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Research Paper

Determining and Evaluating the Efficacy of Antibacterial and Herbal Hand Washing Solutions

Microbiology

KEYWORDS : Mcfarland, MIC, MBC, Nosocomial infections, CFU.

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ABSTRACT

Antibacterial and Herbal Hand Washing Liquid against Escherichia coli, Staphylococcus aureus, Streptococcus pneumoniae, Pseudomonas aeruginosa, Klebsiella pneumoniae. To determine the microbial quality, antibacterial efficacy and dilution effects on activity of hand washes, two brands of Antibacterial (AHWL) and four brands of Herbal Hand Washing liquid (HHWL) were evaluated using susceptibility test by Agar Well Diffusion, Minimum Inhibitory Concentration (MIC), Minimum Bactericidal Concentration (MBC), Time Kill Test, Determination of Reducing Viable Counts against Bacterial Species isolated from unwashed hands of human volunteers.HHWL-1 and AHWL-2 were active on all bacterial species and shown more active against Escherichia coli and Pseudomonas aeruginosa. HHWL-4 and AHWL-1 shown activity against some bacterial species. HHWL-2 and HHWL-3 though are sterile, were least active in all the tests. The Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of most of hand washes at 100% concentration, activity decreased when diluted with more amount of water. Time Kill Test shows that undiluted hand wash has highest effect at 5 and 10 minutes for all test organisms. It is advised that dilution of hand washes a common practice in most eateries must stop as these products are not active when diluted, hands should be washed for 5 to 10 minutes for maximum hand hygiene.

Introduction

"Prevention is better than cure." According to this keeping the hands clean is the most important measure to prevent or reduce the spread of communicable and infectious diseases. This can stop, the spread of disease among people, through the following: fecal-oral transmission,ingesting fecal materials, indirect contact with respiratory secretion, touching or shaking hands with people who cough or sneeze with their hands and contact with body fluids, which the microbes came from urine, saliva, blood and other moist body. Soap and water should be first line of defence when it comes to killing germs. However, hand washing liquid can be an effective as a quick way to disinfect hands when soap are not available[1]. Hands are primary mode of transmission of microbes and infections[2]. Through hand hygiene is a simple and least expensive means of preventing health care- associated infections specially derived from environmental surfaces[3]**.** The CDC(Clean Hands Campaign) as cited in the Mayo Clinic Website 2010[4] recommends washing hands or sanitize the hands before preparing food, eating, treating wounds or giving medicine, touching a sick or injured person, inserting or removing contact lenses, using toilet, changing diaper, touching animals or toys, leashes or waste, blowing your nose, coughing, handling garbage or something that could be contaminated.

Materials and Methods:

Samples were collected from unwashed hands of 5 different volunteers and hand washing liquids are collected from Vasantham Kings Mart, Kumbakonam, Tamilnadu, India. Based on the literature survey [5] sample was collected from the following people: After lavoratory practices, unwashed hands of students, laboratory staff, Staffs on ICU[6]**,** Health care worker in NICU[7]**.** Swab collection[8] and direct finger print impression method was used.

Mcfarland 0.5 turbidity standard was prepared according to National Committee for Clinical Laboratory Standards and verified using spectrophotometer[9]. Inoculum from a pure culture was transferred and suspended with sterile normal saline. The tube was compared with the turbidity standard and density of the organism was adjusted to standard by adding more bacteria or more sterile saline[10]**.**

Agar Diffusion Technique was described and demonstrated[11] [12]. 0.1ml of gel sanitizer was poured in pores (5mm in diameter) in Muller–Hinton agar medium with bacterial sample. 10ml of the undiluted hand washes were added to standardized test organisms for MIC. The tubes were incubated for 24h at 37°C, no growth were plated out on Nutrient agar, absence of bacterial colonies after 24h incubation was recorded as MBC.

Two, Five and Ten minutes was chosen based on length of time people spend in hand washing before eating/after using toilet[13]. To 9ml of standardized cultures in distilled water 1ml of undiluted hand washes is added. The tubes were agitated and plated on Nutrient agar, same procedure was repeated. Plates were incubated; colonies on plates were counted using digital colony counter.

Results

Table-1 shows susceptibility profile of test organisms to hand sanitizers in agar diffusion test. Zone of inhibition indicates degree of susceptibility of test organism to antibacterial agent and zone was measured using kirby-bauer scale.HHWL-1 was only product that showed inhibitory activity against all test organisms with remarkable activity against P. aeruginosa(39.0mm) and E.coli(40.0mm) and lowest against S. aureus(18.0mm).AHWL-2 was only active against E.coli(26.0mm) and S.pneumoniae (29.0mm), whereas HHWL-4 and AHWL-1 shows less activity against microbial isolates, HHWL-2 and HHWL-3 shown least activity against test organisms.

HHWL-1 and AHWL-2 were tested to determine their MIC and MBC, HHWL-1 and AHWL-2 shown inhibition at 100% concentration(Table-2) while determining the bactericidal concentration analysis, plates showed absence indicates bactericidal, antibacterial activity.

Randomly selected human were examined with HHWL-1 and AHWL-2 independently in Nutrient agar plates the same carried out with unwashed hands. Subjects left hands were gently used to make a finger impression on section A of the plate. 3ml of washing liquid was applied to hands. Process was repeated on section B of plates, colonies were counted.

Table-3 represent percentage baseline bacterial counts. Mean reduction after applying HHWL-1 and AHWL-2 was 93.6% and 79.2% respectively. There was great reduction in the organisms at great intervals. Bacteriostatic and bactericidal effect at 5 and 10 minutes, advised that hands be washed for 5 to 10 minutes for maximum result of good hand hygiene.

