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Short Communication

Comparative assessment of antimicrobial efficacy of hand sanitizers as antiseptic solutions: an *in vitro* study

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Abstract

Hand hygiene, particularly hand sanitizing, is essential in reducing infectious disease transmission.With respect to the realization that hand hygiene is a prerequisite for the prevention of diseases, the conventional method of washing hand with soap has became quite non popular. Instead it is the use of hand sanitizer, which has gradually become the method of choice due to its various advantages. In the present study, the invitro bacterial activity of two well known brands of hand sanitizers available in laboratory was conducted by agar susceptibility test, minimum inhibitory concentration test and in-vivo reduction of viable bacteria counts on hands of subject'smethod. Reference bacterial strains like *Pseudomonas aeruginosa* and *Bacillus subtilis* were treated with different concentrations of each sanitizers showed good result. Antibacterial activity of these sanitizers different from each other. Increased concentrations (25µl, 50 µl, 75 µl & 100µl) of avagard showed good results, where as lesser concentrations (0.5µl, 1µl 5µl, 10µl, 15µl, 20µl) haven't showed the antibacterial activity. In the case of dettol all the concentration (from lower to higher) showed good results, The dettolis much stronger than avagard in the antibacterial activity having well established inhibition zones against both gram positive and gram negative bacteria.

Key words: Hand sanitizer, antimicrobial agents, Dettol, avagard antiseptic, inhibitory concentration, susceptibility test.

Introduction

Hands are regarded as a major source oftransmitting infection. It has been estimated thatthere are not less than 10000 organisms per $cm^2$of normal skin. This includes both nonpathogenicresident flora as well as pathogenic transient flora (Carter *et al.*, 2000). As skin is the first line of defense, so most of the bacteria like*Pseudomonas aureginosa* and *Staphylococcus aureus* reside on skinand is the major cause of skin infections. Hand washing withantibacterial is of more importance in accordance with the health careassociates as they may be the main cause of bacterial contaminationeither opportunistic or pathogens (Fluit*et al.*,2001, Higaki *et al.*, 2000). A huge number of chemical compounds are present that have theability to stop the growth of bacteria and can kill them. Thesecompounds are very

large in number possibly 10,000 of which 1000 are being usually used in hospitals and homes. These chemical compoundsexist in the form of solids, liquids and gases. Many groups of chemicalsused to decrease or destroy microbes. Significant groups includehalogens, phenols, soaps, detergents, ammonia compounds, alcohols,heavy metals, acids and certain extraordinary compounds (Lucet*et al*., 2002).

Decontamination of hands can be carried outby various means. This include either by washinghands with soap or by the use of various agentssuch as gloves, skin protectants and waterless hand sanitizers (HS), which reduce contaminationon hands by removal or by killing the organismsin situ. Washing hands with soap is not feasibleall times due to unavailability of resources. It isnot practical to find purified water and soap at allplaces. Similarly the use of gloves is limited tohospitals and that too require use of aseptictechnique before and after using gloves. Thusamongst these, HS have gradually become themost effective means of preventing spread ofdiseases and were the subject of present study.A hand sanitizer is a supplement or alternativeto hand washing with soap and water. HS,sometimes also referred to as rub, can bepresented in the form of either a gel, as foam oras liquid solutions. Further, the vehicle for HS maybe either alcohol (alcoholic) or aqueous (callednon-alcoholic). For preparation of alcoholic hand sanitizers (AHS), ethanol, isopropanol, and/orn-propanol are used. The antimicrobial activity of alcohols is based on itscapacity to induce microbial protein denaturation.These were reported to have excellent and rapidgermicidal activity against vegetative bacteria,fungi, and many viruses. On the other hand, non–alcoholic hand sanitizers (NAHS) incorporate smallconcentrations of the nitrogenous cationicsurface-acting agent such as benzalkoniumchloride or the chlorinated aromatic compoundtriclosan or povidone-iodine (MadanK*et al*., 2012).

Hand sanitizers have beenreported to cause a decrease in infection rates and aregenerally particularly useful in situations whereaccess to water is limited. In addition to being usefulin the absence of water, other advantages of the useof the hand sanitizers include, high antimicrobialactivity in a shorter time, and the lack of requirementfor drying of the hand, which could serve as another source of contamination (Wolfe *et al*.,2017).

