SHORT COMMUNICATION

A Study on the Efficacy of Alcohol Based and Alcohol Free Hand Sanitizers Available in the Market Against *Staphylococcus Aureus* and *Salmonella* Typhimurium

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ABSTRACT

Hand sanitizers can be manufactured in the form of alcohol based hand sanitizer (ABHS) that contains alcohol, or alcohol free hand sanitizer (AFHS) containing benzalkonium chloride. This study determines the efficacy of ABHS and AFHS products against *Staphylococcus aureus* and *Salmonella* Typhimurium. Agar well diffusion assay was performed against the bacterial colonies grown on Mueller Hinton agar in triplicates. Wells were made by punching holes onto the agar and filled with three different brands of hand sanitizers namely 70% ABHS, 95% ABHS, and AFHS. The diameters for zone of inhibition was measured after 24 hours incubation at 37 ℃. Only AFHS product inhibited the growth of *S. aureus* and *S.* Typhimurium, whereas 70% and 95% ABHS did not exhibit any zone of inhibition. Although AFHS was proven to be useful, this finding suggests that ABHS available in the market might not necessarily be effective against *S. aureus* and *S.* Typhimurium.
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INTRODUCTION

Infections acquired either from the hospitals or in the community have become a major public health concerns across the world. Centers for Disease Control and Prevention (CDC) reported that about 2 million people have contracted hospital-acquired diseases each year, with roughly around 90,000 of the patients died because of their infections (1). Face touching including eyes, nose, and mouth area is a common habit and theoretically become a transmission pathway that has an implication for hand hygiene (2). Organizations such as World Health Organization (WHO) and CDC have documented that practicing hand cleanliness is the simplest and efficient technique to prevent infections. Maintaining self-hygiene has a very significant impact in minimizing the possibility of colonization and spread of infectious diseases in the community. Compliance with hygiene recommendation played an important part in reducing the risk of respiratory infections and gastroenteric (3).

Hand sanitizers can largely be classified into two types which is identified as alcohol based or alcohol free (4). Alcohol based hand sanitizer (ABHS) typically contains alcohol or isopropanol, whereas benzalkonium chloride is commonly used in alcohol free hand sanitizer (AFHS). Both ABHS and AFHS acts as antimicrobial products with the aim to decrease the presence of bacteria on the skin that could cause disease or infection. WHO recommends the use of hand sanitizer as quick protection against broad spectrum bacteria and viruses. ABHS acts by destroying the protective coatings on microbes making it non-functional, whilst AFHS offer instant germ-killing action (3). CDC has endorsed the use of AFHS as a safe alternative with less toxic concerns than ABHS. Commonly, the most effective ABHS contains 60-90% alcohol concentration, whilst AFHS contains at least 0.1% benzalkonium chloride (5). The emer-gence of COVID-19 in recent years has seen numerous hand sanitizer products sold in the market based on either alcohol or non-alcohol formulations. Therefore, this study investigates the

effectiveness of selected 70% ABHS, 95% ABHS, and AFHS products available in the market against *Staphylococcus aureus* and *Salmonella* Typhimurium colonies *in vitro*.

MATERIALS AND METHODS

Selection of hand sanitizers

Hand sanitizer products with different brands were bought from the shops around the area of Kajang, Malaysia. The 70% ABHS, 95% ABHS, and AFHS products were selected based on the information stated on the label by the manufacturer.

Samples and materials

S. aureus and *S.* Typhimurium suspensions were acquired from Microbiology Unit, Universiti Kuala Lumpur, Institute of Medical Science Technology. Prior to the tests, Mueller Hilton agar (MHA) (Merck, Germany) medium was prepared on petri dishes and stored in the refrig-erator until further use. The concentrations of the bacterial colonies were prepared according to 0.5 McFarland standard. Test tubes were filled with 0.9% normal saline and several drops of the bacterial suspensions were added before the turbidity was compared to 0.5 McFarland standard.

Agar well diffusion assay

This works was performed according to the standard microbiological practices in order to avoid any possible cross contaminations (6). Before the beginning of the tests, the MHA plates were taken out from the refrigerator and placed at room temperature. The bacterial suspensions with turbidity of 0.5 McFarland standard was spread on the MHA plates using sterile cotton swabs. Four holes of about 1 cm in diameter were made with a puncher on the MHA plates. The holes were labelled as 70% ABHS, 95% ABHS, AFHS, and sterile distilled water. 30 µL from each of the product solution were filled into the holes on the MHA plates respectively. The plates were incubated at 37 ℃ for 24 hours and the diameter of the zone of inhibitions were measured the next day using a ruler. All products were tested in triplicate.

RESULTS AND DISCUSSION

The difference between the effectiveness of 70% ABHS, 95% ABHS, and AFHS is shown in Fig. 1. The results were represented by the measurement of zone of inhibition diameters on the MHA plates as recorded in Table I. Although both colonies of *S. aureus* and *S.* Typhi-murium bacteria was challenged with 70% and 95% ABHS products, interestingly only AFHS product demonstrated zone of inhibitions after the susceptibility tests.

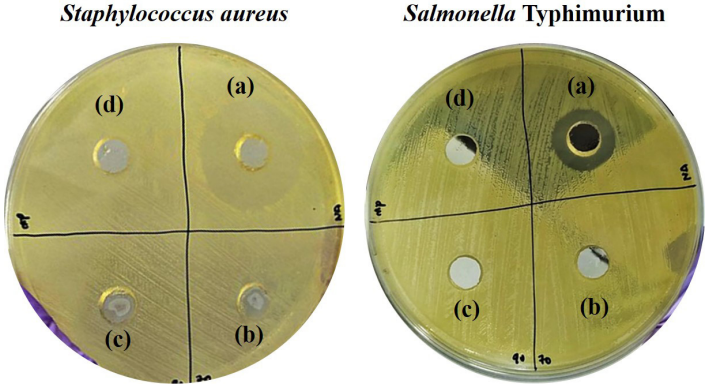

Figure 1 : Zone of inhibitions against *S. aureus* and *S.* Typhimurium colonies. Four holes were made according to the agar well diffusion assay and filled with (a) AFHS, (b) 70% ABHS, (c) 95% ABHS, and (d) distilled water. The efficacy of the 70% ABHS, 95% ABHS, and AFHS products tested was represented by the measurements of the zone of inhibition diameter after 24 hours incubation period at 37 ℃.

