

Evaluation of the efficiency of Non alcoholic-Hand Gel Sanitizers products as an antibacterial

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Abstract:

Companies producing hygiene products have offered a solution—sanitary, antibacterial, antimicrobial, antibiotic, wipes, and soaps to destroy anything that dares to creep into our wholesome lives. These salves will cure us of the demons that dare to grow near us. Antimicrobial hand gel sanitizers provide a greater bacterial reduction than others. However, the link between greater bacterial reduction and a reduction of disease has not been definitively demonstrated. Confounding factors, such as compliance, composition, and frequent use, may all influence the outcomes of studies. Therefore, this research addresses the challenge of improving hand hygiene through using non alcoholic hand gel sanitizers. The antibacterial efficacy of these products was to be evaluated and compared through studying the response of organisms to cleaning regimens in healthcare settings using different responses of various hand gel sanitizers that are sold in Saudi market in Jeddah . [Salha H.M. Al-Zahrani and Afraa A. Baghdadi. **Evaluation of the efficiency of Non alcoholic-Hand Gel Sanitizers products as an antibacterial.** Nat.Sci;2012,10(6):15-20](ISSN:1545-0740) <http://www.sciencepub.net/nature>.4

Key words: Hand gel sanitizers, antimicrobial agent,

1.Introduction:

Hands are primary mode of transmission of microbes and infections ,Mondal and Kolhapure (2004) . Through hand hygiene a simple and least expensive means of preventing health care-associated infections specially derived from environmental surfaces,Pittet et al.,(1999).

A wide variety of active chemical agents (or “biocides”) are found in these products, many of which have been used for hundreds of years for antiseptis, disinfection, and preservation, Block (1991) .It is important to note that many of these biocides may be used singly or in combination in a variety of products which vary considerably in activity against microorganisms. Russell et al (1992;1995) reported that antimicrobial activity can be influenced by many factors such as formulation effects, presence of an organic load, synergy, temperature, dilution, and test method, Interaction of the antiseptic or disinfectant with the cell surface followed by penetration into the cell and action at the target site(s) is the normal mechanism of action. The nature and composition of the surface vary from one cell type (or entity) to another but can also alter as a result of changes in the environment, Brown and Gilbert (1993). Interaction at the cell

surface can produce a significant effect on viability (e.g. with glutaraldehyde), Russell (1994);Power (1995), but most antimicrobial agents appear to be active intracellular, Russell and Chopra(1996). The outermost layers of microbial cells can thus have a significant effect on their susceptibility (or insusceptibility) to antiseptics and disinfectants; it is disappointing how little is known about the passage of these antimicrobial agents into different types of microorganisms. Potentiating of activity of most biocides may be achieved.

Traditionally, microbes incorporating hands are divided into resident and transient flora. Resident flora e.g. *Staphylococcus aureus*, *Staphylococcus epidermidis* and *Streptococcus viridians* which always colonizing deeper skin layers which always resistant to mechanical removing and had lower pathogenic potential. Ttransient flora e.g. *Staphylococcus aureus*, Gram negative bacilli colonizes the superficial skin layers for short periods, Widmer (2000). Therefore we chose these organisms to determine the effect on their susceptibility to tested Sanitizers tested.

Kampf and Ostermeyer (2005) reported that most of sanitizer contains in its composition alcohols. Ethanol can destroy bacteria by causing membrane damage and denaturation of proteins. Ethanol also prevents the spread of microbes by interfering with cell metabolism and cell division. Therefore ,it is effective for hand disinfection against bacteria after 0 and 3 hours of application, Other hand gel sanitizers do not contain alcohol

. Therefore, we aimed at shedding light on the response of these organisms to cleaning regimens in healthcare settings using different responses of using various hand gel sanitizers some of that are sold in Saudi market in Jeddah for education in reducing infectious disease symptoms transmission among consumers.

2.Material and Methods

2.1. Materials:

2.1.1. Samples:

a- Swabs from hand skin of (10) volunteers without any clinical signs of dermal abrasion, trauma and infection were included in this study.

