https:// www.ajol.info/index.php/jasem
and www.bioline.org.br/ja

Vol. 21 (7) 1279-1280

# Evaluation of antibacterial activity of hand sanitizers – an *in vitro* study

***[1]OTOKUNEFOR, K; PRINCEWILL, I**

*Department of Microbiology, Faculty of Science, University of Port Harcourt, PMB 5323, Choba, Port Harcourt, Rivers State.*
*Corresponding Author E. mail: kome.otokunefor@uniport.edu.ng

**ABSTRACT:** Hand hygiene, particularly hand sanitizing, is essential in reducing infectious disease transmission. The recent outbreak of Ebola in Nigeria both increased public awareness of the practice of hand sanitizing and resulted in the introduction of new products to the Nigerian market. This study set out to explore the actual antibacterial activity of these products against key clinical isolates using both dilution and diffusion susceptibility tests methods. Results showed higher inhibitory activity of the products to *Klebsiella pneumoniae* and *Staphylococcus aureus* than *Escherichia coli* and *Pseudomonas aeruginosa*. Overall the only local product tested had the least inhibitory activity. In general however, the sanitizers showed good activities, with inhibition of bacteria noted at concentrations as low as 25%. Products tested in this study showed higher zones of inhibition than previously reported, indicating their overall effectiveness. The variations in diffusion and dilution results highlight the effect of texture of the sanitizing product on testing methods and point at a need to properly assess if this could perhaps have any effect in real time on inhibitory activities. The hand sanitizing products tested in this study are suitable in disease prevention. However, regulatory bodies may need to focus on product texture until the effect of this on activity is determined.

DOI: **https://dx.doi.org/10.4314/jasem.v21i7.9**



Received 10 October 2017; received in revised form 02 November 2017; accepted 15 December 2017

***Keywords***: Sanitizer, Nigeria, dilution, MIC, bacteriocidal

Hand hygiene is well known as one of the most significant of activities essential for the reduction of transmission of infectious diseases, particularly in hospitals (Pittet *et al.,* 2006, Zapka *et al.,* 2017). Hand hygiene generally refers to different methods of eliminating or killing microorganisms which may be present on hands, by either hand washing or sanitizing. Though the concept of hand sanitization has been in place right from the start of the hand hygiene campaign by Semmelweis (WHO 2009, Pires *et al.,* 2017), majority of early reports focused primarily on the role of hand washing as an infection control measure (Garner and Favero 1986). This changed by the early 2000s, when the Centers for Disease Control and Prevention (CDC) issued a guideline recommending that alcohol-based hand rub (ABHR) be routinely used for decontaminating hands (CDC 2002). These ABHRs which are the most commonly used hand sanitizers are often composed of alcohol, ethanol, isopropanol or propanol (Pittet 2001, Pickering *et al.,* 2010). They have a recommended concentration range of 60% to 95% (Reynolds *et al.,* 2006). Hand sanitizers have been reported to cause a decrease in infection rates and are generally particularly useful in situations where access to water is limited. In addition to being useful in the absence of water, other advantages of the use of the hand sanitizers include, high antimicrobial activity in a shorter time, and the lack of requirement for drying of the hand (which could serve as another source of contamination). The use of alcohol-based hand sanitizers has been reported as one of the commonly recommended means of hand hygiene for outbreaks of the Ebola-Virus Disease (Wolfe *et al.,* 2017), particularly for hands that are not visibly soiled. These hand sanitizers have been shown to be effective in various situations such as the reduction of gastrointestinal infection, reducing infection in University hostels and reducing absenteeism in elementary schools (Meadows and Le Saux 2004, Reynolds *et al.,* 2006). And has been previously reported to give better results than hand washing (Pickering *et al.,* 2010)

The 2014 outbreak of EVD in Nigeria led to an increased awareness of the role of hand sanitizers in infection control (Olalekan and Adeola 2014, Nwabueze *et al.,* 2016) and an upsurge of various brands of hand sanitizers into the Nigerian market (Odebisi-Omokanye 2015, Ogoina *et al.,* 2016). Most of these products have made numerous claims, notably their ability to eliminate 99.9% of microorganisms. A number of these claims have not been verified (Odebisi-Omokanye 2015). This study therefore set out to explore the antibacterial activities of a number of hand sanitizers sold in Port Harcourt, Nigeria against bacteria of clinical importance using both dilution and diffusion susceptibility methods.