Susceptibility tests against *S. aureus* and *S.* Typhimurium

AFHS product recorded the biggest zone of inhibition against *S. aureus* with average diameter of 27.5 mm, and an average zone of inhibition of 15.5 mm against *S.* Typhimurium. Both 70% and 95% ABHS products did not show any zone of inhibition formation against the bacterial colonies tested. Although alcohol is known to possess antimicrobial properties, the result from this study suggests that the 70% and 95% ABHS products tested may have less than 60% alcohol content required in hand sanitizer products. This would definitely raise the questions about the quality

Table I : Average diameter measurement of zone of inhibition against *S. aureus* and *S.* Typhimurium growth using different alcohol based and alcohol free hand sanitizer products

No.	Type of hand sanitizer products tested	Average diameter of zone of inhibition against bacterial colonies	
		Staphylococcus aureus	*Salmonella* Typhimurium
1	Alcohol free hand sanitizer (AFHS)	27.5 mm	15.5 mm
2	70% Alcohol based hand sanitizer (70% ABHS)	-	-
3	95% Alcohol based hand sanitizer (95% ABHS)	-	-

and effectiveness of numerous hand sanitizers products sold in the market particularly during COVID-19 pandemic. The use of benzalkonium chloride based hand sanitizer containing was compared to 70% ethanolic based hand sanitizer for a week, and the outcomes proved that hand sanitizer containing benzalkonium chloride was more successful at reducing the presence of *S. aureus* on the healthcare worker's hands (7). Alcohol free hand sanitizers formulation sustained a higher de-germing activity as compared to the alcohol based hand sanitizer formulation (8). It was reported that alcohol based hand sanitizer becomes less effective after continual use and irritates the hands of the test subjects, whereas the alcohol free hand sanitizer appeared to be more effective without further complaints or irritation reported by the volunteers (8).

The active ingredients in the AFHS products used in this study contains 0.3% benzalkonium chloride, surfactant, glycerin, fragrance, and water. The zone of inhibition demonstrated by AFHS products could also be due to the use of surfactant in its formulation. Surfactant helps to lift up any soils and microorganisms from the surface and are a recommended component in products for disinfection and sterilization purpose together with other antimicrobial agents (9). Cationic surfactants have been recognized as the molecules that exhibits antimicrobial action and the antibacterial activity depends strongly on the surfactants structure (10).

The use of hand sanitizers containing alcohol is commonly practiced not just in healthcare facilities but also in other sectors where the cleanliness of hands is utmost important. Ethanol is known to be efficient against an extensive range of bacteria that could stay on the skin (11). Isopropanol, like ethanol, is also used as active ingredients in disinfectant and antiseptic products. Isopropanol is capable to cause damage to the proteins and lipids of bacteria and viruses, which consequently kills them upon contact (12). The outcomes from the 70% and 95% ABHS products tested in this study raised certain doubts on the concentration of active ingredients added into the hand sanitizers. It could be because of the alcohol concentration used is not as high as the manufacturer claims to be, or the products does not contain any form of alcohol. Laboratory tests conducted using various brands of hand sanitizers available in Malaysian market found that some of the products contained less than 20% alcohol as opposed to the minimum 70% concentration suggested by the regulatory body (13). Other possibility that contributed towards the negative results may also include the agar well diffusion assay performed in this test. Both the 70% and 95% ABHS applied during the tests against *S. aureus* and *S.* Typhimurium might have dissipated on the MHA plate throughout the 24 hours incubation period. Alcohol used in sanitizer products could quickly dry out the skin and eventually disrupt the layers of oils that acts as protective barrier on human skin (14).

Alcohol content in hand sanitizer products

The increasing awareness for personal hygiene during COVID-19 pandemic has seen a surge in hand sanitizers sales with numerous brands of hand sanitizers sold in the market. Many big and small manufacturers around the world has taken the opportunity to produce different types of hand sanitizers to combat the spread of COVID-19. Different types of hand sanitizers with active ingredients such as alcohol, isopropyl alcohol, and alcohol free products were manufactured during that period even until today. However, there were safety concerns raised with the reported increase in substandard hand sanitizers products available in the market (15). With the spread of COVID-19 expected to still continue, the use of hand sanitizers will be in growing demand for an extended period of time. Therefore, the availability and purchase of hand sanitizers products that does not meet the required efficiency standard imposed by the regulatory body will certainly create unnecessary public safety problems.

CONCLUSION

Taking care of hands hygiene is part of an essential prevention strategy to minimize the risk of microorganism transmission particularly during the spread of COVID-19. In the situation when water and soap are not available, the use of either alcohol-based or alcohol-free hand sanitizer is advised. Effective infection control technique such as the use of hand sanitizer helps to keep hands clean and free of pathogens. Thus, formulation and active ingredients used to produce hand sanitizers are important in order to ensure that it can effectively eliminate the presence of any unwanted microorganisms and does not irritate the skin. In conclusion, the AFHS product tested in this study was effective against *S. aureus* and *S.* Typhimurium colonies, but both 70% and 95% ABHS were ineffectual and raised critical questions about the actual concentrations of alcohol added into the products.

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