Comparative Studies on the Antibacterial Activity of Alcohol-Based Hand Sanitizers Against Bacteria Isolates from the Hands of Undergraduate Students of University of Agriculture, Makurdi

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Abstract

The study identified the species of bacteria on the hands of undergraduate students of University of Agriculture, Makurdi. Susceptibility tests of the isolates to 5 different alcohol-based hand sanitizers (Lovilee®, Dettol®, Passion®, Y-Senses® and My care®) was done. 50 Hand swabs taken from the hands of male and female students were analyzed microbiologically. The most prevalent bacteria isolated were E. coli 47(94%), Staphylococcus epidermidis 42(84%), Proteus sp 32(64%), Klebsiella sp 31(62%), Shigella sp 25(50%), Staphylococcus aureus 18(36%), Pseudomonas aeruginosa and Salmonella typhi 15(30%) and Enterobacter aerogenes 11(22%) from males and females. Lovilee® and Y senses® hand sanitizers showed better efficacy against the isolates. Passion® sanitizer inhibited the growth of nine isolates with the highest zone of inhibition against Pseudomonas aeruginosa and Staphylococcus epidermidis (10 mm) and least against Salmonella sp. Dettol® inhibited growth E. coli (10 mm) and the least against S. aureus, P. aeruginosa and S. epidermidis were resistant. My care® hand sanitizer showed the least antimicrobial activity inhibited the growth of only 3 isolates, with the highest zone of inhibition against S. aureus and Enterobacter aerogenes (5 mm); E. coli, S. pyogenes, Salmonella sp, Klebsiella sp, Proteus sp, Pseudomonas spp, S. epidermidis were all resistant. The minimum inhibitory concentration (MIC) of the susceptible organisms was (0.5 ml) on Salmonella sp, E. aerogenes, Klebsiella sp and S. epidermidis. There was no significant difference in the antibacterial activity of the sanitizers.

Keywords: Cross-contamination; Sanitizers; Hygiene; Resistance

Introduction

Bacteria are prokaryotic heterogeneous group of unicellular organisms that possess a rigid cell that determines their shape as coccoid (spherical), bacillary (rod shaped), helical or common shaped. They are found almost everywhere in the environment such as air, soil, water, sewage, human body, wounds and other solid surfaces. Some are beneficial in the body and others may cause problems. Normal flora such as the Staphylococcus epidermidis produces antibiotics on the skin for protection against infection meanwhile the transient one acquired from the environment can be pathogenic [1].

There are two types of normal flora on the skin, transient flora which are contacted from the external environment and the resident flora which is permanently found on the skin [2]. Bacteria associated with the hands are termed micro flora of the hands which include Propionibacterium, Staphylococcus and others which could be reduced depending on personal hygiene and environment of humans [3]. In 1847 Dr. Semmelwies Ignaz established a link between infection and unclean hands and demonstrated that washing could reduce transmission of puerperal fever (child birth fever) a dreaded disease which had high mortality previously [4].

The human hands are the parts of human body that are mostly in contact with the outside world. People use their hands for a variety of activities every day. It is extremely easy to meet different microbes and transfer them to other objects like door knobs, pen, pencils, seats and even people. Surprisingly finger nails harbor the most bacteria found on the human hands. Pupils can contaminate their own food by playing with sand, eating with hands unwashed, poor hygienic practices like sucking finger, not washing hands after using the toilets. The hands of a person may get contaminated with Staphylococcus aureus either by contact with genital areas, nose, toilet doors, playing with sand etc [5,6]. Also, long nails of pupils tend to harbor more microorganisms than short nails [7]. Artificial nails harbor greater quantities of pathogenic organisms on its surface than the surface of native nails, these include S. aureus, Acinetobacter baumanii, E. cloacae, E. agglomerans, Klebsiella oxytoca, Pseudomonas aeruginosa, Aeromonas hydrophilia and Gram-negative bacilli [8].

