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Impact of Sanitizer on Normal Human Microflora: A Review on Effect of Frequent Use of Sanitizer on Normal Human Microflora

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Abstract: ***In the current situation of covid 19, uses of sanitizers are increasing rapidly which have direct bad impact on normal human microflora. The human microbiome is the aggregate of all microbiota that reside on or within human tissues and biofluids along with the corresponding anatomical sites in which they reside, including the skin, mammary glands, seminal fluid, uterus, ovarian follicles, lung, saliva, oral mucosa, conjunctiva, biliary tract, and gastrointestinal tract. Uses of sanitizers are increasing in covid pandemic situation due to the fact that it reduces the chances of infection by pathogenic microorganisms including coronavirus. This review paper contains the information and facts about effect of sanitizers on human microflora and advantages and disadvantages of using sanitizers a lot. This paper also includes the effect of sanitizers of different brands on normal human microflora.***

Keywords: Microflora, Sanitizers, Microbiome, Pathogens, Disinfectants

I. INTRODUCTION

Sanitization is known to be the best way to prevent entry of pathogenic microorganisms into human body that may occur due to exposure or coming in close contact to a susceptible pathogenic environment. Sanitizers are the agents that used for sanitization purpose and remove all diseases causing microorganisms. Depending on activity, sanitizers are of two different types 1) alcohol based 2) non alcohol based (alcohol free).

Alcohol based sanitizers contains 60-95% alcohol in the form of ethanol or isopropanol. In the current covid 19 situation, most of the sanitizers used to prevent infection are alcohol based. This is due to the fact that alcohol cause denaturation of cell wall's proteins of pathogenic microorganisms that cause death of pathogens. Alcohol free products are generally made up of disinfectants such as benzalkonium chloride or triclosan.[1]

II. MECHANISM OF ACTION

A. Mechanism of Action of Alcohol Based Sanitizers Against Bacteria

In alcohol based sanitizers, the most important component that used to kill bacteria is n-propanol. The exact mechanism of alcohol's antimicrobial activity is denaturation of proteins, destruction of cell membrane, uncoupling of m RNA and protein synthesis.

The optimal concentration of alcohol for its bactericidal effect lies between 60%to 90% and 100% absolute alcohol is less effective against bacteria. The fact behind this is that pure alcohol cogulate proteins that comes in contact, thus it make a layer of coagulated proteins just inside the cell wall and prevent entering of alcohol into the cell. At this time the cell would become inactive but not dead. 70% alcohol also coagulate proteins but at a slower rate and allowed farther penetration of alcohol into the cell. Therefore, alcohol penetrate all through the cell before coagulation block the way and the organism dies. This point should also be noted that alcohol only kills vegetative cells not spores.[2]

B. Mechanism of action of alcohol based sanitizers against Viruses

For targeting viruses ethanol is the main component used in sanitizers. Ethanol mainly target virus envelope, viral protein capsid that contains and protect genetic material itself. All these materials are necessary for viral life cycle and for its pathogenicity. High concentration of alcohol is highly effective against viruses. It is also interesting to note that adding acids into ethanol increases its viracidal effects.

Despite the fact that alcohol based sanitizers have a potential virucidal effect, most of the non enveloped viruses remains uneffective after the use of sanitizers.[2]

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C. *Mechanism of Action of non-alcohol Based Sanitizers*

Similar to alcohol based sanitizers, non alcohol based sanitizers are also effective against bacteria and enveloped viruses. Though these sanitizers are not effective against non enveloped viruses, a recent study demonstrating its effect against non enveloped human coxsackie virus shows an exception exists. The main targeting sites of these non alcohol based sanitizers is lipid envelope of either bacteria or viruses and the main component of these sanitizers against bacteria and viruses are benzalkonium chlorides (BCs). Benzalkonium chloride has a cationic head group and alkyl chain tail. The cationic head group of BCs adsorb progressively to the phosphate group of phospholipid bilayer and increases fluidity in the membrane and creates pore or gap into the membrane. In addition, alkyl head chain of BCs further penetrate and disrupt cell membranes and destroy its physical and biochemical properties.[2]

III. USE OF SANITIZERS DURING COVID 19 PANDEMIC

Coronavirus, the etiological agent of Covid 19 spreads usually through respiratory droplets and fomites from infectious persons. To prevent this spread or to remains protected from covid infection use of sanitizers is increasing rapidly. In India, almost 74 million people are homeless or live in slum area, therefore controlling infection in such a situation is very difficult and requires heavy usage of hygienic measures and sanitizers. Also in india slum areas also faces the problem of open defecation, which also raises the chances of spread of diseases. In 2017, WHO/UNICEF Joint monitoring programme estimated that only 60% of total population had basic and safe sanitation.

