates Associated With Urinary Tract Infection. *Nigerian Journal of Pharmaceutical and Applied Science Research*. 2017; 6(1): 19 – 25.

19. Magaldi S., Mata-Essayag S., De Capriles C.H., Perez C., Colella M.T., Olaizola C. and Ontiveros Y. (2004). Well diffusion for antifungal susceptibility testing. *International Journal of Infectious Diseases*. 8(1), 39 – 45.

20. Clinical and Laboratory Standard Institute (CLSI). (2012). Methods for Dilution Antimicrobial Susceptibility Testing for bacteria that grow Aerobically. 9th edition. Wayne, PA.

21. Wolfe MK, Gallandat K, Daniels K, Desmarais AM, Scheinman P, Lantagne D. Handwashing and Ebola virus disease outbreaks: A randomized comparison of soap, hand sanitizer, and 0.05% chlorine solutions on the inactivation and removal of model organisms Phi6 and E. coli from hands and persistence in rinse water. *PloS one*. 2017; 12(2): e0172734.

22. Schaffner DW, Bowman JP, English DJ, Fischler GE, Fuls JL, Krowka JF, Kruszewski FH. Quantitative microbial risk assessment of antibacterial hand hygiene products on risk of shigellosis. *Journal of Food Protection*. 2014; 77(4): 574 – 582.

23. Fuls J, Fischler G. Antimicrobial Efficacy of Activated Triclosan in Surfactant-Based Formulation versus *Pseudomonas putida*. *American Journal of Infection Control*. 2004; 32(3): E22.

24. Messager S, Goddard PA, Dettmar PW, Maillard JY. Determination of the antibacterial efficacy of several antiseptics tested on skin by an 'ex-vivo'test. *Journal of Medical Microbiology*. 2001; 50(3): 284 – 292.

25. Riaz S, Ahmad A, Hasnain S. Antibacterial activity of soaps against daily encountered bacteria. *African Journal of Biotechnology*. 2009; 8: 1431 – 1436.

26. Bhat RP, Prajna PS, Menezez VP, Shetty P. Antimicrobial activities of soap and detergents. *Advanced Biomedical Research*. 2011; 2: 52 – 62.

27. Igbeneghu OA. The antimicrobial assessment of some Nigerian herbal. *African Journal of Traditional, Complementary and Alternative Medicines*. 2013; 10(6): 513 – 518.

28. Ikegbunam MN, Metuh RC, Anagu LO, Awah NS. Antimicrobial activity of some cleaning products against selected bacteria. *International Research Journal of Pharmaceutical and Applied Sciences*. 2013; 3: 133 – 135.

29. Obi CN. Antibacterial Activities of Some Medicated Soaps on Selected Human Pathogens. *American Journal of Microbiological Research*. 2014; 2(6): 178 – 181.