EVALUATION OF ANTIBACTERIAL EFFICACY OF DIFFERENT HAND GEL SANITIZERS IN UNIVERSITY OF GONDAR STUDENTS, NORTH –WEST ETHIOPIA

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Abstract

This pilot study was designed to determine the antibacterial efficacy of different hand gel sanitizers on transient bacteria associated with the hands of Gondar university students, which is situated in North-West part of Ethiopia. In Ethiopia, accessibility of water is a problem in many parts of the country including in higher education institutes of the country, therefore hand gel sanitizer will serve as an alternative. A total of ten swab samples were collected to isolate transient bacteria \((E. \text{ coli} \text{ and } P. \text{ aeruginosa})\). Each isolate was tested in series of biochemical test (IMViC) to study its biochemical characteristics. Furthermore, gram staining was performed for each culture to observe whether the bacteria are gram negative or positive. To observe antibacterial activity Agar diffusion (Kirby-Bauer) method was performed. Out of the ten samples taken before applying Tri active and Imaj hand gel sanitizers, six of them were positive in indole and MR test and three of them were positive in citrate test. After applying Tri active hand gel sanitizer two of them were found positive in Indole and MR test while one sample was positive in citrate test. However, after applying Imaj hand gel sanitizer, four of the samples were positive for indole and MR test, and two of the samples were positive in citrate test. In this study we didn’t find any isolate gave a positive reaction for VP test. Therefore based on result of biochemical test we concluded that the isolates were \(E. \text{ coli} \text{ and } P. \text{ aeruginosa}, \) which are transient microflora associated with hands. As compared to individuals who used Tri hand gel Sanitizers, there was a significantly higher chance of being with transient microflora in individuals who didn’t use hand gel sanitizers. Furthermore, all the isolated bacteria are found to be gram negative bacilli. In Kirby-Bauer method 9 mm and 11 mm zone of inhibition was observed for Tri-active hand gel sanitizer against the isolates identified as \(E. \text{ coli} \text{ and } P. \text{ aeruginosa} \) respectively. On the other hand 6 mm and 4 mm zone of inhibition was observed for Imaj hand gel sanitizer against the isolates identified as \(E. \text{ coli} \text{ and } P. \text{ aeruginosa} \) respectively. These results indicate that Tri-active hand gel sanitizer is more effective than Imaj hand gel sanitizer, but both the sanitizers were found effective in reducing transient micro-flora on hands of University of Gondar students. This study shows the need of using these hand gel sanitizer to reduce transient micro-flora on hand of individuals living in areas where there is low access of water and poor hygiene conditions and also health workers who are directly in contact with the patients.

Key words: Hand gel sanitizer, Tri-active, Imaj, transient microflora, \(E. \text{ coli, } P. \text{ aeruginosa}\).

INTRODUCTION

Currently there are different types of hand sanitizers on the market containing ethyl alcohol (62% to 95% concentration). These sanitizers are available in different forms such as fast-drying gels, foams, wipes, and mixed with moisturizing lotions [19]. In the past few decades, several studies were performed to indicate antibacterial effect of hand gel sanitizer in different settings such as extended care facilities, schools and hospitals [6]. However, previous studies showed that not all sanitizers are equally effective in eradicating all germs [7, 9]. While some studies reported high efficacy of hand sanitizers in reducing the microbial flora of hand, other studies failed to show such efficacy of hand sanitizers [3, 8, 17, 13, 2].
Generally hand sanitizers were grouped into alcohol based and non alcohol based. Alcohol-based hand sanitizers exist in liquid, foam, and easy-flowing gel formulations. Although 62% alcohol based sanitizers were commonly used, most alcohol based hand sanitizers contain between 60% and 85% alcohol [12]. Several studies suggested that, sanitizers with at least 70% alcohol were suggested to kill 99.9% of the bacteria on hands [18]. However, the effectiveness of these sanitizers depends on the time of rubbing the sanitizer on hand. For instance, rubbing alcohol based sanitizers for 30 and 25 seconds was reported to kill 99.99% of bacteria on hand, respectively. It has been suggested that, the most important way to prevent the transmission of dangerous diseases is to frequently wash hands with soap and water. If soap and water are not available it is recommended to use a hand sanitizer containing at least 60 percent alcohol to kill pathogenic bacteria on hand [15].