Approximately 0.5 ml of sanitizer were applied to hands and asked to rub hands ensuring that all hand surface were covered by the sanitizer under investigation ,Rubbing continued thoroughly until

hand become dry, process was educated for subjects according to WHO (2006) .

Surface samples were obtained by swabbing each individual surface to take swabs from both hands (dorsal and ventral) including nails and fingers with a sterile polyester fiber-tipped transport system collection swab moistened in transport medium (BBL Culture swabs [Becton Dickinson and Company, Sparks, MD]) .Samples were collected before and after using of the hand gel sanitizer under investigation .

Hand sanitizers used were applied each in a separate day; over a period of 7 days to collect samples concerning the different sanitizers.

B-Bacterial samples:

Bacterial suspension of concentration 10^6 CFU/ml suspension was used.

-Gram-positive bacteria:

Bacillus subtilis ATCC6633; *Staphylococcus aureus* ATCC29213 and *S. epidermidis* was obtained from the laboratory of Jeddah King Fahad Hospital in Saudi Arabia

-Gram negative bacteria:

Escherichia coli ATCC25422, *P. aeruginosa*, were obtained from the library of military Hospital in Riyadh..

2.1.2. Hand gel sanitizers Products

Seven different marketed products of hand gel sanitizers were analyzed to determine the best composition among these products in order to record the optimal antiseptic composition.

2.1.3. Media:

A- Muller–Hinton agar medium (Oxoid CM 41), Hampshire, England) is used in agar diffusion method.

B- Nutrient broth and agar for bacterial isolate preservation

C- MacConkey agar (Oxoid)

2.2. Methods:

2.2.1. Standardization of Inoculum:

The inocula were prepared from the stock cultures, were maintained on nutrient agar at 4°C and sub cultured onto Nutrient broth using a sterile wire loop. The density of suspension inoculated onto the media for susceptibility test was determined by comparison with 0.5 McFarland standard of Barium sulphate solution (Cheesbrough,2002).

2.2.2. Hand gel sanitizers Products preparation for analysis:

Serial double dilution was carried out by adding 1ml of each sanitizer at each serial dilution. Four concentrations were prepared from the original solution. Such that each 0, 01 ml

(Dropped in each agar well) was equivalent to 500µl, 1000 µl, 2000 µl and 4000 µl.

2.2.3. Disc Agar Diffusion Technique:

Technique cited after OIE (2008)

Disc Agar Diffusion Technique described by Bauer et al., (1966) and demonstrated by Cakir et al., (2004) was employed for antibacterial bioassay. The seven different marketed products of hand gel sanitizers were separately determined in plates containing 15 ml of Muller–Hinton agar medium (Oxoid CM 41), Hampshire, England). Each plate was seeded one of the five bacterial culture strains (which was enriched in Nutrient broth). 0.1ml of each gel sanitizer was poured in pores (5mm in diameter) in Muller–Hinton agar medium.

The plates were allowed to stand at refrigerator temperature for 2 h for the compound to diffuse into the agar and then the cultures were incubated at 35 °C for 24 h. After incubation antibacterial effectiveness were determined by measuring the diameter of the inhibition zone formed around the pores for each compound.

2.2.4. Determination of Minimum Inhibitory Concentration (MIC) :

This method was used because the main advantage of its use lies in the fact that it can readily be converted to determine the MBC as well. Dilutions and inoculations are prepared in the same manner as described for the determination of MIC. The control tube containing no hand gel sanitizer is immediately sub cultured (Before incubation) by spreading a loopful evenly over a quarter of the plate on a medium suitable for the growth of the test organism and incubated at 37°C overnight. The tubes are also incubated overnight at 37°C. Note the lowest concentration inhibiting growth of the organisms and record this as the MIC. Subculture all tubes not showing visible growth in the same manner as the control tube described above and incubate at 37°C overnight. Compare the amount of growth from the control tube before incubation, which represents the original inoculum. The test must include a second set of the same dilutions inoculated with an organism of known sensitivity .These tubes are not sub cultured; the purpose of the control is to confirm by its MIC that the drug level is correct, whether or not this organism was killed is immaterial. No growth- if the whole inoculums has been killed or the highest dilution showing at least 99% inhibition is taken as MBC.