The purpose of this study was to evaluate the antimicrobial activity of 2 different brands of hand sanitizers available in the local market of Belthangadytaluk in the Karnataka state against daily encountered bacteria present on the skin. Activities of the sanitizers were studied against the selected strains of bacteria to know their antibacterial effect.

Materials and Methods

Test organisms

Bacillus subtilis and *Pseudomonas aeruginosa*obtained from the culture collection of the Department of biotechnology, SDM Collage ujire, were used as test organisms in this study. These two bacteria are commonly studied in many collage laboratories.

Hand sanitizers (HS)

Two popular brands of HS products commonly sold and used in Belthangady were chosen for the study. The products were selected based on our interactions with consumers and our observation at different retail outlets. Each of the products was stored as recommended by its manufacturer and

they were used well before their expiration dates.

Table 1: Hand sanitizers used in this study and their compositions.

Product	Composition
Dettol	Alcohol IP (denatured) eq.to absolute alcohol 72.34% v/v, water, PEG/PPG-17/6 copolymer, propylene, glycol, acrylates/C 10-30acryl alkylate cross polymer, tetrahydroxypropylethylenediamine, chamomile extract, perfume, ponceau SX
Avagard	Propanol IP 45% w/w, propanol 30% w/w

Inoculum preparation

The nutrient broth preparation is done about 150ml in two separate flasks and the loop full of inoculum is added to it respectively. It is then incubated for 24 hrs.

Susceptibility of test bacteria to hand sanitizers

The well-variant of the agar diffusion method described by Valgas et al. (2007) was modified and adopted in assessing the susceptibility of the test organisms to the sanitizers. Each test organism was seeded onto the surface of a sterile nutrient agar plate using pour plate method.1ml of nutrient broth culture of respective organism is poured on the plate containing nutrient agar before solidification and then it is allowed to solidify. A sterile 4 mm cork borer was used to create wells in the agar for each test organism. Next 100 µL of the sample of each HS with varied concentrations (0.5, 1, 5, 10, 15, 20, 25, 50, 75, and 100) were introduced into the well. All the plates were incubated at 37 ^{0}C for 24 hours in an upright position. The zone of inhibition around each well was measured and the readings were recorded.

Minimum inhibitory concentration (MIC) determination

Minimum inhibitory concentration is the lowest concentration of an antimicrobial agent that completely inhibits the growth of a test organism as seen by the unaided eye (CLSI, 2006). To determine the MIC, increasing concentrations (0.5, 1, 5, 10, 15, 20, 25, 50, 75, and 100) of each HS were prepared in 9 ml tubes of sterile nutrient broth. Exactly 100 µL of each standardized test organism was then introduced into each tube of HS. A tube containing only nutrient broth and bacteria without sanitizer served as negative control while another tube containing just the sanitizer and broth without bacteria served as positive control. Each tube was incubated for 18 hours and then examined for visible growth or turbidity. The concentration of the HS in the tube in which no visible growth was observed when compared with the controls was taken as the MIC.

Minimum bactericidal concentration (MBC) determination

Minimum bactericidal concentration is the lowest concentration of an antimicrobial that can kill the test organism (Cheesbrough, 2006). To determine the MBC for each HS, samples from the test tubes used in MIC test that showed no visible growth after the period of incubation were inoculated on sterile nutrient agar plates (which had no antimicrobial incorporated) in them using pour plate method. The plates were incubated at 37^{o}C for 18-24 hours and were then observed for growth. The concentration at which absence of growth was observed (bactericidal activity) was taken as the MBC.

