EVALUATION OF ANTIMICROBIAL ACTIVITY OF DIFFERENT HAND SANITIZERS AGAINST CERTAIN DAILY ENCOUNTERED BACTERIAL STRAINS

Mrs. JISHA.P.J

Post Graduate Department of Microbiology,

EMEA College of Arts and science, Kondotty-673638 Kerala,India.

ABSTRACT

Hands are the most important in the transmission and spread of pathogens that causes disease. Hand hygiene has been said to be the most important way to avoid these infections. Hand hygiene, particularly hand sanitizing, is essential in reducing infectious disease transmission. This study is set out to evaluate the antimicrobial efficacy of four different hand sanitizers which were selected from ten different sanitizers against *Staphylococcus aureus* and *Escherichia coli*, and as well as to assess and compare the antimicrobial effectiveness among four different hand sanitizers by three different methods. Results showed higher inhibitory activity of the products to *E.coli* than *Staphylococcus aureus*. The hand sanitizing products tested in this study are suitable in disease prevention.

INTRODUCTION

Bacterial inhabitants in hand are considered as an important method for transmission of infection among people or from the patient to the health care worker. So it is very important to take utmost care to practise hand hygiene. Failure to perform appropriate hand cleanliness can cause hospital infections which results in the rise of multi-resistant microorganisms [1]. Hospital infections are caused by opportunistic microorganisms and include species such as *Staphylococcus aureus, Enterococcus* spp*, Pseudomonas* spp. and *Escherichia coli*.Most of the hospital infections occur in central nervous system, urinary tract, respiratory tract, burns skin, blood, gastrointestinal tract, and surgical wounds [2].Hand sanitizer is a disinfectant that works without water [3]. It helps to prevent bacterial transfer and reduces acquired hospital infections. Ethyl alcohol is one of the major components of hand sanitizers (about 60%). Health care industry use hand sanitizer widely against spread of disease.[4].Hand sanitizers are available in diverse forms as gel or as liquid and are formulations such as alcoholic and non-alcoholic [5, 6]. Antimicrobial property of the antiseptics is due to their active ingredients while propylene glycol, glycerin, polyacrylic acid are the inactive ingredients which softens the effect of alcohol containing antiseptics on the skin and moisturizes the skin surface for a long time[5]. Besides these, non-alcoholic hand rubs are also available based on benzalkonium chloride or povidone-iodine chlorinated aromatic compound triclosan and pyroglutamic acids, but studies in these are limited. The mechanism of action of the sanitizers is more or less similar to denaturing the proteins of microbes. Lipids are also denatured by alcohol and causes dehydration in bacteria. On the other hand, Benzalkonium chloride has the ability to denature cell membrane and protein, and effective against wide range of organisms [7].

MATERIALS AND METHODS

The present study is an in vitro study conducted on ten different brands of hand sanitizers were selected out of many available in the market. The selected hand sanitizers to test their antimicrobial efficacy were Q7 Instant Hand Sanitizer(Q7I), Sterimil Plus(SPS), Purest(PRT), Avagard(AGD), Hexigard(HDR), ChlorohexidineGluconate solution IP(NAP), Kainetaz(KAZ), Life Gard(LGD), ChlorohexidineGluconate and

Alcohol Handrub(CGA) and Clean Hands(CHS).In this study, *S. aureus* and *Escherichia coli* were used. These bacterial strains were obtained from the Microbiology department, EMEA College culture collection.

1.Collection of hand sanitizer

To achieve this experimental study different type of common liquid sanitizers were bought from shops and hospitals for testing the activity of this sanitizer to inhibit the growth of bacteria. The hand sanitizers used were Q7I, NAP, CGA, SPS, PRT, KAZ, AGD, HDR, LGD, and CHS.