Several studies have shown the ability of bacteria to survive on hands for differing times. Musa and colleagues demonstrated in a laboratory study that Acinetobacter calcoaceticus survived better than strains of A. lwoffi at 60 minutes after in inoculums of 10⁶ cfu/finger [9]. A similar study by Fryklund and colleagues using epidemic and non-epidemic strains of Escherichia coli and Klebsiella spp. showed a 50% killing to be achieved at 6 minutes, respectively [10]. No skin and colleagues studied the survival of bacteria on hands and the environment: Both Enterococcus faecalis and E. faecium survived for at least 60 minutes on gloved and ungloved fingertip [11]. Furthermore, Doring and colleagues showed that Pseudomonas aeruginosa and Burkholderia cepacia were transmitted for up to 30 minutes when the organisms were suspended in saline and up to 180 minutes when they were suspended in sputum [12]. Shigella dysenteriae type 1 have the capacity to survive on hands for up to 1 hour [13]. Health care workers with psoriatic dermatitis remained colonized with Serratia marcescens for more than three months [14].

Several studies previously undertaken have established that the hands of undergraduate students harbor different types of pathogenic and non-pathogenic bacteria (normal flora which may become opportunistic). For instance [15] undertook a study in Amravati...
University reported high prevalence of bacterial pathogenic isolates from the hands of students due to poor hygiene, games and contact with contaminated surfaces among others. The research findings posited that students don’t wash their hands often well and they play indoor as well as outdoor games and always meet contaminated surfaces and dirt or soil. The study isolated a variety of bacterial species were isolated from the hands of 400 students; *Staphylococcus* sp 135(23%), *E. coli* 121(20%), *Klebsiella* 61(10%), *Micrococcus* sp 52(9%), *Proteus* sp 45(7%), *Citrobacter* sp 42(7%), *Streptococcus* sp 40(7%), *Enterobacter* sp 37(6%), *Enterococcus* sp 27(4%), *Pseudomonas* sp 17(3%), and *Salmonella* sp 13(2%).

Alcohol- based hand sanitizers are antiseptic products used to prevent transmission of pathogens. They may be in liquid, foam or easy flowing gels with varying concentration or level of alcohol ranging between 60% and 95% [16]. They do not require rinsing with water but can be spread over the surface of hands and rubbed until dry [17]. Health care setting prefer hand sanitizers to hand washing with soap and water [18] because it is more effective at killing microorganisms and better tolerated when compared to the use of soap and water [19]. Alcohol based sanitizers show antimicrobial activity against a variety of microorganisms except the spore formers and has been used as an antiseptic at least as early as 1363 though its use became evident in the 1800’s. The evidence that alcohol-based hand sanitizers are effective can be supported by its early use in Europe since the 1890’s and it is recommended by the world health organization [20].

Alcohol-based sanitizer can be used by applying the product to the palm of one hand; rubbing hands together and over the surface of hands and fingers until hands are dry [21]. Alcohol rubs kill different kinds of antibiotic resistant bacteria, tuberculosis bacteria and percentage of 90 kills HIV, flu virus and the common cold virus [22]. 90% alcohol rubs are more effective against most viruses compared to other forms of hand hygiene like hand washing [23]. Isopropyl alcohol kills 99.99 percent or more of all non-spore forming bacteria in less than 30 seconds whether in the laboratory or even on human skin Alcohol rubs/sanitizers with 70% concentration of alcohol (ethyl alcohol) kills 99.99% of the bacteria on hands in 30 second after application and 99.99% to 99.999% in one minute [23].

