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IN VITRO STUDIES ON THE EFFICACY OF HAND SANITIZERS ON PATHOGENIC STAPHYLOCOCCUS AUREUS

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ABSTRACT

Staphylococcus aureus is a common gram positive round shaped facultative bacterium. It is highly pathogenic causing a range of illnesses from minor skin infections to major diseases like pneumonia, meningitis, osteomyelitis and sepsis. The best way to control the spread this pathogen is by proper sanitation of the locality, health centres and personal hygiene. Hand sanitizers are utilized to take care of these highly infectious microbes. In the present study the efficacy of four different commercially available hand sanitizers (Sterilium, Godrej Protekt, Clear Handz and Tree wear) has been tested by comparing their zones of inhibition.

Pure cultures of staphylococcus aureus were isolated from the society sewage treatment plant under the assumption that the bacterium must have gained resistance to the various anti-microbial agents in regular use. Isolation methodology was the standard protocol mentioned in Practical Microbiology Principles and Techniques. Mannitol salt agar was used as the selective media to isolate staph colonies as this medium specifically supports the growth of only gram positive bacteria. Colonies of staph were identified on the plated by observing their luxuriant growth showing yellow/white colonies surrounded by yellow zone.

Clear zones of inhibition that are uniformly circular can be witnessed. In the present study petri plates with confluent lawn of fully grown staphylococcus were punched with three holes. In the holes 20ul of the hand sanitizers were added and the plates were incubated for 36 hrs at 37oc. Different sanitizers showed different zones of inhibition. The diameters were measured at four different orientations by using a regular scale and the average of the four was taken for calculating the zone of inhibition. After a critical analysis, sanitizer showing the greatest zone of inhibition exhibited maximum inhibition on the growth of Staphylococcus aureus.

KEYWORDS : Hand Sanitizers, Satphylococcus, Peptidoglycan, Zone of inhibition

1. INTRODUCTION:

The awareness about the diseases caused by microbes is spreading widely across the globe [Tatem, A.J. Rogers, and S.I Hay]. These microscopic disease-causing organisms are often seen as: bacteria, viruses, fungi and protozoa. They are highly pathogenic that invade, multiply and spread causing a range of illnesses. They may cause minor skin infections like pimples, boils, cellulitis and abscesses or even cause life-threatening diseases such as: pneumonia, meningitis, osteomyelitis and sepsis which may lead to the death of the individual [U.S National Library of Medicine]. Now you know how dreadful these puny creatures can be…So, definitely no one would deliberately consume them. However, because of their microscopic nature the chances of escaping from them is next to impossible. But fortunately, the spread and invasion of microbes can be controlled by adopting proper sanitation. Sanitization or sanitation is basically a type of anti-microbial measure or a process for killing disease causing pathogens. It can be achieved by using certain chemical compounds which have a detrimental effect on microbes. Such compounds having detrimental effect are called sanitizers [WHO – Infection Prevention and control]. Sanitizers kill the microbes by destroying the cell membranes and denaturing proteins in the bacterial cell. The dissolving of the cell membranes is done by the alcohol present in the sanitizers [UCSB ScienceLine]. Our hands are constantly involved in carrying out many activities especially shaking hands while greeting each other, eating, cooking, food processing or while performing surgical procedure; proper sanitation is a must to keep the whole atmosphere germ free. Primarily in hospitals and health centers the probability of diseases spreading is high as people with various illnesses carrying pathogens often check in for medication. So, one wouldn't encourage their invasion into a healthy/cured host or into the doctors. Hence, sanitation is a necessity at every level from personal, community, health centers and hospitals to prevent any disease-causing agents invading into ours or other's bodies.

Thanks to the rapid awareness for personal hygiene and proper sanitation various hand sanitizers were formulated and marketed. And this research attempts to assess the efficacy of four different sanitizers presently available in the market. So that one can know if the sanitizers marketed are performing in equal footing with their claims of anti-microbial activity. The four different hand sanitizers selected for assessing their efficacy are shown in Figure 1 & Table 1.

Table 1: Four commercially available sanitizers

1.	Sterillium	2.	Godrej Protekt
3.	Tree Wear	4.	Clear Handz

Figure 1: Sanitizers tested.

In general, any microbe unless highly pathogenic remains inactive in an individual until the immunity of the individual falls. Yet there are many microbes which are highly pathogenic, causing either dreadful or long persistent diseases. In fact, they may even be antibiotic resistant. And

what's more is, most of such persistent bacteria are very predominantly present in hospitals often being a threat to in house patients [WHO - Attacks on Health Care].

One such bacteria is *Staphylococcus aureus*, a common pathogenic bacterium. First identified in 1880 by Sir Alexander Ogston in pus cells from a surgical-abscess in a knee joint [Etymologia: Staphylococcus]. It is a gram-positive, round-shape bacterium facultative-anaerobe found commonly in the flora of the human body; in the nose, respiratory tract and on the skin [Taylor, Tracey A, Unakal, Chandrashekhar G].