In Ethiopia, accessibility of water is a problem in many parts of the country including in higher education institutes of the country [1]. This shows that, hand washing and drying could be difficult in different part of the country where there is no/low access of water. Furthermore, poor environmental hygiene and group living of students in dormitory of higher educational institutions of Ethiopia seems to increase the risk of contamination with pathogenic bacteria [9]. Although different alcohol based hand sanitizers are available in the Ethiopia. To the best of our knowledge, there is no study that compared the efficacy of the available hand sanitizers in reducing the transient flora on the hands. Therefore, the present study was designed to evaluate the efficacy of Tri-Actice and Imaj hand sanitizers in reducing the transient flora on the hands of UoG students.

MATERIAL AND METHODS

A total of 10 students belongs to University of Gondar were included in this study. Individuals willing to give swab sample from their hand, living in the dormitory of the university and able to fill the questionnaire were included in the study. A total of 10 swab samples were collected before and after using hand gel sanitizer from the hands of students using sterilized normal saline solution during April to June, 2013. The brand name of the hand gel sanitizers used in this study was Tri-Active and Imaj, product of India and Saudia Arabia respectively (Fig. 1).

The swab samples were cultured on nutrient agar media and plates were incubated at 37°C over night to isolate transient bacteria (E. coli and P. aeruginosa) from the hands of the study participants. A series of biochemical test (IMViC) was performed for each isolate to study its biochemical characteristics. Furthermore, gram staining was performed for each culture to observe whether the bacteria are gram negative or positive.

To observe antibacterial activity of each hand gel sanitizer on our isolates Agar diffusion (Kirby-Bauer) method was performed. For this study all the isolates were cultured in nutrient broth at 37°C for 48 hrs. A sterile cotton swab was dip into a suspension culture. The culture was inoculated on the surface of each Muller-Hinton agar plate, first in horizontal direction and then in vertical direction to ensure the even distribution of the organism on the agar surface, using the swab, then allowed to dry for 5 min. Sterile filter paper disks using flamed forceps was dipped into hand gel sanitizers and placed in the centre of the petri plate Each isolate was tested for antibacterial sensitivity using both the hand gel sanitizers. A disks without sanitizer was placed in the centre of the petri plate, which served as control for this experiment.

The collected data will be stored in MS excel and transported to SPSS software. Odds ratio was calculated to see the effect of sanitizers in reducing transient micro flora.

Fig. 1: Showing Tri-Active and Imaj hand gel sanitizer
RESULTS
In this study out of 10 samples isolated from the hands of University of Gondar students 6 were found positive in Indole and MR and 3 were positive for citrate test. After applying Tri active hand gel sanitizer two of them were found positive in Indole and MR test while one sample was positive in citrate test. However, after applying Imaj hand gel sanitizer, four of the samples were positive for indole and MR test, and two of the samples were positive in citrate test. In this study we didn’t find any isolate gave a positive reaction for VP test (Table 2). All of the samples were found Gram negative bacilli after Gram staining.

In Kirby-Bauer method 9 mm and 11 mm zone of inhibition was observed for Tri-active hand gel sanitizer against the isolates identified as E. coli and P. aeruginosa respectively. On the other hand 6 mm and 4 mm zone of inhibition was observed for Imaj hand gel sanitizer against the isolates identified as E. coli and P. aeruginosa respectively (Fig. 2, 3 and 4).

As compared to individuals who used Tri hand gel sanitizers, there was significantly high chance of being with transient microflora in individuals who didn’t use hand gel sanitizers (odds ratio (OR) 13.5, 95% confidence interval (95%CI) 1.19-152.21, P = 0.02) In contrast, there was no significant sanitizers and those who didn’t (OR 2.25, 95%CI 0.376-13.46, P= 0.37) (Table 1).

