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Determining the In Vitro Efficiency of Handwashes

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Abstract: ***Hand washing, is the act of cleaning hands for the purpose of removing soil, dirt, and microorganisms. Though washing hands can be done with bar soap and water but now in this fashionista world, liquid handwash has taken the lead with the advantage, its each drop is free from exposure to organism which are chances with bar soap from people repeatedly washing hands with same soap. The concentration and time required for the handwash to clean hands matters the most. The type and nature of active molecule present in handwashes decide their efficiency for cleaning hands. Among the compared handwashes in the present study, though all exhibited antimicrobial activity, medicated handwash depicted bactericidal activity in less time as compared to ayurvedic and fragrant handwashes. This study provides practical information that medicated handwashes do have an upper hand but organisms are now slowly developing tolerance against the active molecule present in these handwashes.***
Keywords: liquid handwash, pathogenic isolates, resistance, mutation, hygiene

I. INTRODUCTION

Hand washing with soap and water is common, relevant, inexpensive method for prevention of the transmission of infection as it is effective against various contaminants including pathogenic bacteria and/or viruses (Macinga et al., 2008, Obi et al., 2007, Kim et al., 2015, Oranusi et al., 2013). Medical hand hygiene prevails to the hygienic practices related to administration of medicine and medical care that either prevents or minimizes disease and the spreading of it. For infection control, proper and frequent hand washing is must (Carita et al., 2012). It is estimated that simple hand washing and maintaining hand hygiene leads to reduction in rate of infectious diseases and thus could save one million lives per year (Montville et al., 2002). Skin, especially hands are required to be safeguarded from the bacterial pathogens as they are most widely exposed part of the body (Choudhari et al. 2015).

Many viruses and bacteria enter in our body through nose or mouth.

This is because the dirty hands are in constant touch with the mouth or nose while sneezing or coughing. Moreover, various skin infections are now persistent due to unclean hands.

The exposure and unrolling of micro-organisms with decreasing susceptibility to antimicrobial agents is a major public health problem. Hands are the prime tool in our everyday life. They are the primary mode of transmission of infection as they are open to a lot of substances which encompasses of dirt, touching hands during personal hygiene. Hands execute variety of functions of the human body and thus are open to diverse substances that cause various diseases including intestinal infections, SARS, Hepatitis A, skin infections, and food borne infection (Sajed et al., 2014, Shah et al., 2014, Cannon et al., 2012).

One of the ways to kill the micro-organisms and keep the hands clean is by the use of hand wash, which in turn protects the skin from deleterious micro-organisms and thus avoiding the spread of communicable disease (Shah et al., 2014). The only way to keep infection under control is proper and frequent hand hygiene (Macinga et al., 2008).

Various studies have divulge that liquid soaps consist of active antimicrobial ingredients condemn more bacteria as contrast to plain soap. Liquid based hand washes are proved to be more effective than alcohol-based hand rubs (Cannon et al., 2012). Being a rock-bottom method, hand washing with soap and water has been used since coon's age (Kim et al., 2015). Yet, to out caste the old tradition of soaps, on the double; liquid hand washes are used over bar soaps to avoid wastage of soap and the important reason being hygiene.

Therefore, liquid hand washes being need of hour is the easy way and used a lot these days. In this fashionista world, liquid hand washes are given more preferences than the bar soaps adding into the advantage that; liquid hand washes are more hygienic, as the actual soapy content is untouched by the previous user.

Nowadays in market variants of liquid hand washes are available, leaving the common people in dilemma for the appropriate selection. Testing the efficiency of different hand washes including herbal, medicated and fragrant succor people to select the proper hand washes to avoid getting hands exposed to various disease causing organisms. Testing efficiency of these hand washes help to maintain proper hygiene in hospitals thereby preventing nosocomial infections too.

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II. MATERIAL AND METHODS

A. *Types Of Hand Washes Used*

This study was conducted in, Department of Microbiology, R.K.Talreja College, Maharashtra, India. The liquid hand wash sample of three different criteria, i.e, medicated, fragrant and herbal used for the study were purchased from standard cosmetics and pharmacy stores. The expiry dates, batch numbers and if or not the manufacturers seal is present was recorded.