The spread of Staphylococcus from person to person or from surroundings to an individual can be controlled by adopting proper sanitation using sanitizers that kill bacteria by dissolving their cell membranes due to the alcohol present in them; which is generally a usual ingredient. However, *Staphylococcus aureus* being a gram-positive bacterium; has thicker peptidoglycan cell wall, as a result, it is less susceptible to alcohol sanitizers [UCSB ScienceLine].

For effective control of *Staphylococcus aureus* alcohol-based sanitizers might prove to be less effective and its microbial activity might be comparatively more affected by non-alcohol sanitizers [Wikipedia: Gram Stain].

Research query to investigate the effect of both alcohol and non-alcoholic sanitizers on *Staphylococcus aureus* is formulated with a zeal to even carry out a comparative study. Present research is to test the efficacy of four different sanitizers that are commercially available in the local market against *Staphylococcus aureus*. And to find an answer to our research question *"Which among the four available commercial sanitizers is most effective in controlling the growth of Staphylococcus aureus?"* by comparing their zones of inhibition.

1. METHODOLOGY:

After going through Practical Microbiology Principals & Techniques [Kale, Vinita and Kishore Bhusari] and Putting Disinfectants to the test [Dr. Juliana Ansari], two suitable protocols were identified to test the efficacy of the sanitizers chosen (Sterillium, Godrej Protekt, Tree Wear and Clear Handz) for the present study. Among which Turbidimetry estimation of bacteria using McFarland scale is the most efficient method to analyse the competency of disinfectants. However, the 2nd protocol: 'Agar diffusion method' has been selected for this study as it is feasible and does not require sophisticated instruments such as - 'Photo colorimeter' and the observations are clearly noticeable.

Turbidimetry estimation of bacteria using McFarland scale [Kale, Vinita and Kishore Bhusari]:

In turbidimetric method the rate of growth of a bacterium can be measured by subjecting the bacterial cells grown in liquid medium to optical density using photocolorimeter. Higher the number of bacterial cells; higher is the optical density displaced on the monitor of the photocolorimeter instrument. Prior to testing the growth of the bacteria, the photocolorimeter is calibrated to measure the bacterial cells against the same medium in which the cells are grown which represents blank at a wavelength of 600nm. After setting photo-colorimeter instrument to 100% transmission against the nutrient broth as blank at 600nm, the optical density of the test sample is measured. To study the growth pattern , the optical density is measured at different time intervals starting from the time of inoculation taken as zero , with a gap of 30 min each. The respective values are used to plot a graph by taking the optical densities on Y-axis and time interval a on X-axis. Conclusions can be drawn from the graph plotted considering McFarland number of bacteria vs optical density at 600nm. Turbidometric method can be used in the present investigation for testing the efficacy of the sanitizers on the bacteria by adding respective sanitizer selected to four different cultures flasks and then inoculating the bacterial culture.

Agar diffusion method for testing the sensitivity of antibiotics and disinfectants [Kale, Vinita and Kishore Bhusari]:

In this agar diffusion method, the test solution containing the bacteria is spread on solid agar to keep the test pure bacterial culture in contact with the agar medium. Wells are made in the agar medium and a specified amount of antibiotic or disinfectant is added. Upon incubation the antibiotic or disinfectant diffuses into the agar medium and with the increasing distances the concentration of the solute in the agar decreases as the solute (antibiotic or disinfectant) moves away from the interface. An equilibrium is ultimately reached when the solute concentration becomes uniform throughout the whole system. Diffusion method is used in microbiological assays for testing the efficiency of antibiotics in which diffusion of antibiotic, results in the formation of inhibited growth zones. A measurement of the diameter of the zone of inhibition reflects the concentration gradient caused by the diffusion of antibiotic into bacterial cells grown in the area. Factors that may intervene with this method are the concentration of antibiotic, composition of the medium and the test organism.

Among the above-mentioned protocols, 'Agar diffusion method' has been selected for carrying out my investigation as it does not require any sophisticated instruments. Just one simple scale to measure the diameter of zone of inhibition. On the other hand, the turbidometric method requires a photo colorimeter which is not available. However, to tally with my research question, the effectiveness of the commercially available sanitizers (Sterillium, Godrej Protekt, Tree Wear and Clear Handz) on the bacteria – *Staphylococcus aureus* the procedure has been re-designed. For example, instead of antibiotics sanitisers were tested. Pure commercial staph culture was replaced with staph isolated from sewage water, Mannitol Salt Agar medium was used specifically for Staph isolation due to its unique compatibility with gram-positive bacterium like *Staphylococcus aureus* [MicrobiologyInfo.com].

MATERIALS:

- **Sanitizers:**
  Tree Wear
  Sterillium Rub-in Hand
  Godrej Protekt
  Bacterial culture media:
  Mannitol Salt Agar
  Nutrient Broth (LB)
  Clear Handz
- **Glass ware:**
  Petri plates
  Glass spreader
  Conical Flasks and Test tubes
- **Experimental Material:**
  Staphylococcus aureus
  Sewage Water
- **Chemicals:**
  Peptone
  Tryptone
  Beef extract
  NaCl (Sodium Chloride)
  Phenol Red indicator
  Agar Agar
  D-mannitol

Equipment:
- Autoclave
- Incubator
- Laminar Air Flow
- Digital weighing balance

Bacterial medium composition: Mannitol. Salt. Agar medium (M.S.A), Nutrient broth and Nutrient Agar.