2. Agar diffusion test (well variant) to determine susceptibility of test organisms to hand sanitizers

Agar Diffusion Test

To determine the susceptibility test of selected test organisms for each hand sanitizer well variant agar diffusion method was used.[8].This agar diffusion method was done in duplicates for each hand sanitizer by inoculated sterile Mueller Hinton agar plates using sterile cotton swab which was immersed into a tube containing standardized test organisms. After Mueller Hinton agar was inoculated, it was allowed to remain at room temperature to dry for only some minutes and 5 equally spaced holes were bored in the agar plate with the 5th hole at the center of the plate with the aid of a sterile 6mm cork borer. The 4 holes were filled with 50µL of the hand sanitizer at the same time as the central hole (5th) was filled with an equal volume of sterile water for control purposes. The Mueller Hinton agar was incubated for 24 hours at 37° C. The zones of inhibition (susceptibility or resistance) of the hand sanitizers to each test organism were examined with the help of a ruler in millimeter by evaluating the average of 2 readings that were found from duplicates of agar diffusion test for each hand sanitizers.[8]

3. Testing the antimicrobial activity of hand sanitizer by pour plate method

1, 2, 3 and 4 millilitres of the hand sanitizers were placed on the centre of 4 sterile petridish by using a sterile pipette. Sterile molten nutrient agar was added and mixed with the sanitizers by swirling the plates. The plates were allowed to cool at room temperature until solidified. Each nutrient agar plates was marked into two compartments and the test organisms (*E.coli, Stap.aureus*) were streaked on the plates. The plates were incubated for 24 hours at 37 °c. After incubation, then examined for the antimicrobial activity on hand sanitizer against bacteria.Later volume of sanitizers was reduced for getting accurate results.

4. Determination of minimum inhibitory concentration (MIC) – Dilution method

Add 1 ml of undiluted sanitizers to 9 ml of distilled water and shaken thoroughly by proper mixing. The serial dilution was prepared up to 10^{-6}. Add 0.1 ml of standardized test organism (*Staphylococcus aureus* and *E. coli*) to dilution tubes. Each dilution was inoculated on nutrient agar. Accordingly, individual plates contain 6 streaks of dilution. Each dilution is added to its appropriate plates containing test organism. Incubation of plates was at 24 hours at 37 °c. [9] After incubation, it is then examined for growth which is evidenced by turbidity of medium. The MIC was recorded as the lowest concentration of sanitizer which is indicated by lack of turbidity.

RESULT

The selected 10 sanitizers were checked for their effectiveness against *Staphylococcus aureus* and *E.coli*.

SANITIZER	*E coli*		*S.aureus*	
	1	2	1	2
NAP	-	+	+	+
Q7I	++	++	+++	+++
CGA	+	+	-	-
SPS	-	-	+	+
PRT	-	+	+	+
KAZ	+	+	+	+
LGD	+++	+++	++	++
CHS	++	++	++	++
AGD	-	-	+	+
HDR	-	-	+	+

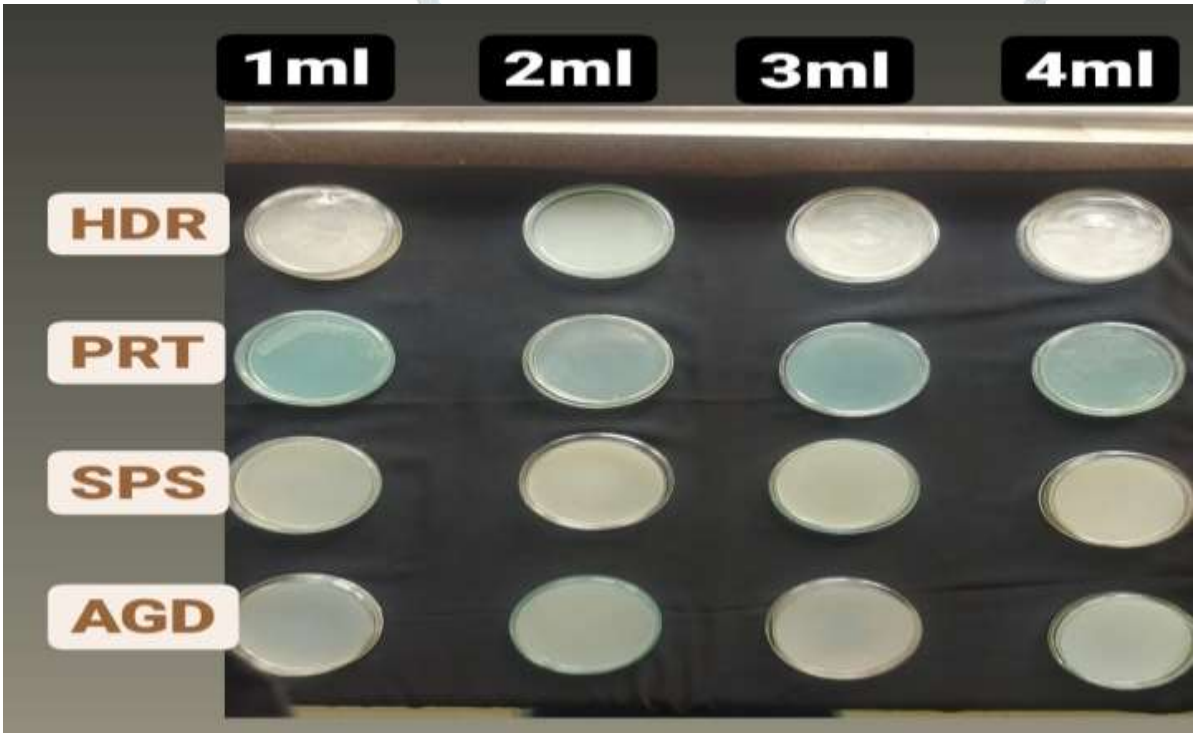
Then from these SPS, HDR, AGD and PRT were selected for further experiments

