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Antibacterial Efficacy of Commonly Available Alcohol-Based Hand Sanitizers on *Escherichia Coli*

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Abstract

Background

The WHO estimates that approximately 600 million people fall ill after consumption of contaminated food and over 420 000 die every year, resulting in loss of 33 million healthy life years. Hand hygiene is considered by the WHO to be the most effective preventive measure for infectious diseases including food borne diseases.

Methods

A laboratory-based study involving convenient sampling of common brands alcohol-based hand sanitizers (ABHS) from retail community pharmacies and local supermarkets was conducted in Ilala District, Dar es salaam, Tanzania. The study was conducted, between December 2018 to January 2019. A modified protocol of The European Norm (EN) 1500 was used for in vivo testing of sampled ABHs. Efficacy was evaluated using standard strain of Escherichia *coli.* A total of 26 healthy volunteers were used for hand sanitization. The percentage of bioburden/microbial reduction was assessed at baseline and after treatment, and the log reduction factor calculated.

Results

A total of 10 gel ABHS were purchased and assayed for antibacterial efficacy. Majority (70%) of ABHS were imported products and contained ethanol as the sole active ingredient. About 60% of them did not correctly indicate the label disclosure information on concentration of active ingredients. Only one product was efficacious against *E. coli* with log reduction of 3.75; while majority (70%) of the samples had poor bacterial efficacy with log reduction ranging from 0.140 -0.664.

Conclusions

Most of ABHS gel products available in the Dar es Salaam market were not efficacious as per FDA and EN 1500 guidelines. Post market surveillance is recommended of the circulating ABH to safe guard consumers.

Keywords: Hand sanitizers, efficacy, *E. coli*, EN 1500.

Introduction

Foodborne diseases (FBDs) are considered an important and growing public health challenge that is associated with significant morbidity and mortality worldwide. Strains of *Escherichia coli* that produce Shiga toxins (Shiga toxin-producing *E. coli*, STEC) are an important cause of FBD worldwide. The World Health Organization (WHO) estimated that foodborne STEC caused more than 1 million infections, resulting in more than 100 deaths and nearly 13 000 disability-adjusted life years (DALYs) in 2010 (1). Lack of water, sanitation, and hygiene (WASH) is linked to the high prevalence of FBDs including diarrheal diseases in low-income countries (LICs) such as Tanzania (2). A major factor contributing to the high prevalence of FBDs is contaminated hands. Contaminated hands play a major role in the transmission of infectious pathogens including those causing FDBs and hospital acquired infections (HAIs). Proper hand hygiene using soap and water or Alcohol-based hand sanitizers (ABHs), has been shown to be an effective means of reducing transmission of infectious disease pathogens on contaminated hands, including multidrug resistant bacteria in the household, clinical, and workplace settings (3-6). Regrettably, studies on hand hygiene show poor compliance among community members and healthcare workers (HCWs) especially in low Income Countries (LICs) (7-10). Barriers to good hand washing practice in LICs, may include limited availability of clean running water, wash stations and lack of alcohol-based hand rub stations (8-11). Consequently, most of the individuals in LICs often resort to use retail sold ABHs as an alternative for washing hands with soap and water (12-13). However, for ABHs to effectively decontaminate hands, they have to be correctly formulated and should contain isopropanol, ethanol, n-propanol, or a combination of the two as active ingredients at concentrations ranging from 60% -95% v/v (14). Regrettably studies on the antibacterial efficiency of retail sold ABHs in some countries have suggested that there are poor quality ABHs circulating in the domestic markets of some LICs (15-18). Thus, this study sought to assess the bacterial efficacy of retail sold ABHS in Tanzania to provide guidance to comsumers by highlighting the efficacy of these products in our markets.

Methods

Sampling, Study Design and Area

An experimental, laboratory-based study involving testing for antibacterial efficacy of ABHs against *E. coli* isolates was carried out at the School of Pharmacy, Muhimbili University of Health and Allied Sciences (MUHAS), Dar es Salaam, Tanzania. Convenient sampling was used to purchase duplicate samples of the most common brands of ABHs from community pharmacies and local supermarkets located in Ilala district, Dar es Salaam between December 2018 to January 2019.