The reason the use of alcohol- based hand sanitizers is preferred to hand washing is that, apart from killing microorganism it dries skin less, leaving more moisture in the epidermis compared to hand washing [24]. Though alcohol may strip the skin of the outer layer of oil, disrupting the barrier function of the skin [25], previous research has shown that it does not eliminate good microorganism naturally present on the skin, but the body replenishes the good microorganisms on the hands quickly, often moving them in from just up the arms where there are fewer harmful microorganisms [26]. Sanitizers are effective against bacteria though may not have the 99.99% germ killing activity as claimed by the manufacturers. A study by Ikekbumun and colleagues in Nnamdi Azikiwe University Awka, Nigeria Indicated that Dettol ® hand sanitizer demonstrated antimicrobial activity against *Staphylococcus aureus* and little inhibition of *E. coli*, *Pseudomonas aeruginosa* and *Streptococcus pneumoniae*. The findings showed decreased activity of sanitizers against bacteria as its concentration is decreased by dilution [27]. In the same vein, a study conducted by Mc Neil and colleagues among health care workers revealed that hand sanitizers proved effective against *S. aureus* isolated from hands and nails of HCWs, reducing it from 28% to 8% after its use [28]. Also, a study by Enwa and colleagues in Delta state University, Nigeria on the comparative activity of alcohol-based hand sanitizers (Dettol® and lovillea®,), dettol antiseptic and toilet soaps against bacterial isolates (*Staphylococcus aureus*, *Staphylococcus epidermidis*, *Streptococcus* species and *Shigella* species). Dettol antiseptic demonstrated the greatest antimicrobial activity, followed by the sanitizers and lastly the soaps (Lux and premier). For the minimum inhibitory concentration, the two hand sanitizers inhibited the growth of the four test organisms at 2.0 ml [29].

The aim of the study was to compare the antibacterial activity of different brands of alcohol-based hand sanitizers (Lovillea®, Dettol®, My care®, Passion® and Y senses®) against bacteria isolated from the hands of undergraduate students of Federal University of Agriculture Makurdi.

The objective of the study was:

- i. To isolate and identify the different bacterial species associated with students’ hands using morphological and biochemical approaches.
- ii. To compare the antibacterial activity of five (5) different alcohol- based hand sanitizers (Lovillea®, Dettol®, My care®, Y- senses®, and Passion®) against the bacteria isolates from hands of the students.

Materials and Methods

The study was carried out in Federal University of Agriculture, Makurdi, Benue state Nigeria. A total of 50 hand swabs sticks consisting of 50% [25] females and 50% [25] males were collected from hands of undergraduate students of Federal University of Agriculture. A sterile swab stick moistened or damped with 0.85% saline water was used to swab both hands of the students beginning from the flexor aspect of wrist, across the palm and up all the 5 fingers (beginning with thumb); including the creases and nail beds ending in the dorsal aspect and then the stick replaced in to the tube [30]. The samples were transported to the Biology Laboratory, Department of Biological Sciences of the Federal University of Agriculture, Makurdi (immediately) between 1-2 hours for examination.

Cultural and morphological characterizations of the bacterial isolates

A Loopful of discrete colonies on nutrient agar (oxoid) medium were selected and aseptically sub cultured on differential media. The inoculated plates were incubated at 37°C for 24 hours after which their cultural characteristics were observed and recorded. Discrete suspected colonies were further subjected to Gram staining to characterize their morphology [30].

Bacterial susceptibility/sensitivity to hand sanitizer

Muller-Hinton agar was prepared according to manufacturer’s specifications, sterilized, cooled, then 20 ml of each poured into Petri dishes and kept for 45 minutes to allow it to solidify. Thereafter, the test organisms aseptically inoculated into different properly labeled Petri dishes containing already solidified Muller -hinton agar by using different sterile swab sticks to pick the organisms from prepared overnight broth and streaking the organisms all over the Petri dishes [29]. A 5 mm corn borer was used to bore holes in the solidified agar on each Petri dish. Using a 2 ml syringe, few drops each of the hand sanitizers was added to their respective holes in the Petri dish. After 5 minutes all the Petri dishes were carefully packed with a masking tape and transferred into the incubator for 24 hours at 37°C. Zones of were observed and recorded after 24 hours [29].
Minimum inhibitory concentration

The determination of the minimum inhibitory concentration of the hand sanitizers was carried out to know the volumes of hand sanitizers that enhance their effectiveness.