B. *Collection Of Sample From Hands*

Swabbed samples from hands were collected from various category like teaching and non-teaching staff, students, doctors, meat sellers, food and vegetable vendors. Suspended samples were transported to Microbiology department in appropriate transport medium and processed immediately.

C. *Isolation, Identification And Maintenance Of Isolated Pathogens (Bergey's Manual Of Determinative Bacteriology)*

The **s**wabbed samples were streaked on sterile Nutrient agar, Thiosulfate Citrate Bile-salts Sucrose agar, MacConkey agar, Salmonella Shigella agar, and Super Imposed Blood agar plates. The plates were incubated at 37°C for 24 hours for observing growth of bacterial pathogens. The pathogens were identified on the basis of morphological, biochemical and cultural characteristics. Further these pathogens were maintained by serial subculture method.

D. *Antibiotic Susceptibility Testing (AST) by Kirby-Bauer disc diffusion method (Bauer et al, 1996)*

The pathogenic isolates obtained from hands of different volunteers were swabbed on sterile Muller and Hinton agar plates. Four antibiotic discs, Cefazolin (30µg), Amikacin (30µg), Ceftazidime (30µg) and Ofloxacin (5µg) for Gram negative pathogens and Oxacillin (5µg), Erythromycin (10µg), Rifampin (30µg) and Ofloxacin (5µg) for Gram positive pathogens were placed on surface of swabbed plates. The plates were incubated at 37°C for24 hrs and results were noted.

E. *In Vitro Determination Of Antimicrobial Activity Of Different Handwashes*

1) *Qualitative Analysis: By Agar Well Diffusion Method (Indian Pharmacopoeia, 2007):* Qualitative analysis by agar well diffusion was done using undiluted handwashes against standard cultures of *E. coli* ATCC 25907 and *S. aureus* ATCC 6538. The plates were incubated for 24 hours at 37°C and observed for zone of inhibition.
2) *Quantitative Analysis*

a) *Minimum Bactericidal Concentration Of Handwashes (Clinical And Laboratory Standards Institute, 2012):* Six different dilutions of test handwashes were made in range of 0.1% to 20% including undiluted using sterile distilled water. Suspension of 24 hour old standard cultures of *E. coli* ATCC 25907 and *S. aureus* ATCC 6538 with O.D 0.1 at 600 nm were inoculated (0.1mL) in handwash. After 30 seconds of exposure at room temperature, a loopful (10 µL) of this mixture was spot inoculated on Nutrient agar in the grid pattern. Standard organisms unexposed to handwashes and sterile distilled water were used as positive and negative controls respectively. The plates were incubated at 37°C for 24 hours, growth and no growth of organism was observed.

b) *Time Required To Kill Standard Pathogens By Handwashes Using Grid Plate Technique (Clinical And Laboratory Standards Institute, 2012):* Two different dilutions of test hand washes were made (10% and undiluted) using sterile distilled water. Suspension of 24 hour old standard cultures of *E. coli* ATCC 25907 and *S. aureus* ATCC 6538 with O.D 0.1 at 600 nm were inoculated (0.1mL) in handwash for varying time period of 0 seconds to 30 minutes at room temperature. A loopful (10 µL) of this mixture using standard nichrome loop was spot inoculated on grids made on Nutrient agar. Appropriate controls were maintained and plates were incubated at 37°C for 24 hours, growth and no growth of organism was observed.

F. *Selection of the best handwash*

On the basis of qualitative and quantitative analysis; using standard cultures, the best handwash was selected and it was used to check its efficiency against pathogenic isolates.

G. In Vitro Efficiency Of Selected Handwash Against The Isolated Pathogens

Selected handwash with 62.5% concentration (Kim *et al*, 2015) was made using sterile distilled water. Suspension of 24 hour old isolated pathogens (O.D 0.1) were inoculated (0.1mL) in handwash. The tubes were incubated at room temperature for varying time period ranging from 0 seconds to 1 minute. A loopful (10 µL) of this mixture using standard nichrome loop was spot inoculated on grids made on Nutrient agar. Appropriate controls were maintained and plates were incubated at 37°C for 24 hours, growth and no growth of organism was observed.