Reference Product Standard

The WHO II formulation, isopropanol 75% (v/v) plus hydrogen peroxide 0.125% v/v) plus glycerol 1.45% (v/v) was used as a reference standard. A freshly prepared solution was made on each test day. The solution was rubbed twice using volumes of 3 mls for 30 seconds each. Within 60 seconds, a total of 6 mls was applied to 2 volunteers that acted as the positive control group.

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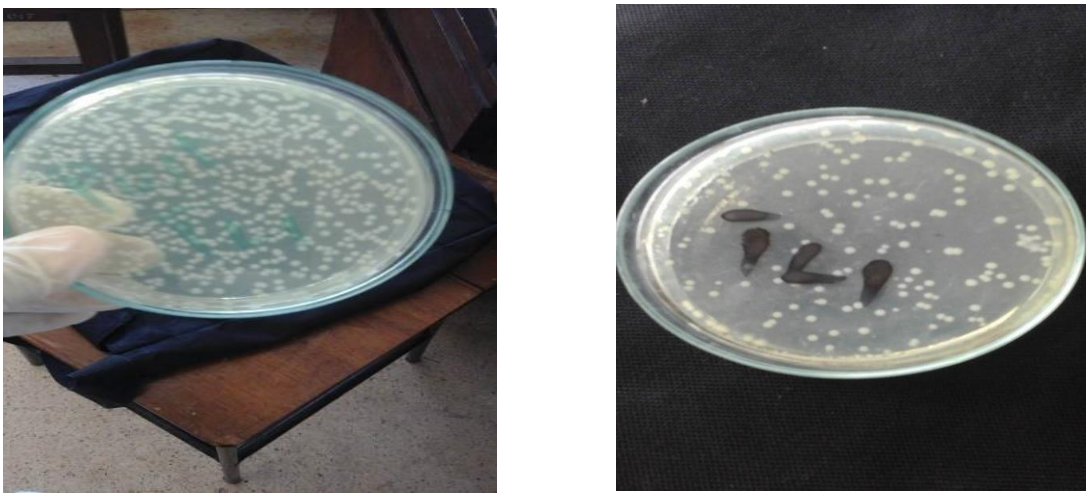
Antibacterial Efficacy Determination

Alcohol based percentage reduction factors (RF) were assessed based on the baseline and after treatment with the ABHs. Each ABHs was compared with the positive and negative controls as per the WHO II recommendations/guidelines. The log reduction factor (LRF) was calculated as (A-B)/A%; where A =the number of viable microorganisms at baseline and B = the number of viable microorganisms after treatment as shown in figure1.



Pretreatment Post-treatment

Figure 1: Bacterial Counts before and after Hand sanitizer Use

Anti-bacterial Efficacy testing

Twenty-six healthy MUHAS undergraduate fourth year Bachelor of Pharmacy students were recruited to act as test volunteers for this study. All volunteers had no previous training on hand disinfection. The volunteers were randomized into twelve groups (10 test groups, 1 positive control group and 1 negative control group). Each of the test groups comprised of two volunteers per group, the positive control group comprised of three volunteers and the negative control group also comprised of three volunteers. A modified protocol of The European Norm (EN) 1500 was used for *in vivo* testing of sampled ABHs. Volunteers were initially required to thoroughly wash their hands with soap and water for 5 minutes followed by air-drying for 5 minutes. The visibly four clean fingers of the left and right hands of each volunteer were then dipped sequentially into a suspension of an overnight culture of *E. coli* ATCC 25922 in of Tryptic soy broth (TSB) adjusted to 0.5 McFarland suspension. Then each contaminated finger was dipped into a separate universal bottle containing 30mls of sterile TSB. The inoculated bottles where mixed using a table top vortex machine. Tenfold serial dilutions were then performed using TSB. A total volume of 100ul was removed from the highest dilutions and plated onto plate count agar (PCA Himedia). The plates were then incubated at 37°C overnight so as to provide the baseline colony forming unit (cful/ml) count for the testing or pre-values, that is before application of ABHs or reference product.

