Evaluation of antibacterial properties of various hand sanitizers wipes used for cosmetic and hand hygiene purposes in Nigeria

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ABSTRACT

Hand sanitizer wipe is formulated and used to reduce bacterial load on the skin but there have been no previous studies on the effectiveness of this product. In this study, the antibacterial activity of selected hand sanitizers was evaluated using three methods and ten wipes commonly used in Nigeria in a volunteer study. EDN® inhibited all the microorganisms tested with highest and least zones of inhibition against Staphylococcus aureus and Enterococcus faecalis, respectively. NYC® also inhibited all the organisms tested except Pseudomonas aeruginosa. All the organisms were resistant to DVN®, FTR® and ACS® wipes. Using the in situ spread method DRB®, DVN®, FTR®, ACS® and TAF® did not inhibit the growth of the microorganisms. Salmonella typhi was inhibited by three of the ten wipes whereas all the ten products showed decrease in bacterial loads although EDN® was the most effective of the tested wipes. This study shows that the wipes have to be complemented by other types of hand disinfection to achieve effective hand hygiene.

Keywords: Sanitizers, pathogens, wipes, hygiene, antibacterial, hand washing.

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INTRODUCTION

Hand washing and hand hygiene are terms that are often used interchangeably in the general public and among healthcare workers (HCWs). Hand hygiene is a means of making hands free of pathogens in particular either by using water always (accompanied by soap), hand rub or waterless sanitizers (Busari et al., 2012). Chemicals used in hand hygiene exhibit bactericidal or bacteriostatic properties depending on their concentrations (Boyce and Pittet, 2002; McDonald, 2003; David and Famurewa, 2006).

Alcohol is used as the main antibacterial component of most waterless antiseptic agents due to its antimicrobial properties (Boyce and Pittet, 2002). Some alcohol-based waterless hand sanitizers (WHS) have been reported to kill up to 99.9% of organisms within 15 s of application (Larson, 1995; Aiello and Larson, 2002); however, they do not possess residual antimicrobial activity due to their high volatility (Boyce and Pittet, 2002). Ethanol has the record of being the oldest skin disinfectant; it acts as a permeation enhancer when applied topically to human skin. However, it has been reported to induce irritation in addition to its carcinogenic properties (Lachenmeier, 2008). Hand sanitizer wipes are not effective when hands are visibly soiled or heavily contaminated (Dharan et al., 2003).

Hand sanitizers are well-adapted to the skin (Boyce, 2000; Pedersen et al., 2005a) and work by stripping away the outer layer of oil on the skin and also remove the cutaneous microflora (Axel et al., 2002). Hand washing and/or the use of hand sanitizer remains the major way of breaking transmission of infection (Pedersen et al., 2005b; CDC, 2009; Alex-Hart and Opara, 2011; Omogbai et al., 2011). Different antimicrobial agents have been incorporated into WHS to increase its performance...
(Matthieu, 2009). WHS has been reported not only to reduce hand bacterial contamination but also enhance hand hygiene compliance among HCWs (Fendler et al., 2002; Kampf et al., 2002; Mody et al., 2003). Despite the widespread use of WHS in Nigeria there is dearth of information in the open scientific literature that could substantiate its use. Therefore, this study was aimed at investigating the antibacterial activity of commonly used WHSs in Nigeria on some selected common pathogenic bacteria.

**MATERIALS AND METHODS**

**Sources of wipes and test organisms**

Fifty different wipes used for hand hygiene from ten different manufacturers were purchased from different supermarkets in Ado-Ekiti, Nigeria. The wipes included the following: ACS® , DVN®, DRB®, EDN®, FTR®, JHS®, LND®, LFC®, NYC® and TAF® as shown in Table 1. They were collected and stored at room temperature in the laboratory. None of the products was sealed or had expired at the time of this investigation but they were all certified by the National Agency for Food and Drug Administration and Control (NAFDAC). The test organisms used were clinical isolates obtained from the Department of Microbiology, Ekiti State University, Ado-Ekiti and Nigeria. They included: *Enterococcus faecalis*, *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Salmonella Typhi*, *Serratia marcescens* and *Staphylococcus aureus*. The organisms were grown in nutrient broth at 37°C for 18 h with shaking. The inoculum was standardized to 0.1 optical density (0.5 McFarland standard) at wavelength of 620 nm.