During covid 19 crisis, sharing of things, touching public surfaces, coming in contact with contaminated fomites increases the risk of exposure to coronavirus. Thus, it is recommended to clean the objects by sanitizers before their use.Therefore usage of sanitizers is increasing rapidly during the spread of covid 19.[3]

IV. NORMAL MICROFLORA

Normal Microflora term used to collection of microorganisms that normally reside in healthy living organisms (human or animal) without providing any harm to them. Instead of getting harmed, humans or animals get the benefit of protection through these microbial collection. In humans, normal microflora constitutes about 10^14 bacteria. These normal microflora resides in different regions of the body with different genera. Other terms used for normal microflora are ‘commensals’ and ‘indigenous microbes’.[4]

A. *Significance of Normal Microflora*

Microorganisms that normally inhabit healthy living organisms may serve as a commensal (lives without causing any harm or benefit to the host) or may act as a competitor for the pathogens.Some human studies suggested that normal microflora may influences human anatomy, physiology, life span and metabolic functions.

Most of the normal microflora inhabiting the human skin, eyes, nails, oropharynx, genitalia and gastrointestinal tract. Normal microflora includes only bacteria not viruses or fungi because they are not commensals and do not aid the host.[4]

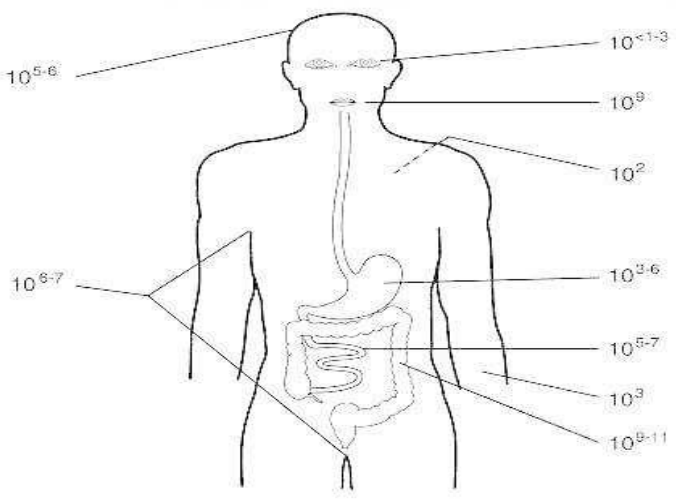


Fig. 1. Numbers of bacteria that colonize different parts of the body. Numbers represent the number of organisms per gram of homogenized tissue or fluid or per square centimeter of skin surface.

B. Normal microflora of skin

Human skin is composed of millions of bacteria that constitutes normal microbiota. These microbiota plays an important role in protecting our skin from invading pathogens, to boost up immune response, breakdown of natural products.In the case of any disturbance in normal microflora of skin, invasion of pathogens can occur which may results in diseases.

Structurally the skin is composed of two layers, dermis(Inner layer) and epidermis (Outermost layer). Human skin has different sites which can be categorised based on their physiological characteristics such as sebaceous(oily), moist or dry. According to a sequencing survey, in healthy adults microflora colonises differently in different sites categorised according to their physiological characteristics. Sebaceous sites are dominated by lipophillic propionibacterium species, whereas staphylococcus and corynebacterium species that lives in humus area reside in moist sites. Fungi of the genus Malassezia predominated at core body and arm sites, whereas foot sites are colonized by a more diverse combination of Malassezia spp., Aspergillus spp., Cryptococcus spp., Rhodotorula spp., Epicoccum spp. and others . Staphylococcus epidermidis is the major inhabitant of skin and contribute 90% of normal aerobic microbiota of skin. Staphylococcus aureus normally resides in nasal and perineum areas.Micrococcus luteus contributes about 20% of normal microflora of skin. Beta hemolytic Streptococci rarely seen on the skin but alpha hemolytic streptococci normally found in mouth from where they can in rare instances spread to skin. Gram negative bacteria make up a small portion of microflora of skin.[4][8]