Two days later the second part of study was conducted. The same 26 volunteers were pre-trained on the proper hand rubbing using the WHO technique. All volunteers were then

required to follow the same procedure as before to contaminate the same four fingers of the left and right hand with a suspension of an overnight culture of *E. coli* ATCC 25922 in TBS. In this second stage, 10mls of sample hand sanitizers were randomly allocated to be n applied to hands of the 20 volunteers while 6mls of reference product was applied to the hands of 3 volunteers and 10mls of sterile water was applied to the hands of three volunteers to act as negative control. The volunteers performed the WHO hand rub technique to sanitize their hands for 60 seconds. After sanitation the tips of four fingers of both the right and left hands were again each sampled in 30ml of sterile TSB by dipping the tips into the broth. Serial dilutions of each samples were then performed. A total volume of 100ul was removed from the highest dilutions and plated onto plate count agar (PCA himedia). The plates were then incubated at 37°C overnight so as to provide the post value mean colony forming unit (cful/ml) count.

Ethical approval

The study protocol was reviewed and approved by the Institutional Review Board (IRB) of the Muhimbili University of Health and Allied Sciences. The IRB granted a waiver of consent for collecting data. Given the low risk nature of the data.

Results

General Characteristics of ABHs

At total of 10 samples of ABHS were purchased from retail outlets in Dar es Salaam Ilala district. A total of seven ABHS products were imported, and the remaining three were locally manufactured. All ten ABHS samples were in the form of gel hand sanitizer (gABHS). All ten products contained ethanol as one of its active ingredients. Seven gABHS products specifically gABHS3, gABHS5, gBHS6, gABHS7, gABHS8, gABHS9 and gABH10 contained ethanol as the sole active ingredient in single form, while three products, namely gABHS1, gABHS2 and gABHS4 contained either ethanol in combination with either triclosan or Isopropanol.

Labelling disclosure conformity

Conformity to standard information disclosure among the tested products ranged from 40 to 100%. Only one product namely gABHS 4, conformed to the National Regulatory Authority (NRA) labelling requirements for antiseptics and disinfectants that requires these products to state the; Product name, Efficacy claim, Direction for use, Warnings and Precautions, Expiry date, Name and Concentration of Active Pharmaceutical Ingredient (API), batch number, Net content, Manufacturer name and address and storage conditions. One gABHS sample namely gABHS 9 had only half of the required items to be on the label as par NRA labelling requirements for antiseptics and disinfectants as shown in figure 2.

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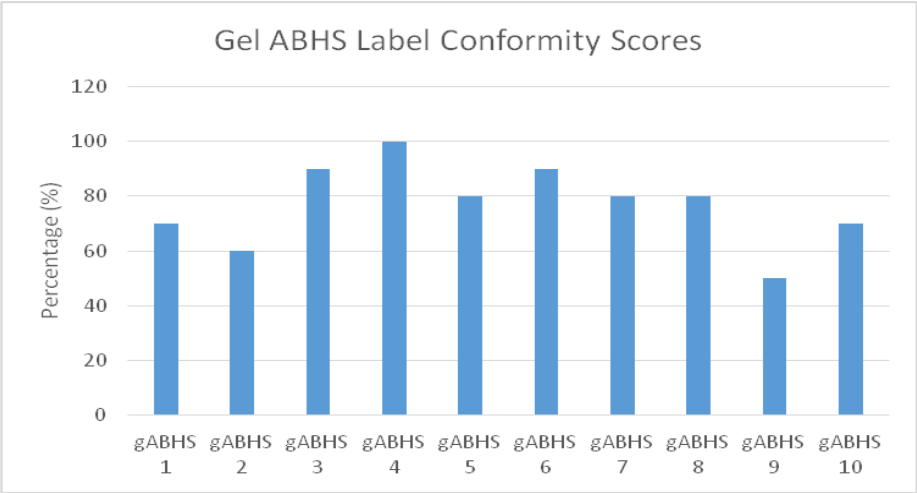

