EFFECTIVENESS OF DIFFERENT BRANDS OF ALCOHOL-BASED HAND SANITIZERS AGAINST SOME SELECTED MICROORGANISMS

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ABSTRACT

Background

Hand washing is one of the key steps to disease prevention and health promotion. Hand sanitizers address the barriers to hand hygiene compliance because they require a fraction of the time for effective hand washing and they are more effective in killing many microorganisms. This study was carried out to evaluate the effects of different brands of alcohol-based hand sanitizers against some selected microorganisms.

Materials and Methods

Four commonly used alcohol-based hand sanitizers within Owerri municipal were used for the study. They were analyzed using standard microbiological procedures. The agar well diffusion method was used to evaluate the efficacy of the hand sanitizers against *Escherichia coli, Staphylococcus aureus, Klebsiella pneumoniae, Candida albicans* and *Aspergillus flavus*.

Results

The susceptibility patterns of the test organisms to the hand sanitizers shows that only Hand Sanitizer A was sensitive to the test organisms; *E. coli* (26.0 mm), *S. aureus* (25.5 mm), *K. pneumoniae* (19.0 mm), *C. albicans* (10.2 mm) and *A. flavus* (17.0 mm). The test organisms were resistant to Hand Sanitizers B, C and D. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of Hand Sanitizer A to the test organisms were determined at the highest concentration (100 mg/ml).

Conclusion

The findings of the study revealed that only Hand Sanitizer A inhibited the growth of all the test organisms in vitro. It was both bactericidal and fungicidal at the highest concentration (100 mg/ml). There is need to verify the claims of the manufacturers of hand sanitizers sold in consumer outlets so as to protect consumers from buying substandard products.

Keywords*: hand sanitizers, effective, susceptibility, consumers, substandard products*

INTRODUCTION

Hand washing is one of the most important steps to avoid spreading germs. Germs are microorganisms such as bacteria, fungi and viruses that may lead to harmful diseases. They can live on the skin, respiratory, intestinal and genitourinary tracts. They can enter the body through openings such as the nose, mouth and also through breaks in the skin. Today, hygiene is associated with disease prevention and health promotion, and the importance of hygiene is universally recognized and evidence based. Physical contact between people and between people and objects is a key vehicle for the transmission of pathogens. Therefore, effective hand hygiene is a key intervention in disease prevention (Aiello *et al*., 2018). It is an integral procedure in the health care environment with healthcare workers receiving regular training about hand hygiene procedures (Hilburn *et al*., 2018). In the community outside of the healthcare environment, studies have reported association between improvements in hand hygiene and reduction in rates of infectious disease. It is estimated that simple hand washing could save one million lives a year (Curtis and Cairncross, 2018). Many public health campaigns worldwide have addressed "hand hygiene" with varying success (Erasmus *et al*., 2015).

Hand sanitizers are alcohol-containing preparations designed for application to the hands for reducing the number of viable microorganisms on the hands (CDC, 2017). They are also used as supplements or alternatives to hand washing with soap and water (Hammond *et al*., 2015). Various preparations of hand sanitizers are available including the gel, foam and liquid solutions. Active ingredients of hand sanitizers include isopropanol, ethanol, n-propanol or providone-iodine while the inactive ingredients usually include a thickening agent (such as polyacrylic acid for gels), humectants (such as glycerin for liquid rubs) or propylene glycol and essential oils of plants.

Hand sanitizers address the barriers to hand hygiene compliance because they require a fraction of the time for effective hand washing (Mody *et al*., 2018). They are less damaging to the skin than soap and water and they are more effective in killing many microorganisms (Larson *et al*., 2016). While alcohol-based hand sanitizers have been demonstrated to be effective against a wide range of Gram-positive and Gram-negative bacteria, multi-resistant pathogens, fungi and many viruses (Kampf *et al*., 2019), they have also been reported to have very poor activity against bacterial spores, protozoan oocysts and certain non-enveloped (nonlipophilic) viruses (CDC, 2017). Despite several reports stating their efficacy, consumers have been warned against false claims of efficacy by some manufacturers (FDA, 2016). This study aimed at evaluating the effects of different brands of alcohol-based hand sanitizers against some selected microorganisms.

MATERIALS AND METHODS

Sample Collection

Four (4) commonly used alcohol-based hand sanitizers commercially sold within Owerri municipal were used in this study. They were labeled Hand Sanitizer A, B, C and D.

Test Microorganisms

The test microorganisms: *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Candida albicans* and *Aspergillus flavus* were isolated from clinical samples of wound, skin and ear swabs of patients attending Federal Medical Center (F.M.C), Owerri, Imo State. The isolates were identified by standard methods. The pure cultures of the isolates were preserved on nutrient agar and Sabouraud dextrose agar slants.