## MATERIALS AND METHODS

*Test Isolates*: Four previously characterised clinical isolates obtained from the culture collection of the Pathogenic Bacteriology group of the Medical Microbiology Unit, University of Port Harcourt were used in this study. Isolates were selected to represent the more common organisms isolated from a clinical

microbiology laboratory and include the Gram positive cocci *Staphylococcus aureus*, and three Gram negative rods (*Klebsiella pneumoniae*, *Escherichia coli* and *Pseudomonas aeruginosa*).

*Test Products*: Three different alcohol based hand sanitizers were analysed in this study. Two of these were imported products (Carex and Bactigel), while Ebecare was a locally made product. All products had alcohol as the active ingredient.

*Antibacterial Activity of Products*: The efficacy of the various hand sanitizers against select clinical isolates was determined using previously described methods (Otokunefor and Dappa 2017, Magaldi et al., 2004; CLSI, 2012); the well-variant of the agar diffusion test and the minimum inhibitory concentration (MIC) dilution technique.

*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 to 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)*: MIC testing was carried out to determine the minimum concentration of test substance which could cause an inhibition of the growth of the test isolates. This involved the inoculation of $5 \times 10^8$ 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)*: 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 AND DISCUSSION

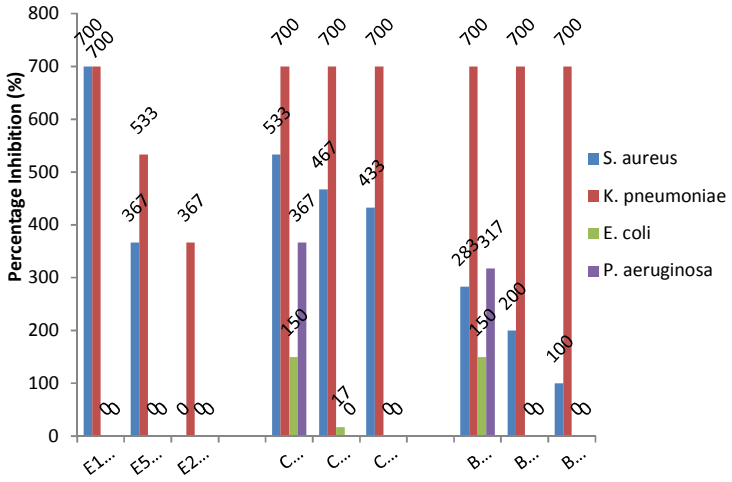
All three handwash products exhibited inhibitory activity against the test isolates (Table 1), with zones of inhibition ranging from 15 mm to 50 mm at concentrations of 100%. This inhibitory activity varied with product concentration. A general reduction in inhibitory activity was associated with a reduction in product concentration, and inhibition was still observed at concentrations as low as 25%, in some cases.

**Table 1:** Inhibitory effect of hand wash as detected by Agar well diffusion technique

| **Antibacterial Agent** | Zone of Inhibition *S. aureus* | *Klebsiella pneumonia* | *Escherichia coli* | *Pseudomonas aeruginosa* |
|---|---|---|---|---|
| Ebecare | | | | |
| 100% | 48 mm | 48 mm | 6 mm | 6 mm |
| 50% | 38 mm | 38 mm | 6 mm | 6 mm |
| 25% | 6 mm | 28 mm | 6 mm | 6 mm |
| NC | 6 mm | 6 mm | 6 mm | 6 mm |
| Carex | | | | |
| 100% | 38 mm | 48 mm | 15 mm | 28 mm |
| 50% | 34 mm | 48 mm | 7 mm | 6 mm |
| 25% | 32 mm | 48 mm | 6 mm | 6 mm |
| NC | 6 mm | 6 mm | 6 mm | 6 mm |
| Bactigel | | | | |
| 100% | 25 mm | 50 mm | 15 mm | 25 mm |
| 50% | 20 mm | 50 mm | 6 mm | 6 mm |
| 25% | 14 mm | 50 mm | 6 mm | 6 mm |
| NC | 8 mm | 8 mm | 6 mm | 6 mm |

