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ANTIBACTERIAL EFFECTIVENESS OF COMMERCIALLY AVAILABLE HAND SANITIZERS

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ABSTRACT

Hand washing is recognized as a key element to prevent the spread of infectious disease. Hand sanitation is the act of cleansing hands with sanitizers to ensure proper hand hygiene. Keeping in view, we conducted the study to evaluate the antimicrobial efficacy of twenty five different brands of hand sanitizers. The susceptibility test was performed by agar well diffusion method against *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa and Micrococcus luteus.* The study revealed the pattern of susceptibility as *Micrococcus luteus >Staphylococcus aureus > Escherichia coli > Pseudomonas aeruginosa.* Every sample showed different degree of antibacterial activity. It is universally recognized that hand hygiene is the best and most cost effective way to prevent infections and illness. The efficiency of sanitizer depends on the concentration and grade of its active ingredient.

Key Words: Hand sanitizer, susceptibility, hand hygiene.

INTRODUCTION

Hand sanitizer is an alternate way to hand washing. It can be used after hand wash or when soap and water are not available. They are available in variety of forms mostly in gel or liquid preparations. Every sanitizer has an active ingredient, may be ethanol or isopropanol. Antimicrobial property of sanitizer is based on its active ingredient. Beside these, some inactive ingredients like polyacrylic acid, glycerin, propylene glycol or extract of plants are added in hand sanitizers (Moses *et. al*., 2013).The use of alcohol based hand sanitizers may reduce the chances to spread infections in the community. It has great demand in health care facilities, schools, food processing areas etc. (Aiello *et. al*., 2008; Bloomfield *et. al*., 2007; Allegranzi and Pittet, 2007). It has been recommended by WHO as preferred method of cleaning hands especially for hospitals and clinics (WHO 2009;Pickering*et. al*., 2013). These hand sanitizers are preferred over antiseptic soaps in hospital environment in two ways, to maintain hand hygiene and to apply before surgical treatments (Boyce *et al*., 2000).

Besides alcohol as a basic ingredient, others may include quaternary ammonium compounds (QACs) and triclosan. Alcohols are broad spectrum disinfectants, kills bacteria and fungi. QACs include benzalkonium chloride or benzthonium chloride are active surfactants and broad spectrum antimicrobials, use for domestic purpose. The third type of hand sanitizers contains triclosan widely used as antibacterial in deodorant soaps, toothpastes and mouth washes.

The study was conducted to evaluate the antibacterial activity of different brands of hand sanitizers available in local market and to see which disinfectant is effective against commensals.

MATERIAL AND METHODS

Sample Collection

Twenty one hand sanitizers of different brands were purchased from local supermarkets of Karachi, Pakistan. All the samples are within their expiry date from date of manufacture. The batch number, manufacture date, product composition were recorded before analysis of samples for antimicrobial activities.

Four hand sanitizers were collected from general practioner doctors given to them complementary by different pharmaceutical companies. These samples were abbreviated as SA1 – SA4.

Bacterial Cultures

Bacterial cultures of *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Micrococcus luteus* were obtained from culture bank of Department of Microbiology, Jinnah University for Women. The bacterial cultures / test organisms were previously isolated from clinical and environmental sources. The cultures were maintained in nutrient broth, prepared as per manufacturer's instructions.

Preparation of Inoculum

Microbial cultures were inoculated in 10mL nutrient broth incubated at 37^0C for 24 h. The cultures were diluted to obtain the maximum count of 10^8 cells mL^{-1}. Nutrient agar plate was seeded with 100µL of standardized culture to check antibacterial activity.

Susceptibility Test of Hand Sanitizers

Wells were made with the help of sterile borer on seeded agar plates. The wells were filled with 100µL sanitizer samples. Sterile distilled water was used as a negative control. The plates were allowed to stand at room temperature for 3h for diffusion of the hand sanitizers and then incubated at 37^0C for 24 hrs. The antimicrobial susceptibility was indicated by the zone diameter of inhibition in millimeter. Each antimicrobial testing was performed in triplicate. The results were compared with standard streptomycin zone of inhibition.