1.Agar diffusion test(well variant) to determine susceptibility of test organism to hand sanitizers

SANITIZER	SPS	HDR	PRT	AGD
E.coli	1.2cm	0.8cm	-	0.6cm
S.aureus	1.6cm	1.2cm	1.0cm	1.1cm

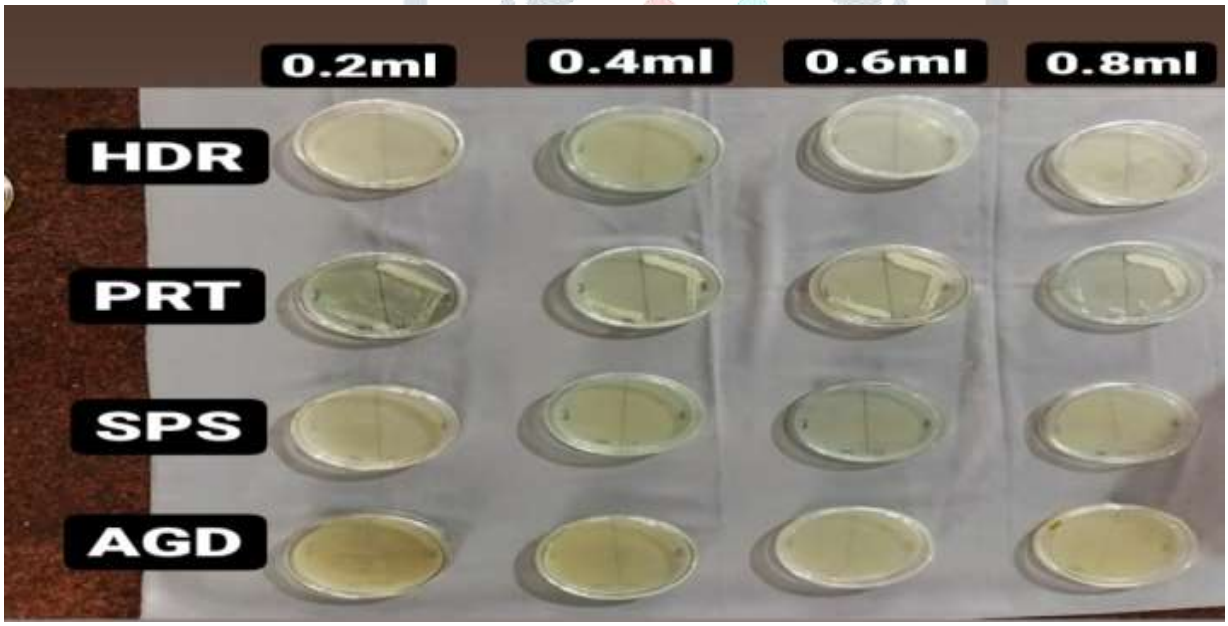
2.Testing the antimicrobial activity of hand sanitizer by pour plate method