Antimicrobial Susceptibility Test

The susceptibility of the test organisms to the hand sanitizers was evaluated using agar well diffusion method as described by Sharif and Ansari (2015) and Ochwoto *et al*. (20170. Sterile Mueller Hinton agar and Sabouraud dextrose agar plates were inoculated with standardized test organisms. A sterile cotton swab was dipped into a tube containing the inoculum and was rotated properly to allow maximum contact. Excess inoculum was removed by pressing and rotating the swab firmly against the inside of the tube above the liquid level. The swab was spread over the surface of the medium and the inoculum left to dry for 5 minutes. With the aid of a sterile 6mm cork borer, four equally spaced holes was bored in the agar plate with a fifth hole in the centre of the plate as control. The agar plugs were discarded using a sterile needle. One hundred microlitres (100µL) of the hand sanitizer was then introduced into each of the four wells. 25 mcg of ciprofloxacin dissolved in 100µL 70% ethanol was pipetted into the fifth hole. This was done for all the test organisms and hand sanitizers. The plates were incubated at 37^{o}C for 24 hours (bacteria) and 25^{o}C for 48 hours (fungi) in an upright position. They were examined for zones of inhibition which indicate the degree of susceptibility or resistance of the test organisms to the hand sanitizers. The test was carried out in duplicates and the average of 2 readings was taken as the zone of inhibition (mm) in each case.

Determination of Minimum Inhibitory Concentration (MIC)

The hand sanitizer which showed activity against the test organisms in the agar diffusion test was subjected to further test to determine its MIC values using the broth dilution method. MIC is the lowest concentration of a specific antimicrobial needed to prevent the growth of a given organism in vitro (Nester *et al*., 2019). Various concentrations of the hand sanitizer were prepared in increasing order (20 mg/ml, 40 mg/ml, 60 mg/ml, 80 mg/ml and 100 mg/ml. One milliliter of the sanitizer was introduced into tubes containing equal volume (1ml) of standardized test organisms. Each of the concentrations of the sanitizer was used in each case. Two tubes, one containing only nutrient broth and bacteria without sanitizer and the other containing Sabouraud dextrose broth and fungi without sanitizer served as control. The tubes were incubated at 37^{o}C for 24 hours (bacteria) and 25^{o}C for 48 hours (fungi) and examined for visible growth or turbidity. The concentration of the sanitizer at which no visible growth was observed when compared with the controls was regarded as the MIC.

Determination of Minimum Bactericidal Concentration (MBC)

To determine the MBC of the hand sanitizer against the test organisms, the lowest concentration which resulted in an inhibition of the test organisms was subcultured unto nutrient agar and Sabouraud dextrose agar plates. The plates were incubated at 37°C for 24 hours (bacteria) and 25°C for 48 hours (fungi) and observed for growth. The MBC was taken as the least concentration which did result in growth of the organism (Chesbrough, 2006).

RESULTS

Susceptibility Patterns of Test Organisms to Hand Sanitizers

The susceptibility pattern of the test organisms to the hand sanitizers shows that only Hand Sanitizer A was sensitive to the test organisms; *E. coli* (26.0 mm), *S. aureus* (25.5 mm), *K. pneumoniae* (19.0 mm), *C. albicans* (10.2 mm) and *A. flavus* (17.0 mm). The test organisms were resistant to Hand Sanitizer B, C and D (table 1).

Table 1. Susceptibility Patterns of Test Organisms to Hand Sanitizers

Test organisms	Zones of inhibition (mm)			
	Hand Sanitizer A	Hand Sanitizer B	Hand Sanitizer C	Hand Sanitizer D
E. coli	26.0	+	+	+
S. aureus	25.5	+	+	+
K. pneumoniae	19.0	+	+	+
C. albicans	10.2	+	+	+
A. flavus	17.0	+	+	+

Key: + indicates growth

Determination of MIC and MBC of Hand Sanitizer on Test Organisms

Table 2 shows the result of MIC and MBC of Hand Sanitizer A on the test organisms only. From the result, MIC and MBC of Hand Sanitizer A to the test organisms were determined at the highest concentration (100 mg/ml). There were no MIC and MBC on Hand Sanitizer B, C and D because they showed no activity against the test organisms in the agar well diffusion test.