Figure 2: Gel ABHS Label Conformity to National authority requirements

Six out of ten gABHS products did not state the concentration of the active ingredient on their label in contrast to NRA labelling requirements for antiseptics and disinfectants as shown in table 1. Seven out of ten products had an efficacy claim of 99% microbial reduction or more on their label. While three gABHS products, did not have a numerical efficacy claim on their label as shown in table 1.

Table 1: Characteristics of the assayed ABHS available in the market

Hand Gel no	Country of Manufacture	Active Ingredient stated	Concentration of API (v/v)	Net volume (mls)	Efficacy claim (%reduction)
gABHS 1	Tanzania	Isopropanol, ethanol	-	60	-
gABHS 2	Turkey	Denat, Ethyl alcohol,Triclosan	-	100	99.9
gABHS 3	India	Alcohol IP (Denatured)	72.34	100	99.9
gABHS 4	India	Ethyl alcohol BP,Isopropanol BP	66.5, 3.5	120	99
gABHS 5	Turkey	Alcohol IP (Denatured), Isopropanol, Triclosan	-	50	99.9
gABHS 6	South Africa	Ethanol	-	75	Most
gABHS 7	Tanzania	Ethanol	66	200	99.9
gABHS 8	Tanzania	Ethanol	-	250	99.99
gABHS 9	China	Ethanol	62	500	-
gABHS10	Ghana	Alcohol	-	100	99

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Antibacterial efficacy against E. coli

Only one product namely gABHS 6 containing ethanol of an unknown concentration was found to be efficacious in terms of attaining a bacterial log reduction (LR) of higher than threefold as par U.S. FDA requirements of reductions of ≥2 log (19). Two gABHS samples, namely gABHS1 and ABHS 8 had low efficacy and they were only capable of reducing the microbial load of E*. coli* by a LRF of 1.189 and 1.024 respectively. Seven gABHS samples were found to be non- efficacious as they achieved log reduction factors below 1 as shown in figure 3. gABHS product indicating containing 70% ethanol did not exhibit a greater efficacy (gABHS 9) than those that contained less than 70% ethanol (gABHS3).