As can be seen from figure 1, 60 % of the study participants were positive for transient microflora in indole test before applying hand gel sanitizer. However, after applying Tri hand gel sanitizer only 10 percent of the participants were positive for transient micro flora in indole test (Fig 1). Only 40 % of individuals who used Imaj hand gel sanitizer were positive for transient miroflora in indole test (Fig 1). However, compared to individuals who did not used hand sanitizer (30%), 10 and 20 percentage of individuals who used Tri and Imaj hand gel sanitizer were positive for transient microflora in citrate test respectively (Fig 2). The results of this experiment revealed that most antibacterial products were effective at reducing bacteria (Fig. 2).

DISCUSSION
The demonstration that Tri hand sanitizer high efficacy of reducing microflora in the hand of the study participants was not in consistent with studies conducted in other parts of the world [5, 4]. For instance, a study conducted by Centre for Disease Control and Prevention [5] showed that alcohol-based hand wipes are not as effective as alcohol-based hand rubs. Furthermore, a study conducted using 30% vol/vol alcohol-impregnated wipes also reported low efficacy of alcohol based hand sanitizers in reducing microbial flora on hand. The contradictory report on the efficacy of the sanitizers could be due to low alcohol content of the sanitizers used in previous studies than the present study.

Similar to the present study, several studies reported significantly high efficacy of hand gel sanitizers in reducing micro flora on the hand of individuals in different settings [14, 11]. In a study high efficacy of isopropyl alcohol based alcoholic hand sanitizer in reducing microbial contaminants was reported [14]. This study also provided strong evidence that alcohol based hand sanitizers have high efficacy in reducing micro flora on hand than non alcohol based hand sanitizer. Furthermore, a study conducted among schools children showed significantly high efficacy of hand sanitizers in reducing micro flora on hand. The finding of this study also showed an overall reduction in infection related absenteeism of 19.8% [10, 20].

In the present study, about 70 % of the study participants were with transient micro flora. This is in agreement with several studies conducted in different parts of the world where there was high percentage of transient micro flora on the hand of the study participants [16]. Although several factors were suggested to be responsible to affect the level of transient micro flora on hand in Ethiopia, lack of clean water and alternative hand disinfectants is the major one. Transient micro flora is suggested to cause gastrointestinal and diharrial disease. Therefore, there is a need of integrated control programme to have a lasting impact on transmission of transient micro flora.

The demonstration that there is high percentage reduction of transient in micro flora using alcohol based sanitizers is in agreement with the report of other studies elsewhere [21, 3]. Several in vitro and in vivo studies have also shown considerable percentage of antimicrobial killing with alcohol based hand sanitizers. For instance, other study reported that using PURELL alcohol based hand sanitizer showed high reduction of transient micro flora on hand [22]. The finding of increased percentage reduction of transient micro flora using alcohol based hand sanitizer in France also supports the
hypothesis that alcohol based hand sanitizers reduces considerable percentage of microbial contamination on hand [11].

CONCLUSION
The present study revealed that there is high percentage of transient micro flora on the hand of the study participants. Furthermore, Tri active hand sanitizer has significantly high efficacy in reducing transient bacteria of hand than Imaj hand gel sanitizer, but both the hand gel sanitizer found to be effective to reduce the load of transient bacteria associated with hand. Since transient bacteria are known to cause several health problems, therefore the use of hand gel sanitizer is important to reduce transient bacteria on hand. In the areas, where there is limited access of water, poor hygiene conditions and for the health workers who are in close contact with the patients, the use of these hand sanitizer will be effective. Furthermore, creating awareness regarding the importance and efficacy of hand gel sanitizers in reducing transient bacteria is necessary to increase use of hand gel sanitizer and reduce the consequences occurred due to transient bacteria.