2.2.5. Microbiological sample processing :

Aerobic and anaerobic media (Nutrient agar and MacConkey agar) was placed in the Petri dishes . All Petri dishes for both aerobic and anaerobic media were marked before application (Bf) and after application (Aft). Swabs were inoculated on the surface of the media. The Petri dishes were incubated at 37 °C for 24-48 hours. Bacterial smear

resulted from the culture was stained by Gram stain and examined for bacterial presence. The same procedure was obeyed for the 7 consecutive days on all subjects.

3. Results:

According to the zone of inhibition formed resulting from each sanitizer against different bacterial isolates, statistical analysis findings in table (1) showed that hand gel sanitizer No. (6)

Was the most broad spectrum antibacterial agent with different response for different bacterial kinds tested, followed by No. 1 although it showed a higher score against Gram negative more than Gram positive, whereas No. 2 was anti Gram negative agent. Other sanitizer gel kinds were effective against certain kind of bacteria with a limited scale. No sanitizer gel inhibited *S. epidermidis*.

Table (1) : Antibacterial effect of Hand Gel Sanitizers products on selected Gram- positive and Gram negative bacteria

Hand Gel Sanitizer	Diameter in mm of Inhibition Zone				
	Gram Negative			Gram Positive	
	<i>Escherichia coli</i> ATCC25422	<i>P. aeruginosa</i>	<i>Bacillus subtilis</i> ATCC6633	<i>S. aureus</i> ATCC 29213	<i>S. epidermidis</i>
1	-	10.67±0.434	9±0.804	4.67±0.161	-
2	-	17.67±0.200	12.33±0.844	-	-
3	-	-	3.67±0.657	8.33±0.289	-
4	-	-	-	7±0.866	-
5	-	-	4.33±0.355	9±0.100	-
6	7±0.100	3.67±0.804	2±0.332	10.33±0.419	-
7	-	-	3±0.165		

Table (2) Comparing effect demonstrated that only hand gel sanitizer No. 2 and 5 were effective as bactericidal agent with all subjects (100%) activity. Followed by No. 5 (70%).

No. (1 and 3) was showed to be bactericidal agent among 70 and 80% respectively of cases whereas,

it was not effective at all among the rest..No. (6) was not promising as anti bacterial agents because bacterial count was either the same or increased in most of cases. No. (4 and 7) Showed bactericidal effect among 50 and 60% of cases respectively and an increase in bacterial number in the rest .

Table 2. Effect of using hand gel sanitizers on volunteers hand skins as bactericidal.

Hand gel Sanitizers Volunteers	Bacterial number before using hand gel sanitizers	Bacterial number after using hand gel sanitizers						
		1	2	3	4	5	6	7
A	34	-	-	-	-	27	-	-
B	24	-	-	9	-	-	22	22
C	10	-	-	34	-	-	-	-
D	5	-	-	-	4	-	5	-
E	6	15	-	2	-	-	4	25
F	20	-	-	-	-	-	25	-
G	25	30	-	2	95	-	30	-
H	50	25	-	-	100	-	50	50
I	10	-	-	25	25	4	50	30
J	20	-	-	4	50	5	30	-
Total		70%	100%	80%	50%	100%	20%	60%

Table (3) showed that sanitizer gel No. (2) was the most effective as broad spectrum bactericidal concerning the higher concentrations (2%,6%,8%). Whereas, No. 3, 4, 5 was considered anti-Gram negative antibactericidal among concentrations 6% and 8% .

No. (1) was anti-Gram positive antibactericidal within all concentrations. Other sanitizer gels were considered bacteriostatic agents.