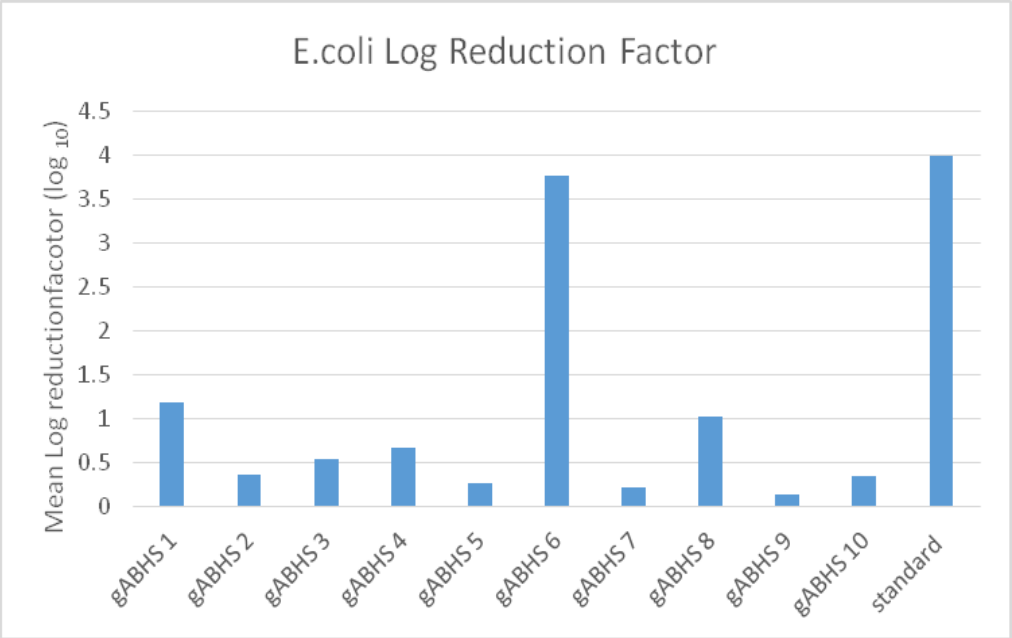

Figure 3. E. coli Log Reduction Factor

Discussion

Contaminated hands are known to be a major vehicle for the transmission and spread of pathogens that causes diseases, food borne illnesses and hospital acquired infections. Alcohol based hand sanitizer products have become increasingly popular in urban areas of low-income countries like Tanzania as an alternative method to hand washing with soap and water to prevent the spread of infection. This study is one of the first investigations in Tanzania on the effectiveness of retail sold gABHS against a common food born pathogen *E. coli*. Our results confirm the findings from other studies, that some ABHS sold in retail outlets may vary in their ability to reduce the microbial burden of contaminated hands (15-17). In contrast, to previous studies conducted, the majority (90%) of the retail sampled gABHS in our study were found not to be efficacious as par The European Norm (EN) 1500 guidelines for ABHS. In fact, it was only one sample product, specifically gABHS 6 that was able to achieve a threefold reduction factor against the standard strain of *E. coli*. The finding that the majority of gel ABHS failed is similar to findings from the study conducted in Kenya that found that all the poor performing AHBS were gel formulations (18). Our results also augment the findings observed by Karmer et al (2002) and Dharan et al (2003) that

suggested that ethanol gel formulations, unless specially formulated and tested for this purpose, are less efficacious than ethanol solution ABHS formulations (19-20). What seems to be clearly suggested from this study is that the active ingredient in gel formulated hand sanitizer may not be readily available within the short contact time prescribed. This hypothesis may corroborate the fact that gABHS products containing 70% ethanol did not exhibit a greater efficacy than those containing less than 70% ethanol as has been shown in other studies irrespective of whether the products were locally manufactured or imported products (21). The possibility that gel based ABHS may be less efficacious than ethanol solution formulations has major implications to consumers who solely rely on ABHS label claim in selecting effective hand sensitizers. What undoubtedly must be determined is whether the gABHS circulating in the Tanzanian market may have formulation issues or counterfeit products. Past studies on counterfeit pharmaceuticals in Tanzania have concentrated mainly on essential medicines and cosmetics, with limited data on antiseptics and disinfectants (22). Our study findings suggest that to safeguard ABHS consumers, policy-makers in Tanzania need to continue to improve the existing post-marketing surveillance system to include the detection of poor-quality antiseptics and disinfectants especially in this era of pandemics associated with infectious microorganisms.

Conclusion and Recommendation

The majority of gel formulated ABHS circulating in the Dar es Salaam, market are of poor antibacterial efficacy as per FDA requirements. Further studies involving larger sample size should be conducted to compare the efficacy of alcohol-based gels versus alcohol-based rinses in reducing the transmission of hospital acquired infections and other bacterial and viral food borne illnesses.

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Declarations

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Conflict of interest

The authors declare no conflict of interest.

Authors' contribution

JSH participated in study concept and design, data acquisition, data analysis, and drafting the manuscript. EM was involved in providing technical help during the laboratory activities. DM, GB and KDM were involved in study concept, study design, and writing the manuscript. All authors read and approved the final manuscript for publication.

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List of Abbreviations

ABHS	Alcohol based hand sanitizers
EN	Comite Europeen de Normalisation
FDA	Food and Drug Administration
LRF	Log reduction factor
MUHAS	Muhimbili University of Health and Allied Sciences
TBS	Tryptic soy broth

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