The agar dilution technique was as follows: 15 ml of sterilized Muller-hinton agar was poured with 0.5 ml, 1 ml, 1.5 ml and 2 ml respectively of the 5 different alcohol-based hand sanitizers being tested into different Petri dishes. The mixture was swirled gently and allowed to solidify, then the test organisms were aseptically streaked onto the different prepared plates seeded with the hand sanitizers using a flamed wire loop and then incubated at 37°C for 24 hours after which the least volume of the hand sanitizers that inhibited the growth of the test organisms was observed and tabulated [29].

Results

Fifty 50 samples collected from the hands of 25 male and 25 female undergraduate students of University of Agriculture, Makurdi, were analyzed microbiologically. Table 1 shows the distribution of bacterial isolates from the hands of students according to gender. E. coli had the highest frequency of 23(93%) and 24(96%) in male and female students respectively. Staphylococcus pyogenes and Pseudomonas aeruginosa both with a frequency of 5(20%) being the least prevalent in male while Salmonella typhi with a frequency of 4(16%) was the lowest of the isolates in the females.

Table 2 shows the susceptibility pattern of the isolates to the different hand sanitizers as well as the ANOVA Dettol® sanitizer inhibited the growth E. coli and Enterobacter sp with a zone of inhibition of 10 mm, Proteus sp and E. coli (5 mm), Shigella sp (4 mm), Salmonella typhi (2 mm), S. pyogenes and Klebsiella sp (1 mm) whereas S. aureus, P. aeruginosa and S. epidermidis were resistant. Lovillea® was more effective against Enterobacter aerogenes (10 mm), Proteus sp and E. coli (6 mm), Salmonella sp, Klebsiella sp, P. aeruginosa and S. epidermidis (2 mm) and least effective against Shigella and S. pyogenes (1 mm). My care® hand sanitizer had the following zones of inhibition; 5 mm, 5 mm and 2 mm against S. aureus, E. aerogenes and Shigella spp respectively whereas E. coli, S. pyogenes, Salmonella sp, Klebsiella sp, Proteus sp, P. aeruginosa and S. epidermidis were all resistant. Passion® hand sanitizer gave the following zones of inhibition; 2 mm, 5 mm, 2 mm, 6 mm, 8 mm, 1 mm, 7 mm and 10 mm against E. coli, S. pyogenes, E. aerogenes, Shigella sp, Klebsiella sp, Proteus sp, P. aeruginosa, and S. epidermidis respectively, least against Salmonella sp which proved resistant. Senses® hand sanitizers inhibited the growth of all isolates with the following zones of inhibition 15 mm (S. aureus, S. Pyogenes and Klebsiella sp) being the highest, 10 mm (E. coli, Salmonella sp, E. aerogenes, Proteus sp), 8 mm (S. epidermidis) being the lowest. Statistical analysis showed no significant difference in the activity of the sanitizers against the various isolates (p>0.05).

Table 3 represents the mean effect of each of the sanitizers against the bacteria isolates. This reveals that there was a significant difference between some sanitizers in their activity against the various isolates. Means with the same letter are not significantly different but means with different letters and widely apart implying a significant difference between the activity of the sanitizers against the isolates. As shown in the Table, the mean effect of senses® (29.25) against E. coli is significantly different from that of Passion® (7.75), Dettol® (2.50), Lovillea® (2.50), and My care® (0.00) but there was no significant difference between the mean effect of Dettol® and Lovillea®. Key: Means with the same letter are not significantly different.