III. RESULT AND DISCUSSION

A. Types Of Hand Washes Selected And Collection Of Sample

A total of three hand washes were selected as per the popularity in the society. The medicated hand wash was with 4.8% Chloroxylenol and Ayurvedic hand wash containing Azadirachta indica and Ocimum sanctum and fragrant handwash with essential oils were selected for this study.

From the hands of 16 different volunteers (two of each category), total 14 isolates were obtained. In all 6 isolates were obtained from volunteers of both fish seller and chicken seller category. Whereas single pathogenic isolate was obtained from category of vegetable seller and student. No pathogens were isolated from volunteers of chat seller, ward boys, nurses and doctor's category. Maximum number of isolates were obtained from chicken and fish sellers.

B. Identification Of Organisms

On the basis of cultural, morphological and biochemical characteristics among the isolates, maximum were *Salmonella spp* (42.85%) followed by *Vibrio spp* (28.57%), *Shigella spp* (14.28%), *E.coli* and *Staphylococcus spp* (7.14% each). Sajed *et al*. in 2014 reported somewhat similar isolates from swabbed hand samples.

C. Determining The Antibiotic Susceptibility Of Various Pathogenic Isolates

All Gram negative and Gram positive pathogens isolated from volunteers were sensitive to antibiotics used in this study except *S.aureus*. It was resistant to Ofloxacin (5 µg). There was no MDR among 14 different isolated pathogens.

D. In Vitro Determination Of Antimicrobial Activity Of Different Handwashes

1) *Qualitative Analysis: By Agar Well Diffusion Method (Indian Pharmacopoeia, 2007):* Qualitative analysis by agar well diffusion method revealed that all the hand washes used in the present study had antimicrobial activity against both standard cultures. The average zone of inhibition for standard cultures *S. aureus* ATCC 6538 and *E. coli* ATCC 29522 was in range of 12-18 mm and 12-20 mm respectively. Jayant *et al*, 2015 reported zone of inhibition in the range of 9.8-12.8 mm against *Staphylococcus aureus* by using 10% Dettol. Chaudhari *et al*, 2016, also reported inhibition in range of 14-19 mm and 12-21 mm against *S. aureus* and *E.coli* respectively with lower concentrations of herbal handwash.
2) *Quantitative Analysis:* Minimum Bactericidal Concentration (MBC) of handwash for standard organisms Standard cultures of *E.coli* ATCC 25922 and *S.aureus* ATCC 6538were subjected to different concentrations of handwash for 30 seconds and MBC was determined. The MBC of different handwashes were as mentioned in table 1

Table 1: Minimum Bactericidal Concentration In Percentage (V/V) Of Different Handwashes

	Minimum Bactericidal Concentration in percentage (v/v)		
Type of Handwash →	Medicated	Ayurvedic	Fragrant
E.coli ATCC 25922	5.0	5.0	10.0
S.aureus ATCC 6538	5.0	1.0	1.0

All three categories of handwashes were found to be effective against both standard pathogens. Among three, medicated handwash was found to be effective against both Gram positive and Gram negative standard pathogens with MBC of 5%. Ayurvedic and fragrant handwashes had more activity against Gram positive organisms than Gram negative. Whereas fragrant handwash was the least effective against Gram negative standard pathogen. Overall all three handwashes were more effective against Gram positive than Gram negative. Obi. C. N., 2014 also reported Crusader medicated soap was more effective for Gram positive organisms than Gram negative.