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Table-1: Susceptibility pattern of Test organism to Hand sanitizers in Agar Diffusion Test.

Sample No	Test organisms	Zone of inhibition (mm)					
		HHWL 1	HHWL 2	HHWL 3	HHWL 4	AHWL 1	AHWL 2
1.	E. coli	40.0	_	_	11.0	14.0	26.0
2.	S.pneumoniae	16.0	_	_	15.0	13.0	29.0
3.	S.aureus	18.0	_	_	10.0	14.0	16.0
4.	P.aeroginosa	39.0	_	_	_	11.0	28.0
5.	K.pneumoniae	33.0	_	_	12.0	15.0	20.0

HHWL-Herbal Hand Washing Liquid. AHWL-Antibacterial Hand Washing Liquid.

Table-2: Minimum Inhibitory Concentration of Test organism to Hand washing liquid in Diffusion Test.

Name of the sanitizers	Concentration	Turbidity				
		E.coli	S.pneumoniae	S.aureus	P.aeroginosa	K.pneumoniae
HHWL-1	20%	+	+	+	+	+
	40%	+	+	+	+	+
	60%	+	+	+	+	+
	80%	MG	+	MG	+	+
	100%	-	-	-	-	-
AHWL-2	20%	+	+	+	+	+
	40%	+	+	+	+	+
	60%	+	+	+	+	+
	80%	+	MG	+	+	+
	100%	-	-	-	-	-

Key: + Growth appeared , - no growth , MG Mild Growth.

Figure 1: Susceptibility pattern of Test organism to Hand sanitizers in agar diffusion test.

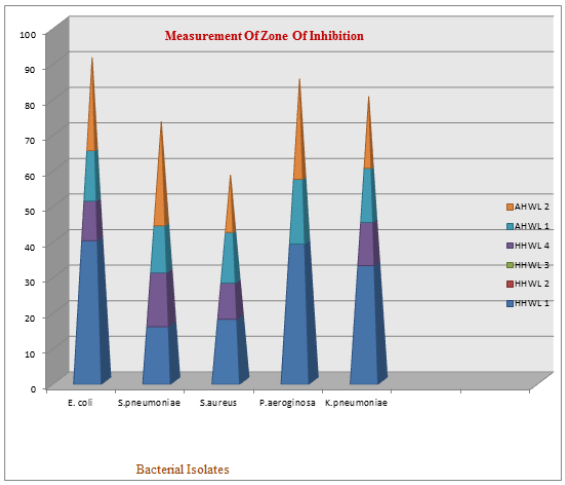
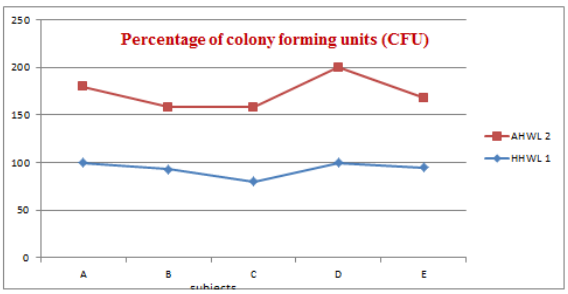


Figure 3: Percentage CFU Reduction of Viable Bacterial Count Reduction on Hands of Subjects by HHWL1 and AHWL2



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 hands to food, objects, or people plays an important role in the spread of diseases[14]. All the hand sanitizer showed antibacterial activity and efficacy in conformity with submission of Randon[15], hand washes bactericidal or bacteriostatic. Among six hand washing liquid, four (HHWL1,2,3,4) has herbal extracts along with chemical constituents. Among four, HHWL1 shows good bactericidal properties, Triclosan the major ingredient in HHWL1. These results illustrate that liquid soap significantly depressed bacterial population than plane soaps[16]. Triclosan may be responsible for very good bactericidal property than other three hand sanitizers. In antibacterial hand-washing liquid (AHWL 1&2), AHWL-2 shows higher bactericidal property than AHWL-1. Actually the liquid soaps hold ingredients (Iodophor and Triclosan). From 1950 till 2009 when Nho et al [17], suggested that glycerol derivatives e.g. propylene glycol and trimethylene glycol was investigated as antimicrobial agents to certain extent. Antimicrobial activity was increased with the number of substituent, AHWL-2 has propylene glycol, may be the substituent responsible for good antibacterial activity.

Our study suggests that liquid hand washes are more efficient against Gram-negative and Gram-positive bacteria. Comparison between the efficacies of the six chased hand gel sanitizers showed different concentrations ie.100% could inhibit Gram negative and Gram positive bacteria under investigation[18]. Activity is decreased when diluted with more amount of water.

Two, Five and Ten minutes was chosen for this test based on

questionnaire interview on length of time people spend in hand washing before eating/after using the toilet and also to accommodate for sustenance effect[19][13]. Despite a label claim of reducing "germs and harmful bacteria" by 99.9%, observed an apparent increase in the concentration of bacteria in handprints impressed on agar plates after cleansing. Our finding demonstrated that only two sanitizers HHWL-1 and AHWL-2 showed 100% effectiveness. AHWL-2 and HHWL-4 was 80 and 70%, two other HHWL-2 & HHWL-3 contributed less 20%. The results clearly proved that the herbal hand wash is more effective than the commercially available antibacterial synthetic hand washes as stated by AVS Praveen Kumar et al[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[5].

Conclusion

Most of the people do not bother to use hand washes in their daily life. Nobody knows the consequences of liquid hand washes on skin of hands and it is suitable for all type of skin. Mostly hand washes protect us from many daily encounter bacteria. We have evaluated the antibacterial efficacy of the most popular brands of hand washes sold in Tamil Nadu. Only two of the products inhibited growth of all the test organisms in vitro.

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