Table 2. MIC and MBC (mg/ml) of Hand Sanitizer A on the test organisms

Test organisms	Concentrations (mg/ml)						
	100	80	60	40	20	MIC	MBC
E. coli	-	+	+	+	+	100	100
S. aureus	-	+	+	+	+	100	100
K. pneumoniae	-	+	+	+	+	100	100
C. albicans	-	+	+	+	+	100	100
A. flavus	-	+	+	+	+	100	100

Keys: + indicates growth

- indicates no growth

DISCUSSION

This study was carried out to evaluate the effects of different brands of alcohol-based hand sanitizers against some selected microorganisms. The findings of the study showed that Hand Sanitizer A was the only product that showed inhibitory activity against all the test organisms with the highest activity against *E. coli* (26.0 mm) and the lowest activity against *Candida* sp. (10.2 mm). Hand Sanitizer B, C and D showed no activity against all the test organisms.

Hand Sanitizer A showed both bactericidal and fungicidal activity at the highest concentration (100 mg/ml) against all the test organisms. This is attributable to the presence of alcohols as the main active ingredients in the product. Alcohols are known to exert disinfectant activity in bacteria by causing protein denaturation, disruption of tissue membranes and dissolution of several lipids (Kar, 2008). Hand Sanitizer A which had the highest activity against the organisms in this study contained 62% ethanol as the main active ingredient while Hand Sanitizer B contained alcohol denat as the active ingredient. Isopropyl alcohol was the main active ingredient in Hand Sanitizer C and D (in addition to ethyl alcohol).

Although isopropanol has been reported as being superior to ethanol as an antiseptic, the non activity of Hand Sanitizer B, C and D observed in this study is probably due to the negative interactive effects of the additional ingredients such fragrance, emollients, humectants and thickening agents added to them and which are not present in Hand Sanitizer A. These could probably limit the cidal effect of the alcohol from reaching the microbial 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). Generally, the lack of antimicrobial activity observed among the other products could be due to poor or prolonged storage of the products which could lead to increased temperature causing evaporation of the active ingredient.

Hand Sanitizer A showed considerable antimicrobial activity against *C. albicans* and *A. flavus*. It is noteworthy here that fungal species such as *C. albicans* and *A. flavus* have accounted for a high frequency of opportunistic fungal infections implicated in hospital-acquired infections.

CONCLUSION

The findings of the study revealed that only one of the four products (Hand Sanitizer A) inhibited growth of all the test organisms. It showed both bactericidal and fungicidal activity. There is need to verify the claims of the manufacturers of hand sanitizers sold in consumer outlets so as to protect consumers from buying substandard products. Regulatory authorities and manufacturers should enforce stringent quality control measures during production and routine inspections to ensure the efficacy of these products. Finally, consumers should be alerted on the existence of substandard sanitizers on the shelves of some retail outlets.

REFERENCES

Aiello, A.E., Coulborn, R.M., Perez, V. and Larson, E.L. (2018). Effect of hand hygiene on infectious disease risk in the community setting: a meta analysis. *American Journal Public Health*, 98: 1372-1382.

Ansari, S.A., Sattar, S.A., Springthorpe, V.S., Wells, G.A. and Tostowaryk W. (2019). In vivo protocol for testing efficacy of hand-washing agents against viruses and bacteria: Experiments with rotavirus and *Escherichia coli*. *Applied Enviromental Microbiology*, 55(12): 3113–8.

Bore, E., Hebraud, M., Chafsey, I., Chambon, C., Skjæret, C. and Moen, B. (2017). Adapted tolerance to benzalkonium chloride in *Escherichia coli* K-12 studied by transcriptome and proteome analyses. *Microbiology*, 153(4): 935–46.

Bush, L.W., Benson, L.M. and White, J.H. (2016). Pig skin as test substrate for evaluating topical antimicrobial activity. *Journal Clinical Microbiology*, 24(3): 343–8.

Centers for Disease Control and Prevention (2012). Guideline for Hand Hygiene in Health-Care Settings: Recommendations of the Healthcare Infection Control Practices Advisory Committee and the HICPAC/SHEA/APIC/IDSA Hand Hygiene Task Force. MMWR; 51 (No. RR- 16): 1-56.

Cutis, V. and Cairncross, S. (2013) Effect of washing hands with soap on diarrhea risk in the community:

A systematic a review. *Lancet infectious Disease*, 3: 275-81

Dastider, D., Jyoti, S.D., Kumar, M.S, Bose, S., Ray, S. and Mahanti, B. (2020). Hand sanitizers bid farewell to germs on surface area of hands. European *Journal Pharmaceutical Medical Research*, 7(4): 648–56.

Di Muzio, M., Cammilletti, V., Petrelli, E. and Di Simone, E. (2015). Hand hygiene in preventing nosocomial infections: a nursing research. *Ann Ig*, 27(2): 485–91.