RECOMMENDATION
The present study showed high efficacy of Tri hand gel sanitizer. Therefore, it should be used as an alternative in areas where there is low access of water. Although not significant, high percentage reduction of transient micro flora was observed in individuals who used Imaj hand gel sanitizer. This may be due to our small sample size. Therefore, similar study with larger sample size is recommended to determine the exact effect of Imaj hand gel sanitizer.

ACKNOWLEDGEMENT
We are highly thankful to Department of Biotechnology, Faculty of Natural and Computational Sciences, University of Gondar for providing necessary facilities to conduct this research. The assistance and help from the staff members of the department during the present course of investigation are kindly acknowledged.
Table 1. Odds ratio and P values of individuals using Tri and Imaj hand gel sanitizers in University of Gondar students, April-June 2013

*Significant difference; ≠ Values shown are odds ratio value, □ Reference range

<table>
<thead>
<tr>
<th>Tests</th>
<th>Sample</th>
<th>Number of hands positive (%)</th>
<th>Number of hands negative (%)</th>
<th>Odds ratio, [95% CI]</th>
<th>P value#</th>
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<tbody>
<tr>
<td>Indole/MR</td>
<td>Before</td>
<td>6 (60)</td>
<td>4 (40)</td>
<td>2.25, [0.376-13.46]</td>
<td>0.37</td>
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<td>tests</td>
<td>sanitizer</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Imaj</td>
<td>4 (40)</td>
<td>6 (60)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Tri</td>
<td>1 (10)</td>
<td>9 (90)</td>
<td>13.50, [1.19-152.21]</td>
<td>0.02*</td>
</tr>
<tr>
<td>CT</td>
<td>Before</td>
<td>3 (30)</td>
<td>7 (70)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>sanitizer</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Tri</td>
<td>1 (10)</td>
<td>9 (90)</td>
<td>3.86, [0.33-45.60]</td>
<td>0.26</td>
</tr>
<tr>
<td></td>
<td>Imaj</td>
<td>2 (20)</td>
<td>8 (80)</td>
<td>1.80, [0.20-13.40]</td>
<td>0.60</td>
</tr>
</tbody>
</table>

Fig. 2: Percentage reduction of transient microflora before and after using Tri and Imaj hand sanitizers in University of Gondar students, April - June 2013.
### Table 2. Showing results of IMViC test

<table>
<thead>
<tr>
<th>Condition</th>
<th>IMViC Test</th>
<th>Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Indole</td>
<td>1 2 3 4 5 6 7 8 9 10</td>
</tr>
<tr>
<td>Before using sanitizer</td>
<td>MR</td>
<td>+ + + - - - + + -</td>
</tr>
<tr>
<td></td>
<td>VP</td>
<td>- - - - - - - - -</td>
</tr>
<tr>
<td></td>
<td>CT</td>
<td>- - - + - + - - -</td>
</tr>
<tr>
<td>After using Tri-Active</td>
<td>Indole</td>
<td>- - + - - - - + -</td>
</tr>
<tr>
<td></td>
<td>MR</td>
<td>- - + - - - - + -</td>
</tr>
<tr>
<td></td>
<td>VP</td>
<td>- - - - - - - - -</td>
</tr>
<tr>
<td></td>
<td>CT</td>
<td>- - - - - - - + -</td>
</tr>
<tr>
<td>After using Imaj</td>
<td>Indole</td>
<td>- + + - - - - + -</td>
</tr>
<tr>
<td></td>
<td>MR</td>
<td>- + + + - - - + -</td>
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<tr>
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</tr>
<tr>
<td></td>
<td>CT</td>
<td>- - - - - - + - -</td>
</tr>
</tbody>
</table>

**Fig. 2:** Antibacterial activity of Tri-active and Imaj hand gel sanitizer on the isolates identified as *E. coli* by using disc diffusion method (Plate-a: Control; plate-b: disc with Imaj; plate-c: disc with Tri-active)

**Fig. 3:** Antibacterial activity of Tri-active and Imaj hand gel sanitizer on the isolates identified as *P. aeruginosa* by using disc diffusion method (Plate-a: Control; plate-b: disc with Imaj; plate-c: disc with Tri-active)
REFERENCES