**Determination of in-use potency of hand sanitizer wipes**

The modified method of David (2009) was used to determine the in-use potency of the wipes. Briefly, twenty subjects without clinical evidence of dermatoses, chapping of hands or forearms of volunteers were chosen. The subjects confirmed not to have used any of the products nor be on antibiotic medication for at least two weeks prior to the test. Informed consent was obtained from the subjects that participated in this study. Two areas of 4 cm² were swabbed on the hands of subjects after washing with sterile distilled water only and after using sanitizers respectively, inoculated onto trypticase soy agar, incubated at 30°C for 48 h and examined for growth. This temperature was chosen for incubation to ensure detection of both normal aerobic skin microflora and slow growing bacteria from the environment that required a lower incubation temperature. Percentage reduction in the bacterial load was calculated as % R = \[ \frac{BAW - BBW}{BBW} \times 100 \]; where BAW is bacterial load after wipe use and BBW is bacterial load after hand wash.

**Assessment of antibacterial potency of sanitizers wipes**

The assessment of the bacterial potency was carried out using the disc diffusion method according to Oloke (2000) with a slight modification. Briefly, the test organisms were revived in Mueller-Hilton broth and incubated for 24 h at 37°C. The organisms were standardized as described earlier and seeded onto Mueller-Hilton agar after which a 6 mm disc of each of the wipes was placed on the agar at equidistance to each other. The plates were incubated at 37°C for 24 h. The average width (W) of the zone of inhibition on either side of the wipe disc was calculated as follows: \( W = \frac{T-D}{2} \), where T is total diameter of wipes and clear zone (in mm) and D is diameter of the wipes (in mm). Sterile wipe without biocide was used as control.

**Table 1. Chemical composition of different wipe screened for antibacterial activity.**

<table>
<thead>
<tr>
<th>Wipes name</th>
<th>Chemical composition</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACS®</td>
<td>Aloe vera gel, acetate, propylene glycol, polypropylene, methylparaben, benzalkonium chloride.</td>
</tr>
<tr>
<td>DVN®</td>
<td>Aloe vera gel, vitamin, ethyl acetate, propylene glycol, propyl paraben, methyl paraben, benzalkonium chloride.</td>
</tr>
<tr>
<td>DRB®</td>
<td>Propylene glycol, quaternary ammonium salt, cortexamipropyl bentine, phenoxyethanol, methylparaben, propyl paraben, ethyl paraben.</td>
</tr>
<tr>
<td>EDN®</td>
<td>Glycerin, cocomidorpropyl betaine, quaternary ammonium salt, methyl chloroisothiazoline, methyl isothiazoline, lanoline, tea tree oil.</td>
</tr>
<tr>
<td>FTR®</td>
<td>Propylene glycol, polysorbate 20, Disodium cocoaphodiacetate, 2-Bromo-2-nitropropane-1,3-diolcitric, methylchloroisothiazolinone and methylisothiazolinone.</td>
</tr>
<tr>
<td>JHS®</td>
<td>Disodium, Lauroamphodiacet e, methylparaben, methyl chloroisothiazoline and methylisothiazolinone.</td>
</tr>
<tr>
<td>LND®</td>
<td>Propylene glycol, polysorbate 20, Disodium cocoaphodiacetate and sodium lanrethsulfate, citric acid, acetate, methylchloroisothiazolinone, methylisothiazolinone and benzene methanol, 2-Bromo-2-nitropropane-1,3-diol.</td>
</tr>
<tr>
<td>LFC®</td>
<td>Potassium sorbate, potassium benzoate, tridosodium EDTA, triclosan, citric acid, lanolin, aloe vera.</td>
</tr>
<tr>
<td>NYC®</td>
<td>Propylene glycol, polysorbate 20, sodiumcocoamphoacetate, phenoxyethanol, methylparaben, 2-Bromo-Nitropropane-1,3-aloe-barbadensis, citric acid.</td>
</tr>
<tr>
<td>TAF®</td>
<td>Potassiumsorbate, potassiumbenzoate, trisodium EDTA, triclosan, citric acid, lanolin, aloe vera.</td>
</tr>
</tbody>
</table>
Figure 1. In use potency of selected wipes against normal bacterial flora. Key: STP = wipes without biocides.