Generally, all products showed significantly higher activity against the Gram negative *K. pneumoniae* than all other organisms (Figure 1). The widest variation was observed with Bactigel which showed a 700% inhibition in growth of *K. pneumoniae* as opposed to 283% inhibition of *S. aureus* growth. Of all the three products, the local product 'Ebecare' was the least effective. This product showed no activity at all against the Gram negative *E. coli* and *P. aeruginosa*.

had an MIC of 25% (Table 2). Bactigel appeared to be the more effective handwash as it showed an MIC less than 50% in 75% of cases as opposed to the other 2 products which showed an MIC of >50% only in 50% of cases. Generally though, MIC values were similar, falling within a 2 fold difference of each other.




E: Ebecare, C: Carex, B: Bactigel

Further testing of the products to determine the MIC and MBC values, showed that majority of products had an MIC of 25% (Table 2). Bactigel appeared to be the more effective handwash as it showed an MIC less than 50% in 75% of cases as opposed to the other 2 products which showed an MIC of >50% only in 50% of cases. Generally though, MIC values were similar, falling within a 2 fold difference of each other.

All three products showed bacteriocidal activity against the test isolates, with MBC values of 50% and more noted in 11 of 12 cases.

**Table 2:** MIC of hand sanitizers against test isolates

| | *S. aureus* | | *Klebsiella pneumoniae* | | *Escherichia coli* | | *Pseudomonas aeruginosa* | |
|---|---|---|---|---|---|---|---|---|
| | MIC | MBC | MIC | MBC | MIC | MBC | MIC | MBC |
| Ebecare | 25% | 100% | 25% | 50% | 50% | 100% | 50% | 100% |
| Carex | 25% | 50% | 25% | 100% | 50% | 100% | 25% | 100% |
| Bactigel | 25% | 50% | 25% | 100% | 25% | 50% | 25% | 50% |

The impact of hand hygiene in disease prevention has been well established (Kampf and Kramer 2004, Mathur 2011). Hand sanitizing has more recently been the proscribed method of hygiene, possibly due to the higher compliance rates associated with it (Kampf and Kramer 2004) and its particular usefulness in areas lacking adequate water supply. With this increase in compliance in use of hand sanitizers, there is a need to access the efficacy of products available in the market (Nwabueze *et al.,* 2016). Over the years, *S. aureus* and *E. coli* have been documented as the two most common pathogens isolated in the clinical microbiology laboratory. These organisms are notorious for their ability to cause a wide variety of diseases, exhibit a wide repertoire of virulence factors and a high level of antibiotic resistance. Additionally, along with *K. pneumoniae* and *P. aeruginosa*, these organisms can be spread via the hands. An effective hand sanitizer therefore should, exhibit significant levels of inhibitory activity against these isolates.

This study found variable efficacy of the hand sanitizers assessed. While similar levels of inhibition were noted against both *S. aureus* and *K. pneumoniae*, one of the products was unable to inhibit the other two isolates, based on the results of the agar well diffusion technique. This variable level of activity of hand sanitizers in the market, have previously been widely reported. Sharif and Ansari, analysing the efficacy of various hand sanitizing products, noted that one of their products was only effective against 6.5% of the isolates tested (Sharif and Ansari 2015). A more recent study carried out in Kenya (Ochwoto *et al.,* 2017) noted that 25% of tested products were effective against only 33% of the test isolates and an unspecified number were not effective against any of the test isolates at all. The Ochwoto study 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. As well as 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. These act by disrupting tissue membranes, denaturing proteins and dissolving lipids (Oke *et al.*, 2013). It therefore follows that products with higher alcohol concentrations (up to 90%) would be more effective

than products with lower alcohol concentrations (below 60%).

Similar to a previous report (Odebisi-Omokanye *et al.,* 2015), our study noted a lower level of susceptibility to all the tested products in 2 of the 3 Gram negative organisms tested. Both *S. aureus* and *K. pneumoniae* however, showed similar levels of susceptibility. In general however, results of our study showed higher effectiveness of tested products with much higher zones of inhibition than previously published (Oke *et al*., 2013, Odebisi-Omokanye *et al.,* 2015). And unlike both studies which reported a total lack of bacteriocidal activity possibly due to improper storage, all hand sanitizing products in this study exhibited bacteriocidal activity. These point at the general effectiveness of the products assayed in this study.