Tables 4 and 5 shows the interaction effect of different brands of hand sanitizers and different volumes on bacterial isolates. For minimum inhibitory concentration, Dettol® and Lovillea® hand sanitizers inhibited the growth of S. aureus, S. pyogenes, S. typhi, E. aerogenes, Shigella sp, Klebsiella sp, Proteus sp, P. aeruginosa and S. epidermidis at 2.0 ml. My care® hand sanitizer inhibited the growth of S. typhi, E. aerogenes, Shigella sp, Klebsiella sp and S. epidermidis at 0.5 ml, S. aureus at 1.0 ml whereas Proteus sp and Pseudomonas were resistant even at 2.0 ml. Passion® hand sanitizer inhibited all the

<table>
<thead>
<tr>
<th>Sex</th>
<th>N (%)</th>
<th>X</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>25(100)</td>
<td>25(100)</td>
</tr>
<tr>
<td>Females</td>
<td>25(100)</td>
<td>25(100)</td>
</tr>
</tbody>
</table>

Key: N: number of samples

Table 1: Distribution of bacterial isolates according to sex.

<table>
<thead>
<tr>
<th>sanitizers</th>
<th>E. coli</th>
<th>S. aureus</th>
<th>S. pyogenes</th>
<th>Salmonella sp</th>
<th>E. aerogenes</th>
<th>Shigella sp</th>
<th>Klebsiella sp</th>
<th>Proteus sp</th>
<th>P. aeruginosa</th>
<th>S. epidermidis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dettol®</td>
<td>10</td>
<td>--</td>
<td>1</td>
<td>2</td>
<td>10</td>
<td>4</td>
<td>1</td>
<td>5</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>Lovillea®</td>
<td>5</td>
<td>5</td>
<td>1</td>
<td>2</td>
<td>10</td>
<td>1</td>
<td>2</td>
<td>6</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>My care®</td>
<td>--</td>
<td>5</td>
<td>--</td>
<td>--</td>
<td>5</td>
<td>2</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>Passion®</td>
<td>2</td>
<td>5</td>
<td>2</td>
<td>--</td>
<td>6</td>
<td>8</td>
<td>1</td>
<td>7</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Senses®</td>
<td>10</td>
<td>15</td>
<td>15</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>5</td>
<td>15</td>
<td>10</td>
<td>5</td>
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<tr>
<td>LSD (0.05)</td>
<td>0.49</td>
<td>0.56</td>
<td>0.49</td>
<td>0.59</td>
<td>0.91</td>
<td>0.57</td>
<td>0.62</td>
<td>0.66</td>
<td>0.53</td>
<td>0.54</td>
</tr>
<tr>
<td>CV (%)</td>
<td>7.84</td>
<td>5.06</td>
<td>4.56</td>
<td>5.31</td>
<td>7.50</td>
<td>5.94</td>
<td>5.97</td>
<td>8.00</td>
<td>6.26</td>
<td>3.26</td>
</tr>
</tbody>
</table>

LSD=least significant difference at 5% level of probability; CV(%)=coefficient of variation®=Trade name Zones of inhibition are in mm.

Table 2: Sensitivity/susceptibility of bacterial isolates to different hand sanitizers.
isolates at 0.5 ml except *P. aeruginosa* which was inhibited at 1.0 ml. Y- senses® hand sanitizer inhibited the growth of *E. coli*, *S. aureus*, *S. pyogenes*, *S. typhi*, *E. aerogenes*, *Klebsiella sp*, *P. aeruginosa*, and *S. epidermidis* at 0.5 ml, *Proteus sp* at 1.5 ml whereas *Shigella sp* was resistant even at 2.0 ml.

### Discussion

Bacteria isolated from the hands of the undergraduate students of University of Agriculture Makurdi include both normal flora and transient species as also stated by Jackson [2]. The dominant species
identified includes E, coli, S. epidermidis, Proteus sp, Klebsiella sp, Shigella sp, S. aureus, Pseudomonas aeruginosa, Salmonella typhi and Enterobacter aerogenes. According to a normal human body always harbors bacteria between 10^8-10^10 CFU/cm². The present studies showed that the hands alone fell within this range as part of the body, which is high. The hands of female students were more contaminated compared to that of male counterpart which could be attributed to factors such as artificial nails, frequent hand shaking and lack of hand hygiene facilities, contact with phones, other surfaces use of pets and the type of products used on hands. The results also corroborates the findings of Tambekar and colleagues in Amravati. In the study, S. epidermidis was more prevalent on the hands of females which agree with the findings of the transfer of bacteria from the hands to foods, objects or people promotes the spread of diseases.