Table 1. Standard Zone of Inhibition of Streptomycin.

Zone diameter(mm)	**Interpretation**
≥ 15	Susceptible
11-14	Intermediate
≤ 10	Resistant

RESULTS AND DISCUSSION

The purpose of hand sanitizer is to establish hand hygiene and thus preventing spread of infection. Hands are the pathways of transmission and spread of pathogens that causes diseases, food borne illness and nosocomial infections. The antimicrobial susceptibility pattern of hand sanitizers of each organism are tabulated in Table 2. The interpretation of results was according to the Clinical Laboratory Standard Institute (CLSI) methodology as sensitive, intermediate and resistant using streptomycin as standard.

Our results showed that microorganisms were highly sensitive to five sanitzers Dial, Dettol, Just cleanse, Carex and Nuvo.The active ingedient of all sanitzers was alcohol.It kills an organism by denaturing microbial proteins.It was found thatrapid evaporation of alcohol minimizes the chances for developing resistance by a microbial cell (Mondal and Kolhapure, 2004).

Other sanitizers were effective against microorganisms showing variable degree of susceptability. Forever sunshine was not effective against *Ps. aeruginosa* and *Micrococcus leutus*butit gave intermediate zone with*E.coli* and *Staph aureus*. Lifeboy was only effective to kill *Micrococcus leutus* while no effect was found on other organisms. Cool n cool, Safegaurd, Purell, Fresh up, Insta foam were effective against Gram positive organisms while Gram negative organisms were found resistant. Mediwash, Inca and Kleen Hanz inhibits all organisms except Pseudomonas spp. The sanitizers collected from doctors were found effective against all bacterial species except SA4 which was not effective against Gram negative organisms. Their active ingredient was ethyl alcohol except for Nuvo which was triclosan**.** It should be recognized that ethanol is not effective against bacterial spores and some enveloped viruses. Ethanol denatures protein in infectious organisms while triclosan kills microorganisms by damaging the cell membrane.

The organisms were found resistant to Cleanor, Capri, Blue king, Germ X and Kidz n klenz.The overall percentage of resistance and susceptability was also calculatedas shown in Fig. 2. Among Gram negative organisms, *Escherichia coli* and *Pseudomonas aeruginosa*are the causative agents of gastrointestinal and nosocomial infections. The results indicated that *E.coli* was 48% resistant and 52% susceptible to all the hand sanitizers while *Pseudomonas aeruginosa* was 64% resistant and 36% susceptible to all the hand sanitizers. Pseudomonas is an emerging threat due to its biofilm forming ability and antibiotic resistance. The lower activity of hand sanitizers against *Pseudomonas aeruginosa* could be explained by the hardy nature of *Pseudomonas*, it has been reported to survive in disinfectants and resistant to a wide variety of antibiotics. It is known to have prolific ability to degrade a wide variety of substance due to its natural endowment with degradative enzymes and plasmids and high protein repair and regeneration mechanisms. The contaminated hands and other environmental factors are the source of transmission of this organism especially in neonates (Jefferies *et al.,* 2012).

Table2.Susceptibility Pattern of Organisms Using hand Sanitizers by Agar Well Diffusion Method.