Table 2. Qualitative antibacterial effect of the different wipes.

<table>
<thead>
<tr>
<th>Isolates</th>
<th>Hand sanitizers/zones of inhibition (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>EDN</td>
</tr>
<tr>
<td>S. aureus</td>
<td>17.0 ± 2.2</td>
</tr>
<tr>
<td>E. coli</td>
<td>14.0 ± 3.1</td>
</tr>
<tr>
<td>P. aeruginosa</td>
<td>10.0 ± 2.0</td>
</tr>
<tr>
<td>K. pneumonia</td>
<td>13.0 ± 3.7</td>
</tr>
<tr>
<td>Salmonella typhi</td>
<td>15.0 ± 2.3</td>
</tr>
<tr>
<td>E. faecalis</td>
<td>9.0 ± 1.5</td>
</tr>
<tr>
<td>S. marcescens</td>
<td>10.0 ± 1.6</td>
</tr>
</tbody>
</table>

Data is the mean of three determinations.

Determination of in situ antibacterial effect of sanitizer wipes

Antibacterial activity of sanitizer wipes was determined in situ by gently spreading 10 µl of bacterial suspension containing $5.0 \times 10^6$ cfu/ml on a ply of wipe and immediately covered with another from the same wipe. The inoculated wipes were incubated at 37°C for 24 h. Thereafter, the seeded plies of the wipes were placed in inverted position on a Mueller-Hinton agar and carefully removed with sterile forceps after 2 h. The plate was later incubated at 37°C for another 18 h. The number of colonies that developed was counted and recorded.

RESULTS

Figure 1 shows the microbial load on the hand after washing with sterile distilled water. For EDN®, there was no growth after wiping of hand indicating possession of effective antibacterial property while the performance of ACS® was comparatively very poor.

The antibacterial properties of the different wipes using the disc method are shown in Table 2. In all the brands of wipes examined, EDN® was the most potent inhibiting all the bacteria tested with the zone of inhibition ranging from 9.0 ± 1.5 to 17.0 ± 2.2 mm. EDN was most effective against S. aureus with the widest zone (17.0 ± 2.2 mm) while it showed the poorest activity against E. faecalis (9.0 ± 1.5 mm). NYC inhibited all the test bacteria except E. coli and P. aeruginosa, following EDN in activity. JHS, LND, LFC and TAF were each effective against three of the test bacteria, while DRB was effective against only one organism (S. marcescens). In contrast, all the
organisms grew in the presence of DVN®, FTR® and ACS® and were therefore considered resistant to the products indicating lack of activity of the wipes.

The antibacterial effect of the different wipes is presented in Table 3. The wipes had varied degree of effects on the pathogens. There was no growth observed when the bacteria were tested against EDN® indicating that the wipe inhibited the growth of all the test organisms. Salmonella typhi was inhibited by four of the ten wipes tested whereas EDN® and NYC® were the only wipes that inhibited P. aeruginosa. These results show that only EDN® had substantial or absolute effect on the test organisms after contact.