C. Normal Microflora of Gastrointestinal Tract

The microflora present in stomach is usually contains microorganism swallowed with food and those dislodged from mouth. Due to high acidic environment, number of microorganisms is much lower in stomach and intestine as compared to oral parts. After meals bacterial count is highest (approximately 10^3 to 10^6 organisms/g of contents) which lowers after digestion due to acidity. *Helicobacter pylori* is the main potential pathogen cause different type of ulcer in stomach. In normal host, the duodenum part contains least no of microorganisms (0 to 10^3/g of contents), ileum part contains moderate number of microbes and mixed microflora (10^6 to 10^8/g of content) and large bowel is dense and contains highest number of microbes (10^9 to 10^11/g of content). The microflora of the large intestine is composed mainly of anaerobes. In the upper gastrointestinal tract , normal microflora is less dense due to rapid peristalsis and presence of bile.[4][8]

D. Normal microflora of Respiratory Tract

The human respiratory tract spans from the nostrils to lungs consist of a niche specific community of bacteria. The anterior nasal parts of the upper respiratory tract mainly consist of *Actinobacteria, Firmicutes,* and, in lower abundance, anaerobic *Bacteroidetes* . The pharynx and trachea consist mainly of those bacteria that present in nasal parts (hemolytic streptococci). Beside this anaerobic bacteria, *Staphylococci, Neisseria, Diptheroids,* and others are also present.

Some potential pathogenic microorganisms such as *Hemophilus, Mycoplasmas* and *Pneumococci* may also be a part of microflora of pharynx. The upper respiratory tract of humans is main site for colonization of pathogens, on the other side, lower respiratory tract (bronchi and alveoli of lungs) is often sterile because of large size of pathogens. Due to their large size, microbes are unable to cross the pharynx but if they do reach into alveoli then host defense mechanisms will encounter them.[4][8]

E. Effects of Sanitizers on Microflora

The main purpose of using sanitizer is killing germs for a better healthy hygiene but its overusage can cause various side effects. During covid 19 pandemic, most people rely on hand sanitizers to prevent infection.This led to excessive use of sanitizers during covid 19 pandemic. Along with killing pathogens, they can also kill microbes of our body that constitute our normal microflora. Excessive use of sanitizer can cause rupturing of skin by denaturation of stratum corneum. It can also cause skin reactions like irritation, inflammation, allergies etc. As normal microflora play an important role in maintenance of human health, its depletion can cause imbalance between good and bad microbes in our body and result in improper functioning of our body.Example:- Disturbance in the normal microflora of intestine can cause improper digestive system and results in inflammatory bowel diseases, obesity etc.

Sanitizers consist of chemicals such as alcohol, hydrogen peroxide, antiseptics (chlorhexidine, chloxylenol, triclosan, iodine and iodophors, quaternary ammonium compounds. Alcohol cause denaturation of plasma membrane of microorganism, hydrogen peroxide cause spores inactivation and chlorhexidine cause disruption of arrangement of cytoplasmic membrane. Chloxylenol cause inactivation of some enzymes involved in cell wall synthesis. Both iodine and iodophores cause killing of both gram positive and gram negative bacteria. Triclosan cause killing of bacteria in low concentrations.[5]

F. Over Usage of sanitizers

Repeated or frequent use of hand sanitizers can result in skin irritation, improper digestion and chronic irritation. Normal microflora is good for our health and it is the first lie of defence against pathogenic microorganisms. Excessive use of sanitizers can also kill beneficial microbes and can cause depletion in immunity. It can also result in improper functioning system of our body because some part of normal microflora plays an important role in human body functions. Improper body functioning can results in digestive problems, obesity, liver diseases, cardiovascular diseases, cancers etc.[5]

G. Adverse effects of Hand Sanitizers

1) Sanitizers cause development of antibiotic resistance ability of bacteria. Example:- *Clostridium difficle* and *Enterococci* begins tolerating alcohol and it become difficult for alcohol to kill them.
2) Its excessive use can result into skin irritation and damage.
3) Its use can results in denaturation of skin and depletion in normal microflora.
4) Sanitizers have dry effect which can cause peel or crack into the skin.
5) They can also cause gastric and intestinal infections.
6) Some sanitizers contain phthalates that are endocrine disrupters and could change genital development.[5]

V.COMPARATIVE ASSESSMENT OF EFFECTS ON NORMAL MICROFLORA OF DIFFERENT BRANDS OF HAND SANITIZERS

In maintaining good hygienic life, sanitization is a necessary step that includes killing of germs by using sanitizers or disinfectants. During the early stage of covid 19 pandemic, demand of sanitizers were increasing very rapidly and it was very difficult to fulfill the demand at that time, but upto the later stage of pandemic sanitizers of different brands were came in to the market to fulfill the demand. These different brands of sanitizers have different composition and therefore have different impact on normal human microflora and also on pathogenic microorganisms.