Sanitizers	1 ml		2 ml		3ml		4 ml	
	S.aureus	*E.coli*	*S,aureus*	*E.coli*	*S.aureus*	*E.coli*	*S,aureus*	*E.coli*
HDR	-	-	-	-	-	-	-	-
PRT	+	-	-	-	-	-	-	-
SPS	-	-	-	-	-	-	-	-
AGD	-	-	-	-	-	-	-	-



For better testing 0.2, 0.4, 0.6 and 0.8 ml of sanitizers were used

Sanitizers	0.2 ml		0.4 ml		0.6 ml		0.8 ml	
	S.aureus	*E.coli*	*S,aureus*	*E.coli*	*S,aureus*	*E.coli*	*S,aureus*	*E.coli*
HDR	-	-	-	-	-	-	-	-
PRT	+	+	+	+	+	+	+	+
SPS	-	-	-	-	-	-	-	-
AGD	-	-	-	-	-	-	-	-



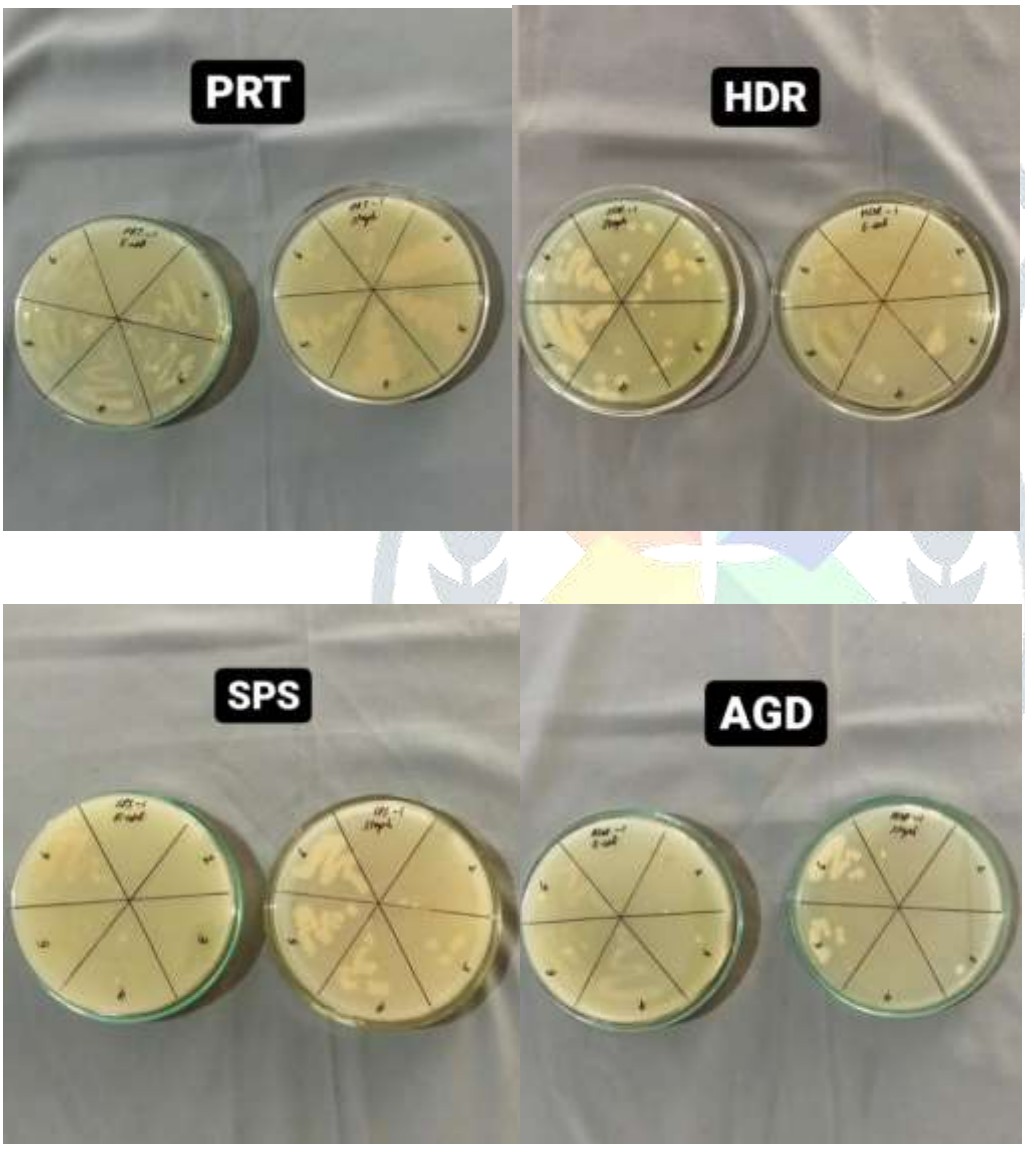
3. Determination of minimum inhibitory concentration (MIC) dilution method