S.No	Samples Name	Active Ingredient	Zone Diameter of Inhibition (mm)			
			E. coli	***Staph. aureus***	***P .aeruginosa***	***M. luteus***
1	Cleanor	-	3[R]	3 [R]	3[R]	3[R]
2	Forever sunshine	Ethyl Alcohol	12 [I]	14 [I]	NZ[R]	NZ[R]
3	Capri	Alovera&neem	NZ [R]	NZ [R]	NZ[R]	NZ[R]
4	Cool n cool	Ethyl alcohol	NZ [R]	14 [I]	NZ[R]	NG[S]
5	Lifebuoy	Ethyl alcohol	NZ[R]	NZ [R]	NZ[R]	NG[S]
6	Safeguard	Alcohol Denat, Isopropyl alcohol	NZ [R]	10 [R]	NZ[R]	NG[S]
7	Blue king	Ethyl Alcohol	NZ [R]	NZ [R]	NZ[R]	NZ[R]
8	Germ X	Ethyl alcohol	NZ [R]	NZ [R]	NZ[R]	NZ[R]
9	Purell	Ethyl alcohol	NZ [R]	12 [I]	NZ[R]	13[I]
10	Fresh up	Ethyl alcohol	NZ [R]	10 [R]	NZ[R]	16[S]
11	Kidz n klenz	Alcohol	NZ [R]	NZ [R]	NZ[R]	NZ[R]
12	Instant foam	Ethyl alcohol	NZ [R]	NG [S]	NZ[R]	NG[S]
13	Mediwash	Alcohol Denat., Triclosan	NG [S]	19 [S]	NZ[R]	18[S]
14	KleenHanz	Benzalkonium chloride, benzithonium chloride	15 [S]	18 [S]	NZ[R]	16 [S]
15	Lana	Ethanol, propanol	14 [I]	14 [I]	16 [S]	16 [S]
16	Inca	Ethyl Alcohol	16 [S]	15 [S]	NZ[R]	15 [S]
17	Dial	Ethyl Alcohol	NG [S]	NG [S]	NG[S]	NG[S]
18	Dettol	Alcohol Denat.,	NG [S]	NG [S]	NG[S]	NG[S]
19	Just cleanse	Alcohol	NG [S]	NG [S]	NG[S]	NG[S]
20	Carex	Alcohol Denat.	NG [S]	NG [S]	NG[S]	NG[S]
21	Nuvo	Triclosan	NG [S]	NG [S]	NG[S]	NG[S]
22	SA1	Not mentioned	NG [S]	NG [S]	NG[S]	NG[S]
23	SA2	Not mentioned	NG [S]	NG [S]	NG[S]	NG[S]
24	SA3	Not mentioned	14 [I]	17 [S]	18[S]	NG[S]
25	SA4	Not mentioned	NZ [R]	25 [S]	NZ[R]	NG[S]

Key: NG = no growth, NZ= no zone, Resistant = [R], Intermediate= [I], Susceptible= [S]

Among Gram positive bacteria, *Staph.aureus* can cause gastrointestinal, skin, systemic and nosocomial infections. Our results showed that *Staph aureus* was resistant 32% and susceptible 68% to all the hand sanitizers. *Micrococcus luteus* transmit through contact with contaminated objects and surfaces. They are frequently found on the exposed skin of face, arms, and hand. *Micrococcus luteus* was found to be 24% resistant and 76% susceptible to all the hand sanitizers.