*Conclusion*: While the results of this study show that the products assayed have a higher efficacy than other previously studied products in Nigeria, not all products tested were active against all the test organisms using the dilution method. More stringent checks of products introduced into the Nigerian market may therefore be necessary to ensure that they meet set international standards both in composition of inhibitory substance and texture to ensure uniformity in activity against pathogens.

## REFERENCES

Pittet, D; Allegranzi, B; Sax, H; Dharan, S; Pessoa-Silva, CL; Donaldson, L; Boyce, JM (2006). Evidence-based model for hand transmission during patient care and the role of improved practices. *The Lancet Infectious Diseases*. 6(10): 641 – 652.

Zapka, C; Leff, J; Henley, J; Tittl, J; De Nardo, E; Butler, M; Griggs, R; Fierer, N; Edmonds-Wilson, S (2017). Comparison of Standard Culture-Based Method to Culture-Independent Method for Evaluation of Hygiene Effects on the Hand Microbiome. *mBio*. 8(2): e00093-17.

World Health Organization (2009). *WHO guidelines on hand hygiene in health care. First Global Patient Safety Challenge*. Clean Care is Safer Care. Geneva: WHO.

Pires, D; Tartari, E; Bellissimo-Rodrigues, F; Pittet, D (2017). Why language matters: a tour through hand hygiene literature. *Antimicrobial Resistance & Infection Control*. 6(1): 65.

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

Centers for Disease Control and Prevention (2002). 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.

Pickering, AJ; Boehm, AB; Mwanjali, M; Davis, J (2010). Efficacy of waterless hand hygiene compared with handwashing with soap: a field study in Dar es Salaam, Tanzania. *The American Journal of Tropical Medicine and Hygiene*. 82(2): 270 – 278.

Reynolds, SA; Levy, F; Walker, ES (2006). Hand sanitizer alert. *Emerging Infectious Diseases*. 12(3): 527 – 529.

Meadows, E; Le Saux, N (2004). A systematic review of the effectiveness of antimicrobial rinse-free hand sanitizers for prevention of illness-related absenteeism in elementary school children. *BMC Public Health.* 4(1): 50.

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

Nwabueze, SA; Amah, CC; Azuike, EC; Anene, JO; Kadiri-Eneh, NP; Anameje, OA; Akudu, AC (2016). Ebola viral disease prevention: Perception of secondary school students in two districts in Anambra State, Nigeria. *Issues in Scientific Research.* 1(1): 1 – 9.

Odebisi-Omokanye, MB; Ahmed El-Imam, AM; Arshad, SO; Oke, MA (2015). Comparative Assessment of Antibacterial Efficacy of four popular hand sanitizers sold in Nigeria. *Fountain Journal of Natural and Applied Sciences*. 4(1): 1 – 9.

Ogoina, D; Oyeyemi, AS; Ayah, O; Midia, A; Olomo, WT; Kunle-Olowu, OE (2016). Preparation and Response to the 2014 Ebola Virus Disease Epidemic in Nigeria—The Experience of a Tertiary Hospital in Nigeria. *PloS One*. 11(10): e0165271.

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

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

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

Mathur P (2011). Hand hygiene: back to the basics of infection control. *The Indian Journal of Medical Research*. 134(5): 611 – 620.

Sharif, M; Ansari, F (2015). Hand Sanitizers: Efficiency against Microbes from Currency Notes and Coins in Local Circulation. *Pakistan Journal of Molecular Medicine*. 2(2): 75 – 83.

Ochwoto, M; Muita, L; Talaam, K; Wanjala, C; Ogeto, F; Wachira, F; Osman, S; Kimotho, J; Ndegwa, L (2017). Anti-bacterial efficacy of alcoholic hand rubs in the Kenyan market, 2015. *Antimicrobial Resistance & Infection Control*. 6(1): 17.

Oke, MA; Bello, AB; Odebisi, MB; El-Imam, AA; Kazeem, MO (2013). Evaluation of antibacterial efficacy of some alcohol-based hand sanitizers sold in Ilorin (North-Central Nigeria*). Ife Journal of Science*. 15(1): 111 – 117.