Dettol® sanitizer inhibited growth of the following species: E. coli as well as Enterobacter aerogenes by 10 mm, Proteus spp 5 mm, Shigella spp 4 mm, Salmonella spp 2 mm, both Streptococcus spp and Klebsiella by 1 mm, but did not inhibit the growth Staphylococcus aureus, Pseudomonas aeruginosa and Staphylococcus epidermidis, this corroborates the findings of Tambekar, Shirsat and Surdkar [27] who showed that Dettol® sanitizer was ineffective against Staphylococcus aureus, Streptococcus and Pseudomonas. On the other hand, the result of this study does not agree with the findings of Kimura et al., [29] who showed that Dettol® hand sanitizer was effective against S. aureus, S. epidermidis with zones of inhibition of 5 mm against each of them. In the study, Dettol® inhibited growth of Shigella sp and Streptococcus with 4 mm and 1 mm zone of inhibition respectively which disagrees with the findings of [29] in which Shigella sp was inhibited by 3 mm and Streptococcus by 2 mm.

In the study, lovivella® hand sanitizer inhibited the growth of S. aureus, S. epidermidis, S. pyogenes, Shigella with the following zones of inhibition (5 mm, 2 mm, 1 mm and 1 mm) respectively. This disagrees with the findings of [29] who showed that S. aureus was inhibited with a zone of inhibition of 3 mm, S. epidermidis (4 mm), S. pyogenes (5 mm) and Shigella (2 mm) but agrees with the findings of McNeil and colleagues who revealed hand sanitizers proved effective as it inhibited the growth of all the ten isolates (E. coli, S. aureus, S. pyogenes, Salmonella sp, E. aerogenes, Shigella sp, Klebsiella sp, Proteus, P. aeruginosa, and S. epidermidis), followed by Passion® which inhibited growth of 9 of the isolates (E. coli, S. aureus, S. pyogenes, E. aerogenes, Shigella sp, Klebsiella sp, Proteus, P. aeruginosa, and S. epidermidis), then Dettol® which inhibited growth of only 7 isolates(E. coli, S. pyogenes, Salmonella sp, E. aerogenes, Shigella sp, Klebsiella sp, Proteus sp) and lastly My care® hand sanitizer which inhibited the growth of only three isolates (S. aureus, E. aerogenes,and Shigella sp).

Use of hand sanitizers can help reduce both transient and pathogenic bacteria present on the hands.

### Conclusion

Hands greatly serve as a means by of transmission of pathogens, thus effective hand hygiene can reduce infection and prevent diseases. The study reveals that the dominant species of bacteria present on the hands of undergraduate students of University of Agriculture, Makurdi are the Enterobacteria and a few Gram-positive bacteria which were more prevalent on hands of females than the males. The study revealed that lovivella® and Y- senses® hand sanitizers showed better efficacy as it inhibited the growth of all the ten isolates (E. coli, S. aureus, S. pyogenes, Salmonella sp, E. aerogenes, Shigella sp, Klebsiella sp, Proteus, P. aeruginosa, and S. epidermidis), followed by Passion® which inhibited growth of 9 of the isolates (E. coli, S. aureus, S. pyogenes, E. aerogenes, Shigella sp, Klebsiella sp, Proteus, P. aeruginosa, and S. epidermidis), then Dettol® which inhibited growth of only 7 isolates(E. coli, S. pyogenes, Salmonella sp, E. aerogenes, Shigella sp, Klebsiella sp,Proteus sp) and lastly My care® hand sanitizer which inhibited the growth of only three isolates (S. aureus, E. aerogenes,and Shigella sp).

References