E. Time Required To Kill Standard Pathogens

Standard cultures of *E. coli* ATCC 25922and *S. aureus* ATCC 6538 were subjected to different time of exposure to handwash. Samples were withdrawn at various time period with 10% and undiluted concentrations of hand wash. The results were as mentioned in table 2

Table 2: Time Required To Kill Standard Pathogens By Various Hand Washes

Org. →	*E.coli* ATCC 25922						*S.aureus* ATCC 6538					
Conc. →	10% (v/v)			Undiluted			10% (v/v)			Undiluted		
Handwash→ Time	A	B	C	A	B	C	A	B	C	A	B	C
0sec	+	+	+	+	+	+	+	+	+	+	+	+
10 sec	-	+	+	-	-	-	-	-	-	-	-	-
20 sec	-	+	+	-	-	-	-	-	-	-	-	-
30 sec	-	-	-	-	-	-	-	-	-	-	-	-
1 min	-	-	-	-	-	-	-	-	-	-	-	-
5-30 min	-	-	-	-	-	-	-	-	-	-	-	-

Key:- A- Medicated, B-Ayurvedic, C- Fragrant; (+): Growth (-): No growth

The outcome of the time required to kill standard pathogens by grid plate technique revealed that all handwashes were succeed to inhibit organisms post 20 seconds. Gram positive got inhibited by all handwashes within 10 seconds of time exposure even at lower concentration of handwashes. Whereas, Gram negative organism took more time to get inhibited by ayurvedic and fragrant handwashes at lower concentrations. Kim *et al* in 2015, Riaz *et al* in 2009 and Oranusi *et al* in 2013 also reported that Gram positive organisms were more susceptible to plain and antibacterial soap than Gram negative.

F. Selection Of Handwash

Since, medicated handwash was found to be more effective and efficient in killing both Gram positive and Gram negative organisms in minimum time period at low concentration, it was selected for studying efficacy against isolated pathogens.

G. Efficacy Of Medicated Handwash Against Isolated Pathogens

Using appropriate concentration and time of exposure, the efficiency of medicated handwash against 14 different isolated pathogens and standard cultures was as depicted in table 3

Table 3: Efficacy of Medicated Handwash Against Pathogenic Isolates.

Concentration of handwash →	10% (v/v)				62.5% (v/v)			
Time (sec.) →	0	10	30	60	0	10	30	60
E. coli ATCC 25922	+	-	-	-	+	-	-	-
S. aureus ATCC 6538	+	-	-	-	+	-	-	-
Escherichia coli(n=1)	+	+	+	+	+	+	+	+
Vibrio cholera (n=4)	+	+	+	+	+	+	+	+
Salmonella paratyphi A (n=2)	+	+	+	+	+	+	+	+
Salmonella paratyphi B(n=2)	+	+	+	+	+	+	-	-
Salmonella typhi (n=2)	+	+	+	+	+	+	+	+
Shigella flexneri (n=2)	+	+	+	+	+	+	+	+
Staphylococcus aureus (n=1)	+	+	+	+	+	+	+	+

Key: - (+): Growth (-): No Growth

All pathogenic isolates sustained effect of medicated handwash at 10% concentration at various exposure period whereas 90.72% pathogens at higher concentration. This may be due to resistance developed towards active molecule present in this handwash. Development of resistance may be due to over exposure of micro-organisms to handwash and/or to various unfavorable environmental conditions ultimately resulting in reduced efficacy of handwash.

IV. CONCLUSION

Handwashing is the prime need of an hour to get rid of bacteria and germs present on the surface of hands. Merely, washing with only water isn't sufficient to clean hands. Soap has cleansing action and thus aids in handwashing. In this process of handwashing, liquid handwashes are always feasible as each drop of handwash is fresh and pure. All handwashes used in present study were bactericidal with activity against both the standard Gram positive and Gram negative pathogens, with more activity against Gram positive pathogens.

Among different handwashes, medicated handwash was the most efficient with respect to concentration and time required to kill pathogens. Fragrant handwash was the least bactericidal in action and ayurvedic handwash had better antibacterial activity but longer exposure time to exhibit its effectiveness. The majority of pathogenic isolates were not inhibited even by the most efficient medicated handwash at the concentration and time of exposure which is commonly used for washing hands. Resistance among pathogenic isolates against active molecule in this handwash is a point of major concern. Thus, in future combination of medicated and ayurvedic handwash can be formulated which may have synergistic activity and low probability of developing resistance among the pathogens.