Erasmus, V., Kuperus, M.N., Richards, J.H., Vos, M.C. and Oenema, A. (2010). Improving hand hygiene behavior of nurses using action planning. a pilot study in the intensive care unit and surgical ward. *Journal Hospital Infection*, 76: 161-4.

FDA (2011). Consumer Health Information. Hand Sanitizers Carry Unproven Claims to Prevent MRSA Infections.

Fendler, E.J., Ali, Y., Hammond, B.S., Lyons, M.K., Kelley, M.B., Vowell, N.A. (2012). The impact of alcohol hand sanitizer use on infection rates in an extended care facility. *American Journal Infectious Control*, 30(4): 226–33.

Fleur, P. and la Jones, S. (2017). Non-Alcohol Based Hand Rubs: A Review of Clinical Effectiveness and Guidelines [Internet]. CADTH Rapid Response Report: Summary with Critical Appraisal. Canadian Agency for Drugs and Technologies in Health.

Foddai, A.C.G., Grant, I.R. and Dean, M. (2016). Efficacy of instant hand sanitizers against foodborne pathogens compared with hand washing with soap and water in food preparation settings: A systematic review. *International Association for Food Protection*; 10(79): 40–54.

Fung, I.C.H. and Cairncross, S. (2016). Effectiveness of handwashing in preventing SARS: A review. *Tropical Medicine and International Health*, 11: 1749–58.

Gerberding, J.L., Director David Fleming, M.W., Snider, D.E., Thacker, S.B., Ward, J.W. and Hewitt, S.M. (2012). Morbidity and Mortality Weekly Report Guideline for Hand Hygiene in HealthCare Settings Recommendations of the Healthcare Infection Control Practices Advisory Committee and the HICPAC/SHEA/APIC/IDSA Hand Hygiene Task Force Centers for Disease Control and Prevention.

Grayson, M.L., Melvani, S., Druce, J., Barr, I.G., Ballard, S.A. and Johnson, P.D.R. (2019). Efficacy of Soap and Water and Alcohol-Based Hand-Rub Preparations against Live H1N1 Influenza Virus on the Hands of Human Volunteers. *Clinical Infectious Disease*, 48(3): 285–91.

Greenaway, R.E., Ormandy, K., Fellows, C. and Hollowood, T. (2018). Impact of hand sanitizer format (gel/foam/liquid) and dose amount on its sensory properties and acceptability for improving hand hygiene compliance. *Journal Hospital Infection*, 100(2): 195–201.

Hammond, B., Ali, Y., Fendler, E., Dolan, M. and Donovan, S. (2010). Effect of hand sanitizer use on elementary school absenteeism. *American Journal Infection Control*, 28(5): 340-346

Jain, V.M., Karibasappa, G.N., Dodamani, A.S., Prashanth, V.K. and Mali, G.V. (2016). Comparative assessment of antimicrobial efficacy of different hand sanitizers: An in vitro study. *Dental Research Journal*, 13(5): 424–31.

Kampf, G., Rudolf, M., Labadie, J.C. and Barrett, S.P. (2012). Spectrum of antimicrobial activity and user acceptability of the hand disinfectant agent Sterillium® Gel. *Journal Hospital Infection*, 52(2): 141–7.

Kampf, G., Ruselack, S., Eggerstedt, S., Nowak, N. and Bashir, M. (2013). Less and less-influence of volume on hand coverage and bactericidal efficacy in hand disinfection. *BMC Infect Disease*: 13(1): 472.

Kampf, G. and Kramer, A. (2014). Epidemiologic background of hand hygiene and evaluation of the most important agents for scrubs and rubs: Clinical Microbiology Reviews. *American Society for Microbiology* (ASM), 17: 863–93.

Kampf, G. (2018). Efficacy of ethanol against viruses in hand disinfection. *Journal of Hospital Infection*, 98: 331–8.

Kampf, G., Höfer, M. and Wendt, C. (2019). Efficacy of hand disinfectants against vancomycin-resistant enterococci in vitro. *Journal Hospital Infection* 42: 143-150.

Kar, A. (2008). *Pharmaceutical Microbiology*. New Age International (P) Ltd., Publishers, Delhi; 216-217.

Larson, E.L., Aiello, A.E. and Bastyr, J. (2011). Assessment of two hand hygiene regimens for intensive care unit personnel. *Critical Care Medicine*, 29: 944-951.

Larson, E.L., Cohen, B. and Baxter, K.A. (2012). Analysis of alcohol-based hand sanitizer delivery systems: Efficacy of foam, gel, and wipes against influenza A (H1N1) virus on hands. *American Journal Infectious Control*, 40(9): 806–9.