**DISCUSSION**

As the skin protects the internal organs and tissues, it also harbours microorganisms ranging from normal flora to pathogenic species (Cogen et al., 2008). Human skin provides nutrients and suitable growth conditions for most pathogens as well as opportunistic bacterial pathogens and these bacteria evidently have the ability to be resistant to most of the cleaning regimen, thus contributing to their persistence in an ecosystem (CDC, 2008). The use of wipes is one of the means of reducing the bacterial load on the surface of the skin; hence reducing the pathogen load especially on the skin.

Comparatively, the wipes reduced the bacterial load on the hands of the subjects than the controls. From the results obtained in this study, there is an indication that the reduction in the bacterial load may not just be ascribed to the mechanical action of the wipes on the surface of the skin but could probably be due more to the action of the biocides (active ingredients) present in the wipe products examined.

Of the ten wipes investigated in this study, EDN® was the most effective in the inhibition of the growth of the test bacteria followed by NYC®. The skin is the largest organ in the human body (Cevc and Vierl, 2010) with its structure and physiology varying from person to person due essentially to genetic, physiological and environmental factors (Giacomoni et al., 2009; Cevc and Vierl, 2010). The results of this study further confirm the antibacterial activity of the wipes that are commonly used in the study area. EDN® evidently had the highest zones of inhibition and hence was the most effective on the test pathogens.

Compliance to hand hygiene is recognized to break the cycle of transmission both in the hospital and at home (Pessoa-Silva et al., 2007; Burnett, 2009; Nicol et al., 2009). Application of wipes could therefore, be very useful and accepted to play a significant role in the health care facilities and households, as it reduces the bio-burden on the hand and as well as prevents irritation due to constant hand washing. The in situ assessment of the wipes, using the spread method, showed a complete inhibition of the test bacteria by Eden®. The usage of the wipes could prevent infection especially salmonellosis as four of the ten wipes tested inhibited the growth of the organism, which is the causative agent of typhoid fever.

The high activity of EDN® and its superiority over the other wipe brands observed in this study may most probably be ascribed to the chemical components present, especially the tea tree oil which was absent in the others wipes. Tea tree oil has been reported to have strong antibacterial activity against pathogens such as methicillin-resistant *S. aureus* (Carson et al., 2002) and other pathogenic bacteria (Sikkema et al., 1995; Chan and Loudon, 1998; Hada et al., 2003).

Some of the wipes examined did not inhibit the growth of the test bacteria probably as a result of low concentrations or lack of biocides in them and or non-compliance to stringent condition (good manufacturing practices) during production among other reasons. Gross contamination of hand sanitizer wipes during manufacturing may also compromise their effectiveness and or quality and possibly lead to infection of the users eventually (Voss and Widmer, 1997).

Conclusively, wipes could be used for cleaning of hands and/or any other part of the skin/body surface as they characteristically reduce microbial load. The use could also aid compliance to hand hygiene both at home and in the health care facility since they do not easily

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### Table 3. In situ antibacterial effect of the different sanitizers tested against selected organisms.

<table>
<thead>
<tr>
<th>Isolates</th>
<th>Hand sanitizers/cfu</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>EDN</td>
</tr>
<tr>
<td><em>S. aureus</em></td>
<td>NG</td>
</tr>
<tr>
<td><em>E. coli</em></td>
<td>NG</td>
</tr>
<tr>
<td><em>P. aeruginosa</em></td>
<td>NG</td>
</tr>
<tr>
<td><em>K. pneumoniae</em></td>
<td>NG</td>
</tr>
<tr>
<td><em>Salmonella typhi</em></td>
<td>NG</td>
</tr>
<tr>
<td><em>E. faecalis</em></td>
<td>NG</td>
</tr>
<tr>
<td><em>S. marcescens</em></td>
<td>NG</td>
</tr>
</tbody>
</table>

Key: NG = No Growth
cause skin irritation and or dryness. From this study, wipes are shown to be relevant for cosmetic purposes (complementary) and not alternatives to hand washing with soap and water. Manufacturers should therefore, maintain the quality of their products in order to avoid or prevent false safety assurance.

REFERENCES