Different brands of sanitizers includes Dettol, Lifebuoy, purehands and sterillum. Many studies have been conducted to compare antimicrobial efficacy of different brands of hand sanitizers, but very few papers are available to assess their efficacy difference. One study was conducted to compare antimicrobial efficacy of Dettol, Lifebuoy, purehands and sterillum sanitizers which have different ingredients and compositions.[6]

Hand Sanitizer	Ingredients
Sterillium	Propan-2-ol, Propan-1-ol, Mecetronium ethyl sulfate, Glycerol, Tetradecan-1-ol, fragrances, Patent blue V, Purified water
PureHands	Hrivera, Coriander, Lime, Ushira, Neem
Dettol	Denatured Alcohol- 69.4% w/w, Water PEG/PPG-17/6 copolymer, Propylene glycol, Acrylate/C10-30 alkyl acrylate, cross polymer, Tetrahydroxpropyl ethylenediamine, Perfume.
Lifebuoy	Ethyl alcohol 95% v/v IP 55% w/w, Isopropyl alcohol 10% IP w/w, Tocopheryl acetate IP 0.05% w/w, Perfumed gel base: qs to 100% w/w

Fig.2. Composition of different brand sanitizers

Disk agar diffusion method were used in this study to evaluate the efficacy of these sanitizers against *S. aureus*, *S. epidermidis*, *E. faecalis*, *E. coli*, and *P. aeruginosa*. By comparing zone of inhibition of different hand sanitizers, it was concluded that sterillum is the most effective sanitizer against all bacteria present in study. This may be due to the presence of 75% propanol (alcohol) in it in liquid form that penetrates the skin easily. On the other side, purehands are the least effective against sanitizer among all which may be due to the presence of coriander, lime and neem in it which has low antimicrobial potency. Thus it can also be concluded that among all these four sanitizers (Dettol, Lifebuoy, purehands, sterillum) Sterillum can cause most harmful effects on normal microflora and purehands can cause least harmful effects on normal microflora.[6][7]

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Test Organism	(Mean±SD)				ANOVA	*P*
	Group A	Group B	Group C	Group D		
S. aureus	27±1.414	3.5±4.95	8.5±0.707	7.5±0.707	F=31.921	0.003
S. epidermidis	22±1.414	7±0.0	8.5±0.707	7.5±0.707	F=138.0	0.001
P. aeruginosa	19.5±0.707	7.5±0.707	10.5±0.707	8.5±0.707	F=120.0	0.001
E. coli	15.5±0.707	7.5±0.707	9.5±0.707	8.5±0.707	F=51.667	0.001
E. faecalis	16.5±0.707	7±0.0	8.5±0.707	7.5±0.707	F=106.11	0.001

Group A-Sterillium; Group B: PureHands; Group C: Dettol; Group D: Lifebuoy ** *P*<0.001, HS

Fig. 3. Zone of inhibition (in mm) measured at the end of 24 h of different hand sanitizers against particular test organism