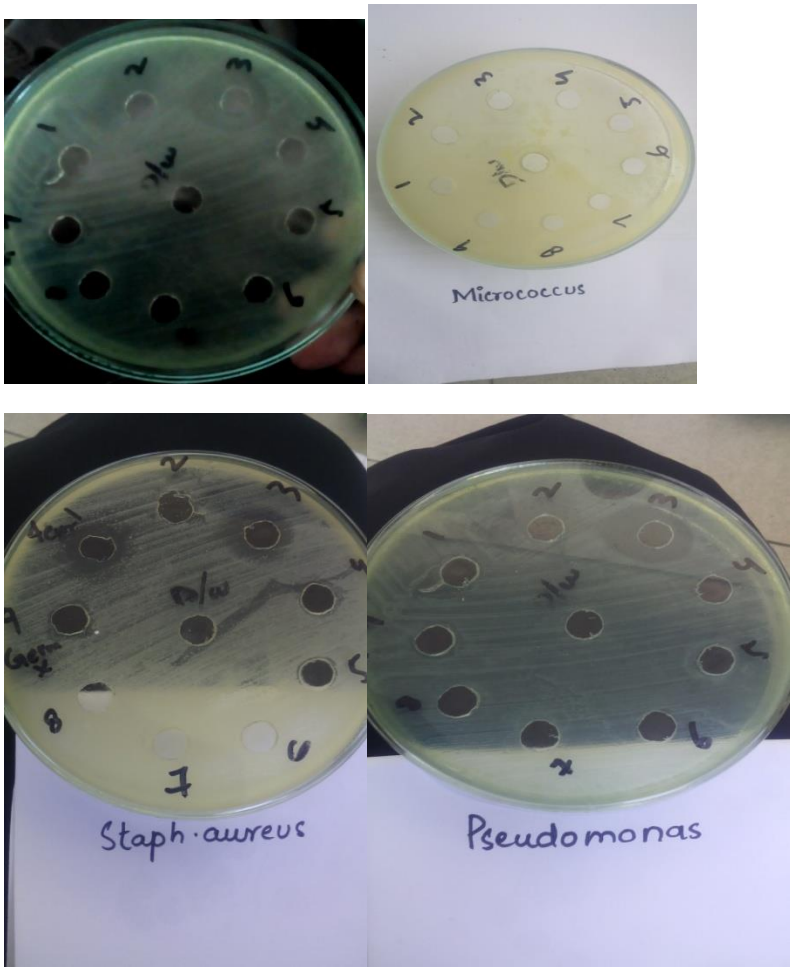

Fig. 1.Zone of Inhibition of Microorganisms using different hand sanitizers.

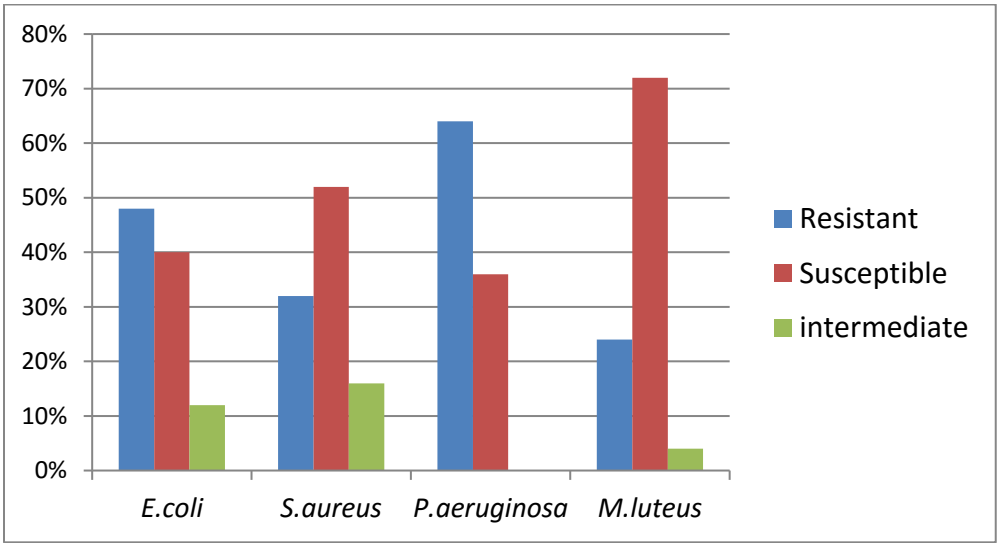

Fig 2.Susceptibility pattern of microorganisms against different hand sanitizers.

The purpose of hand hygiene is to reduce significantly microbial load on the skin which prevents transmission of pathogens among patients. Besides environmental surfaces, hand to mouth route, hand shaking with volunteers,

hand to food handlers, sneezing and coughing increase the risk of spread of infections. Use of hand sanitizers was evolved after washing hands or when soap and water was not available but hand washing with soap should be emphasized instead for routine purpose. The risk of transmission of infectious disease can be reduced by educating people about hand hygiene and to use procedures for cleaning hands at proper time (Bloomfield *et. al*., 2007).

Conclusion

The efficiency of hand sanitizers is based on its active ingredient which should be in appropriate concentration. Every sanitizer is not efficient in killing the microorganism. Therefore, awareness of choosing effective alcohol based hand sanitizers is important to reduce the transmission of infection especially when dealing with patients in hospitals, clinical laboratories, among school children, etc.

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