In vivo reduction of viable bacteria counts on hands of subjects

The products were further evaluated for their efficacy in reducing baseline bacterial counts on hands of subjects. Threeindividuals were selected for each product and verbal informed consent was obtained from each subject prior to the conduct of the experiment. Subjects did not apply any antimicrobial substance to their hands prior to the experiment. Sterile nutrient agar plates were divided into two halves with one half labelled BF (before) and the other labelled AF (after). Subjects were asked to gently make an impression on the surface of the BF side of the agar plate with the three unwashed fingers. After this, 3 ml of the HS was then applied to the hands and then rubbed thoroughly on the palms, fingers, and the back of the hands until the hands became completely dry. Subjects were then asked to repeat the finger impression on the AF part of the plate. This was done by all subjects. The plates were incubated at 37 0C for 24 hours and the numbers of colonies were counted. The percentage cfu reduction was calculated as follows:

$$\% \text{ cfu reduction} = \frac{\text{cfu count of BF section- cfu count on AF section}}{\text{cfu count on BF section}} \times 100$$

(Oke et al., 2013).

Results

In the test for the susceptibility of test bacteria to HS the Avagard HS has started to show the zone of inhibition from the 15% concentration where as the Dettol HS has started to show its effect from 0.5% concentration itself for *Pseudomonas aeruginosa*. For *Bacillus subtilis* the Avagard HS has started to show the zone of inhibition from 15% concentration where as the Dettol HS has showed its effect from 5% concentration itself. The MIC and MBCof Avagard HS for *Bacillus subtilis* is 15% and for *Pseudomonas aeruginosa* is 25%. Dettol HS showed its MIC and MBC for *Bacillus subtilis* at 5% and for *Pseudomonas aeruginosa* at 0.5%. The test for In vivo reduction of viable bacteria counts on hands of subjects has showed 55.02% of cfu reduction by using Avagard HS and 64.28% of cfu reduction by using Dettol HS.

Fig.1: *Pseudomonas aeruginosa* showingzone of inhibition with Avagard and Dettol HS

Fig.2: *Bacillus subtilis* showingzone of inhibition with Avagard and Dettol HS

Table 2: Length of zone of inhibition for the susceptibility test in cm.

HS	Dettol HS							Avagard HS									
%	0.5	1	5	10	15	20	25	0.5	1	5	10	15	20	25	50	75	100
Pseudomonas aeruginosa	0.6	0.7	1.1	1.3	1.4	1.5	1.6	no	no	no	no	0.3	0.4	0.7	0.9	1	1.2
Bacillus subtilis	no	no	0.5	0.6	0.8	1	1.2	no	no	no	no	0.4	0.5	0.6	0.8	1	1.1

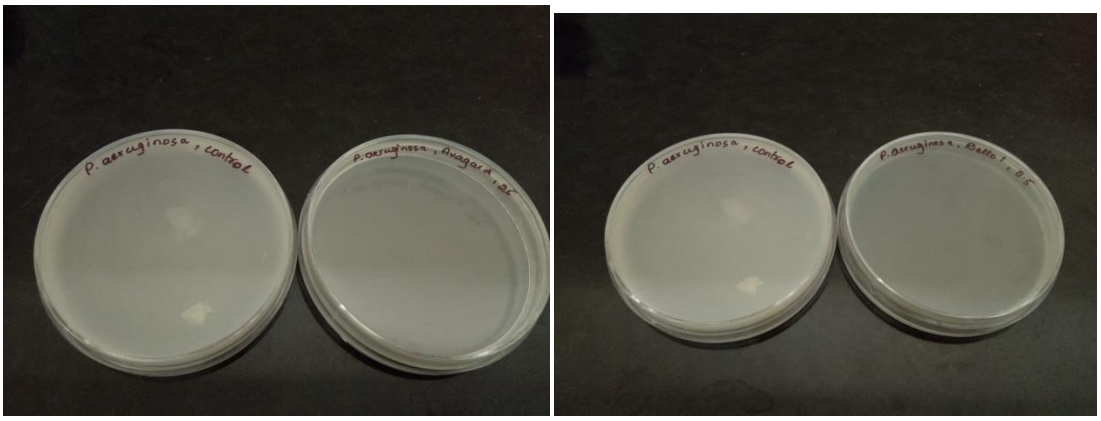

Fig 2: MBC of *Pseudomonas aeruginosa* shown at 25% in Avagard and at 0.5% in Dettol HS