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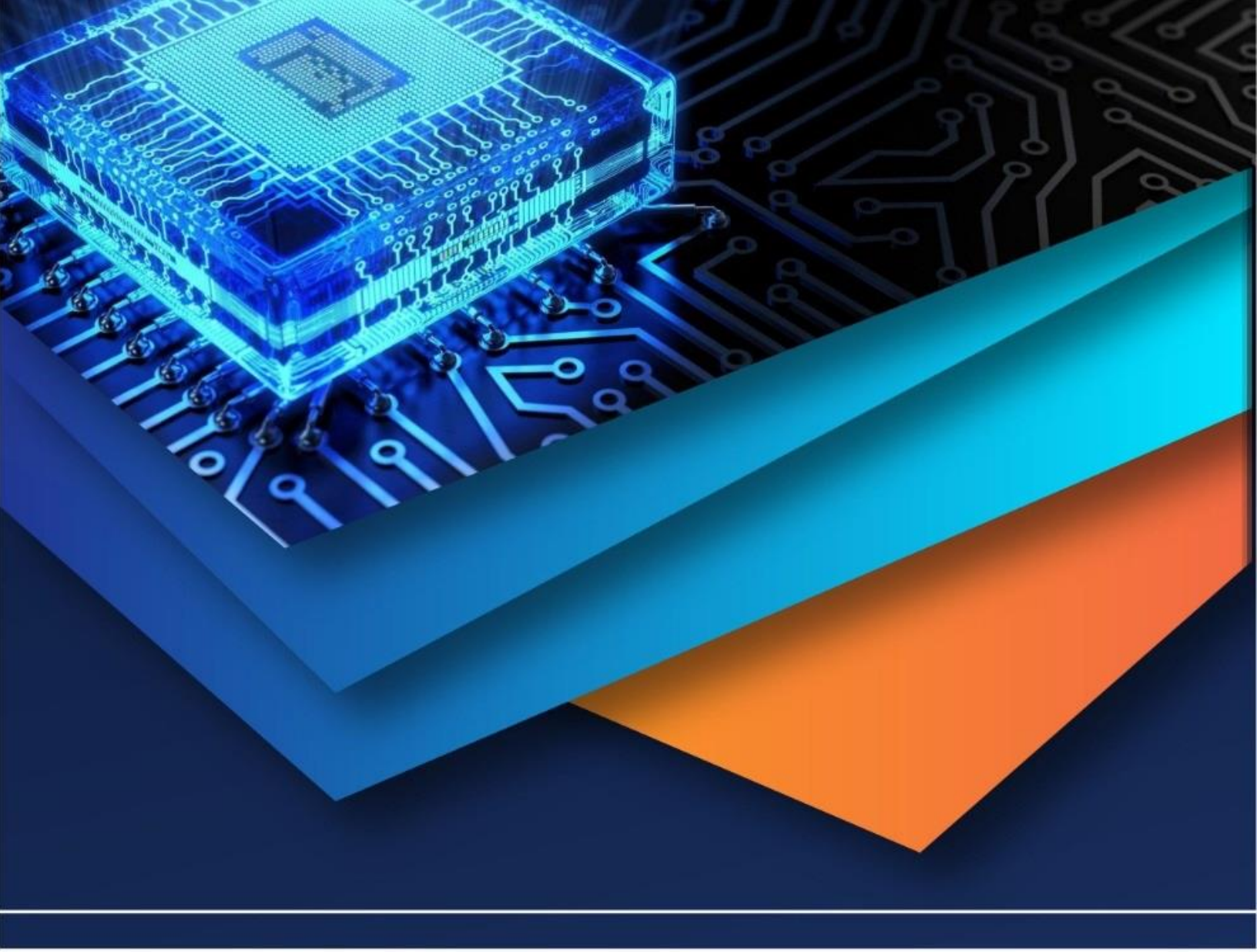

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