Lauharanta, J., Ojajärvi, J., Sarna, S. and Mäkelä, P. (2011). Prevention of dryness and eczema of the hands of hospital staff by emulsion cleansing instead of washing with soap. *Journal Hospital Infection*, 17(3): 207–15.

Lens, C., Jerome L.J. and Grascha, P. A. (2011). Scientific study that

proves alcohol hand sanitiser is more efficacious when dispensed onto the hands as foam rather than as gel. *Canadian Journal Infectious Control*, 26(1): 21–5.

Liu, P., Yuen, Y., Hsiao, H.M., Jaykus, L.A. and Moe, C. (2010). Effectiveness of liquid soap and hand sanitizer against Norwalk virus on contaminated hands. *Applied Environmental Microbiology*, 76(2): 394-399

Malizia, W.F., Gangarosa, E.J. and Goley, A.F. (2010). Benzalkonium Chloride as a Source of Infection. *New England Journal Medicine*, 263(16): 800–2.

Mcdonnell, G. and Russell, A.D. (2019). Antiseptics and disinfectants: Activity, action, and resistance. Clinical Microbiology Reviews. *American Society for Microbiology* (ASM), 12: 147–79.

Mody, L., McNeil, S.A., Sun, R., Bradley, S.F. and Kauffman, C.A. (2013). Introduction of a waterless alcohol-based hand rub in a long-term-care facility. *Infectious Control Hospital Epidemiology*, 24: 165-171.

Morton, H.E. (2010). The relationship of concentration and germicidal efficiency of ethyl alcohol. *Ann New York Academic Science*, 53(1): 191–6.

Nester, E.W., Anderson, D.G., Roberts, C.E. and Nester, M.T. (2019). *Microbiology: a Human Perspective*, 6th edition. The McGraw-Hill Companies, Inc., New York; 480-481.

Ochwoto, M., Muita, L., Talaam, K., Wanjala, C., Ogeto, F., Wachira, F. et al. (2017). Antibacterial efficacy of alcohol hand rubs in the Kenyan market. *Antimicrob. Resist. Infect. Control.* 6: 17.

Peeri, N.C., Shrestha, N., Rahman, M.S., Zaki, R., Tan, Z. and Bibi, S. (2020). The SARS, MERS and novel coronavirus (COVID-19) epidemics, the newest and biggest global health threats: what lessons have we learned? *Epidemiology Association International Journal*, 34-56

Pidot, S.J., Gao, W., Buultjens, A.H., Monk, I.R., Guerillot, R. and Carter, G.P. (2018). Increasing tolerance of hospital Enterococcus faecium to handwash alcohols. *Science Translation Medicine*; 1:10(452).

Rabenau, H.F., Kampf, G., Cinatl, J., Doerr, H.W. (2015). Efficacy of various disinfectants against SARS coronavirus. *Journal Hospital Infection*, 61(2): 107–11.

Sattar, S.A., Abebe, M., Bueti, A.J., Jampani, H., Newman, J. and Hua S. (2010). Activity of an AlcoholBased Hand Gel Against Human Adeno-, Rhino-, and Rotaviruses Using the Fingerpad Method. *Infectious Control Hospital Epidemiology*; 21(8): 516–9.

Sharif, M. and Ansari, F. (2015). Hand Sanitizers: Efficiency against microbes form currency notes

and coins in local circulation. *Pak. J. Mol. Med*, 2: 75-83.

Steinmann, J., Becker, B., Bischoff, B., Paulmann, D., Friesland, M. and Pietschmann, T. (2010). Virucidal activity of 2 alcohol-based formulations proposed as hand rubs by the World Health Organization. *American Journal Infectious Control*, 38(1): 66–8.

Steinmann, J., Paulmann, D., Becker, B., Bischoff, B., Steinmann, E. and Steinmann, J. (2012). Comparison of virucidal activity of alcohol-based hand sanitizers versus antimicrobial hand soaps in vitro and in vivo. *Journal Hospital Infection*, 82(4): 277–80.

Thomas, P. (2012). Long-term survival of bacillus spores in alcohol and identification of 90% ethanol as relatively more spori/bactericidal. *Current Microbiology*, 64(2): 130–9.

Vogel, L. (2011). Hand sanitizers may increase norovirus risk. Vol. 183, CMAJ : Canadian Medical Association journal = journal de l'Association medicale canadiennea. *Canadian Medical Association*; E799.

Wentworth, A.B., Yiannias, J.A., Davis, M.D.P. and Killian, J.M. (2016). Benzalkonium chloride: A known irritant and novel allergen. *Dermatitis*, 27(1): 14–20.