SANITIZER	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}
PRT	-	+	+	++	++	++
HDR	-	-	-	-	+	+
SPS	-	-	-	+	++	++
AGD	-	-	-	-	+	++

Staphylococcus aureus

SANITIZER	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}
PRT	-	+	+	++	++	++
HDR	-	-	-	-	+	+
SPS	-	-	-	-	-	+
AGD	-	-	-	+	+	+

E.coli



DISCUSSION

The average number of micro-organisms on the hands needed for the spread of pathogens remains unknown. It may depend on various factors like the type and duration of contact, the type of micro-organism, the patient's resident flora, and their colonization resistance [10].So in order to overcome the negative impact of microbial contamination in health-care sectors, hand sanitizers are normally recommended as an adjunct to plain hand washing [11]. Alcohol was the main active ingredient in alcohol-based hand sanitizer which exerts antimicrobial activity by causing denaturation of protein, disruption of cell membranes, and

dissolution of lipid components [12]. Alcohol is supposed to have increasing effectiveness from 60% to 90% with 1-propanol being the most effective followed by 2-propanol and finally by ethanol, whereas Coriander, Lime, and Neem are the active ingredients responsible for antimicrobial activity in herbal hand sanitizers.

Hand sanitizers used were effective against all the test organisms. After doing the dilution method with 10 sanitizers used, 4 sanitizer's were selected for further examination. First the antimicrobial effectiveness was assessed by measuring the zone of inhibition against the particular test organism. Maximum inhibition was seen in sanitizer SPS against both test organisms. Testing the antimicrobial activity of hand sanitizers by pour plate method was done by 1ml, 2ml, 3ml, and 4ml dilutions. Where *Staphylococcus aureus* growth is seen only in PRT sanitizers (1ml). In *E.coli*, there is no growth observed in any of the sanitizer. For better testing 0.2, 0.4, 0.6, 0.8 ml of sanitizers were used. The growth of both organism was only showed in PRT sanitizer. So, least antimicrobial activity was shown by PRT sanitizer.

Determination of Minimum Inhibitory Concentration (MIC) is done by dilution method. MIC testing was carried out to determine the minimum concentration of test substance which could cause an inhibition of the growth of the test isolates. PRT had MIC of dilution 10^{-2}while SPS, HDR and AGD had MIC of dilution 10^{-4}.

CONCLUSION

The potency of hand sanitizers is very important to enhance the antimicrobial activity of these hand sanitizers towards controlling microbial population which includes prevention of diseases, transmission and infection. Determination of antimicrobial effectiveness of hand sanitizers is essential to achieve total destruction of pathogens. This project assessed the antimicrobial effectiveness of popular brands of hand sanitizers showed varying level of inhibition against the test organisms. The products show almost equal level of inhibition against *E.coli* and *Staphylococcus aureus*. After testing the activity of 10 brands of hand sanitizers the result showed that the sanitizers HDR and AGD possessed high antimicrobial activity , as all the test isolates were sensitive to them, and sanitizer PRT showed least effectiveness against the test organisms. All the sanitizers tested showed more effectiveness against the test organism *E.coli* than *Staphylococcus*. Apart from the findings, these products do not fulfil the claim of their manufactures as having 99.99% germicidal.

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