Table (3): Differentiation of sanitizer activity as bactericidal or bacteriostatic effect

Sanitizer gel	Sanitizer gel Conc. %	Bacterial Isolates				
		Gram Negative			Gram Positive	
		<i>Escherichia coli</i> ATCC25422	<i>P. aeruginosa</i>	<i>Bacillus subtilis</i> ATCC6633	<i>S. epidemidis</i>	<i>S. aureus</i> ATCC 29213
1	2	-	-	+	+	-
	4	-	-	+	+	-
	6	-	-	+	+	-
	8	-	-	+	+	-
2	2	-	-	-	+	-
	4	+	-	+	+	-
	6	+	-	+	+	-
	8	+	-	+	+	-
3	2	+	-	-	-	-
	4	+	-	-	-	-
	6	+	-	+	-	-
	8	+	-	+	-	-
4	2	-	-	-	-	-
	4	-	-	+	-	-
	6	+	-	+	-	-
	8	+	-	+	-	-
5	2	-	-	-	-	-
	4	+	-	+	-	-
	6	+	-	+	-	-
	8	+	-	+	-	-
6	2	-	-	-	-	-
	4	-	-	-	-	-
	6	-	-	-	+	-
	8	-	-	-	+	-
7	2	-	-	-	-	-
	4	-	-	-	+	-
	6	-	-	-	+	-
	8	-	-	+	+	-

4. Discussion:

Infection with environmental microbes is increasing alarmingly.

Opportunistic microorganisms can cause different infections and multidrug-resistant pathogens that are commonly involved in some infectious diseases are difficult to treat. The transfer of bacteria from the hands to food, objects, or people plays an important role in the spread of diseases, Kimura et al., (2004). Normal human skin always harbors bacteria (10^2 and 10^6 CFU/cm²), Mondal and Kolhabure (2004). The critical density of microorganisms on the hands needed for the spread of pathogens remains unknown and it may depend on the type of micro-organism, the patient's resident flora and their colonization resistance, Mondal and Kolhabure (2004).

Well-controlled studies in the health care setting and home setting pose numerous challenges, which can affect the findings. The test parameters used in

published studies have not been consistent, and therefore, the effect that they may have had makes drawing definitive conclusions on the comparative activities of hand wash products problematic.

To reduce infections in healthcare settings, alcohol-based hand sanitizers are recommended as a component of hand hygiene, Boyce and Pittet, (2002). Food and Drug Administration (FDA) recommends a concentration of 60% to 95% ethanol or isopropanol, the concentration range is of greatest germicidal efficacy, OIC (2008).

Some products marketed to the public as antimicrobial hand sanitizers do not contain alcohol despite a label claim of reducing "germs and harmful bacteria" by 99.9%.

Their main composition were fragrance ingredients, glycerol and /or carbamore. In this study, their antibacterial activity were checked assuming the therapeutic efficacy.

Fragrance ingredients are many including ketones, aldehyde, lactone, phenol derivatives, aliphatic alcohol and quinolone, others are natural. They all proved from ancient times as antiseptics and preservatives, as well as, aromatic agents, Cowan (1999). Antimicrobial activity against bacteria and fungi has been demonstrated for many kinds of fragrance ingredients, Suppakul et al.,(2003); Kalemba and Kunicka(2003);Burt(2004);Carson et al.,(2006).From 1950 till (2009)when Nho et al., suggested that glycerol derivatives e.g. propylene glycol and trimethylene glycol was investigated as antimicrobial agents to a certain extent. They found that antimicrobial activity was increased with the number of substituent on the nucleus and to a certain extent was a function of the position of substitution.. While Carbomer derivatives has proved to improve skin absorption and formulation viscosity, effect can give sustained release of drugs onto the skin.

Statistical analysis for the comparison between the efficacies of the seven chased hand gel sanitizers showed that only one in different concentrations could inhibit Gram negative and Gram positive bacteria under investigation (that always harbor hands). Other sanitizers showed a limited action against either Gram negative or Gram positive used

Despite a label claim of reducing “germs and harmful bacteria” by 99.9%, we observed an apparent increase in the concentration of bacteria in handprints impressed on agar plates after cleansing. Our finding demonstrated that only two of sanitizers under investigation showed 100% effectiveness and the higher % for the two most potent was 80 and 70% ,the three other sanitizers contributed a less percentage (40,50 and 20%) .This indicated that Hand hygiene promotion interventions did not result in sustained improvement in hand hygiene associated with the use of the tested hand gel sanitizers, and an assessment of their impact on individual infection risk are lacking therapeutic effectiveness.

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