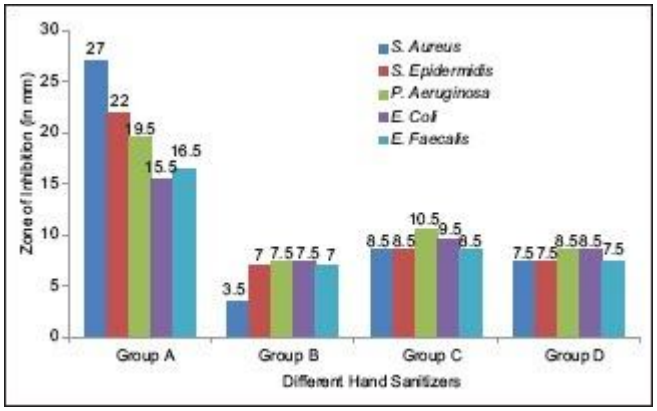


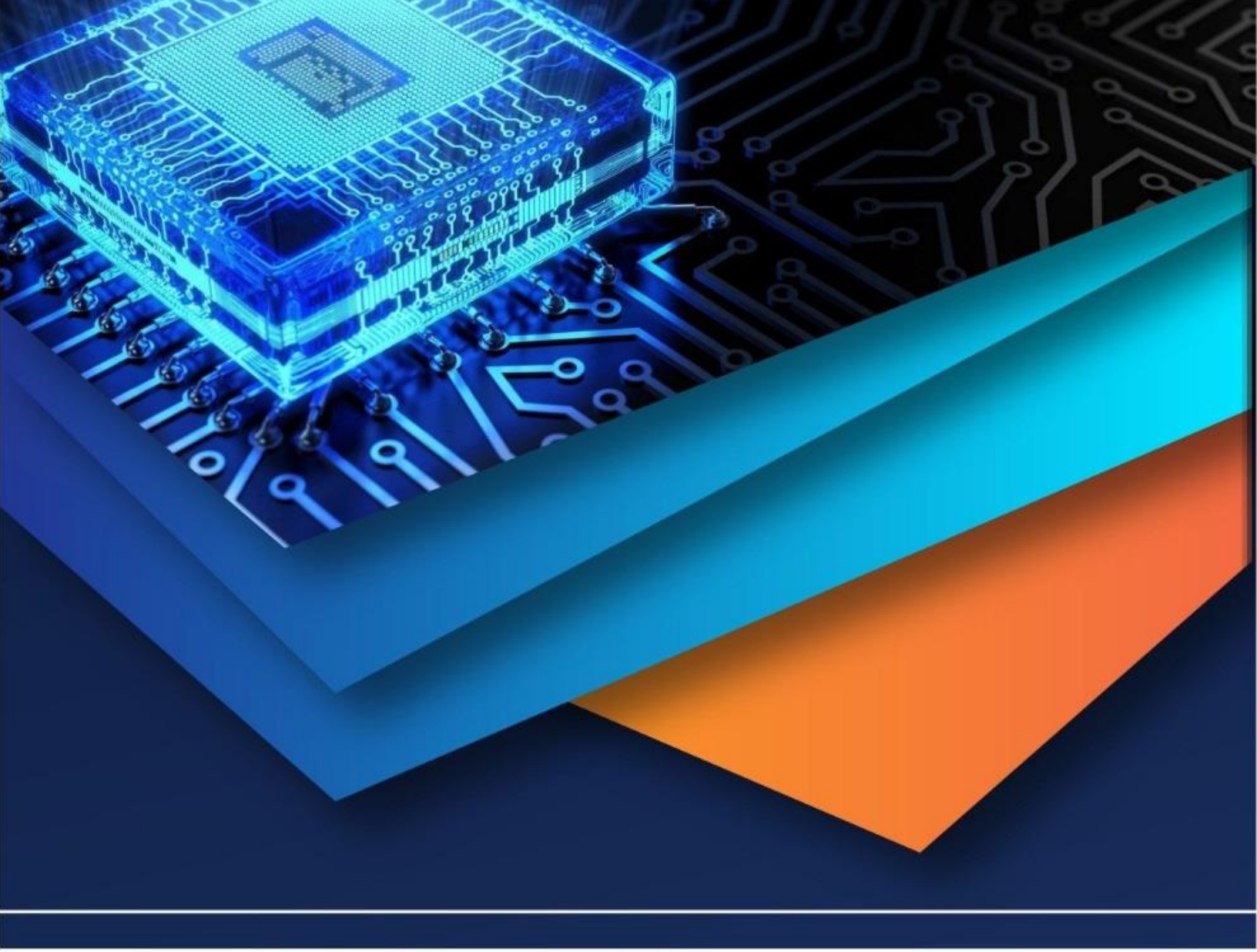
Fig.4. Analysis of zone of inhibition to evaluate antimicrobial efficacy of different hand sanitizers. Labelling on the side of respective zone of inhibition as A, B, C, D etc.

VI.CONCLUSION

Although it is clear that sanitizers are more effective in killing pathogenic microbes and prevent infection but some research also shows that some bacteria are best killed or cleaned by soap or hand washing. Similarly sanitizers cannot removed pesticides and heavy metals like lead. It is completely understandable that during current situations usage of sanitizers cannot be avoided completely but their adverse effects also cannot be neglected. Therefore we should avoid excessive use of sanitizers and also avoid their use at the places where soap and water is available.

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