Fig 3: MBC of *Bacillus subtilis* shown at 15% in Avagard and at 5% in Dettol HS

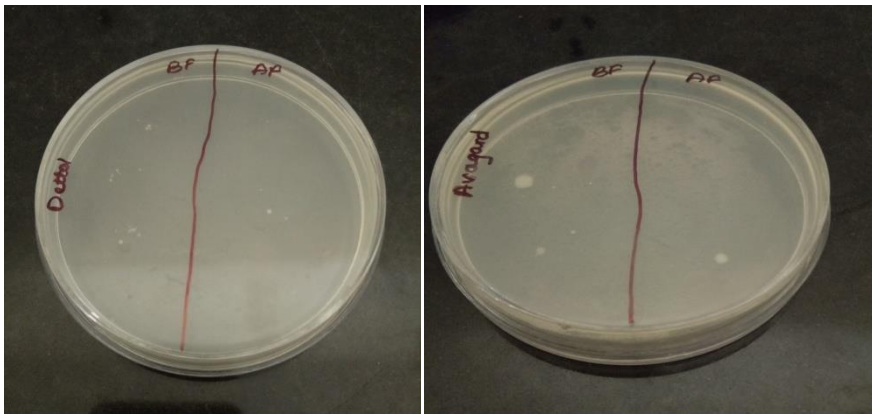

Fig 4: In vivo reduction of viable bacteria counts on hands of subjects using Dettol and Avagard HS.

Table 3: % reduction of cfu count

	BF	AF	%	Mean
Dettol HS	26	16	38.46	55.02%
	34	12	64.70	
	21	8	61.90	
Avagard HS	33	11	66.66	64.28%
	36	12	66.66	
	42	17	59.52	

Discussion

Hand sanitizing has more recently been the prescribed method of hygiene, possibly due to the higher compliance rates associated with it (Kampf and Kramer 2004) and its particular usefulness in areas lacking adequate water supply. With this increase in compliance in use of hand sanitizers, there is a need to access the efficacy of products available in the market (Nwabueze*et al.*, 2016). The Avagard and Dettol HS showed moderate efficacy of reducing microflora in the hands of studied participants as well as

it showed good effect on inhibiting studied microorganism.A study conducted by Centre for Disease Control and Prevention (CDC, 2002) 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 (Madan et al., 2012 &Hiburn et al., 2003). In a study high efficacy of isopropyl alcohol based alcoholic hand sanitizer in reducing microbial contaminants was reported (Madan et al., 2012). 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% (Hammond et al., 2000 &Vessey et al., 2007). Sharif and Ansari, analysing the efficacy of various hand sanitizing products, noted that one of their products was only effective against 6.5% of the isolates tested (Sharif and Ansari 2015). A more recent study carried out in Kenya (Ochwoto*et al.,* 2017) noted that 25% of tested products were effective against only 33% of the test isolates and an unspecified number were not effective against any of the test isolates at all. The Ochwoto study reported a possible link of efficacy to composition and noted that the ethanol based products resulted in a higher efficacy than the isopropyl based products. As well as the type of alcohol present, the difference in efficacy of the various hand sanitizers could also arise from the actual composition of alcohol present in the product. For most alcohol based hand sanitizers, the alcohol components are the major active ingredients. These act by disrupting tissue membranes, denaturing proteins and dissolving lipids (Oke*et al.*, 2013). 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 (Zaragoza et al., 1999). 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 (Deepak et al., 2013).

Conclusion

This research evaluated the antibacterial efficacy of popular brands of hand sanitizers. The products showed varying level of inhibition against the test organisms. HS performed best in terms of inhibitory action against the test organisms and in reducing mean log counts of bacteria on the hands of subjects. Even though the products showed bactericidal effect the hand sanitizers failed to achieve 99.9% killing of bacteria as was claimed on their labels. Antibacterial activity of these sanitizers different from each other. Increased concentrations (25µl, 50 µl, 75 µl & 100µl) of avagard showed good results, where as lesser concentrations (0.5µl, 1µl 5µl, 10µl,

15µl, 20µl) haven't showed the antibacterial activity. In the case of dettol all the concentration (from lower to higher) showed good results, The dettolis much stronger than avagard in the antibacterial activity having well established inhibition zones against both gram positive and gram negative bacteria. Furthermore, creating awareness regarding the importance and efficacy of hand sanitizers in reducing transient bacteria is necessary to increase use of hand sanitizer and reduce the consequences occurred due to transient bacteria.

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