- **Mannitol. Salt. Agar medium:** 20 ml of selective media: Mannitol Salt Agar (M.S.A) media for the growth of *Staphylococcus aureus* was prepared by adding the following components to 20ml of distilled water in a conical flask plugged with a cotton plug.

- Peptone – 0.2gm/20ml
- Tryptone – 0.1gm/20ml
- Beef extract – 0.02gm/20ml
- NaCl (Sodium Chloride) – 1.5gm/20ml
- D-mannitol – 0.2gm/20ml
- Phenol Red (pH indicator) – added until the media turned red
- Agar Agar – 0.36gm/20ml

- **Nutrient broth :** Nutrient broth was prepared with the following composition for 20ml in a conical flask plugged with a cotton plug:
- Peptone – 0.1 gm/20ml
- Beef extract – 0.06gm/20ml
- NaCl – 0.1gm/20ml

- **Nutrient Agar plates:** Nutrient agar plates were prepared by just dissolving agar the gelling agent to the normal nutrient broth.

Isolation and Growing of *Staphylococcus aureus*:
Before testing the efficiency of the sanitizers, the test bacterium – *Staphylococcus aureus*, was collected from the nearby sewage treatment plant, isolated and grown. *Staphylococcus aureus* was specifically isolated from the sewage under the assumption that the bacterium collected might have gained resistance to various anti-microbial agents in regular practice [Foster, Timothy J]. The resistance gained against anti-microbial agents like sanitizers serves as a major drawback for any sanitation methods practiced. Hence, the decision to extract *Staphylococcus aureus* from sewage water was opted. The isolation of the target bacterium was performed as per the standard protocol mentioned in Practical Microbiology Principals & Techniques [Kale, V. Kishore Bhusari].

- Mannitol Salt Agar (M.S.A) media prepared was autoclaved along with petri plates at 121°C, 15psi for 15 minutes.
- The sterile media was transferred into the sterile petri plate in Laminar Air Flow under sterile conditions (Fig 2).
- The agar in the plate was left for solidification and upon solidification 100 μl of the sewage water was spread.
- The Mannitol Salt Agar plates with sewage water were incubated in an incubator maintained at 37°C for 36 hours giving enough incubation time for bacteria in the sewage water to grow.
- As Mannitol-Salt Agar medium specifically supports the growth of only gram-positive bacteria [MicrobiologyInfo.com]. After 36 hours following bacterial colonies were seen (Fig 3). Analysing their growth pattern and colour as shown in Table 2.

Table 2: Morphological identification of Bacteria

Bacteria	Growth	Observation
Staphylococcus aureus	Luxuriant	Yellow/White colonies surrounded by yellow zone.
E. coli	Inhibited	Not seen
Staphylococcus epidermis	Fair-good	Red
Enterobacter aerogenes	Inhibited	Not seen

Before Incubation	After Incubation
	
Figure 2: M.S.A media – Red colour	*Figure 3: Staphylococcus aureus colonies – Circled in black*

After the growth of *Staphylococcus aureus* in M.S.A media, 2 colonies were isolated and grown in 2 test tubes of nutrient broth respectively after the test tubes are autoclaved for 15 minutes at 15psi at 121°C (Figure 4) as shown below:

Figure 4: 2 Nutrient Broth test tubes

- After autoclaving, the sterile broth was transferred equally into the 2 sterile test tubes in a sterile environment supported by Laminar Air Flow.
- An inoculation loop was flame sterilized and used to pick up a single *Staphylococcus aureus* colony separately to culture in two different test tubes containing nutrient broth the cultures were incubated for 24 hours at 37°C. To obtain a pure culture of *Staphylococcus aureus*.
- To carryout the study on efficacy of sanitizers (Sterillium, Godrej Protekt, Tree Wear and Clear Handz) nutrient agar medium was prepared by following the standard procedure [Kale, V. Kishore Bhusari] and distributed into four different media plates of 20 ml each.

Upcoming is the efficacy test of the 4 different sanitizers (Figure 1: Sterillium, Godrej Protekt, Tree wear and Clear Handz) via Agar diffusion method.

Procedure (Agar diffusion method) [Kale, V. Kishore Bhusari]:
Principle: Antimicrobials like sanitizers can diffuse out into the agar medium and interact in a plate freshly seeded with the test organisms. The plates on incubation result in the formation of zones of inhibition that are uniformly circular as there will be a confluent lawn of growth. The diameter of zone of inhibition can be measured in millimetres which gives us the understanding of the effect of a sanitizer on the growth of *Staphylococcus aureus*.

Steps:

- Bacterial media plates were made ready prior to the start of the experiment.
- On to the sterile Bacterial media plates, the fresh overnight (24 hours) culture of *Staphylococcus aureus* was inoculated with the help of a spreader for obtaining uniform culture on the surface of the petri plates.
- Cork borers that help to make wells in the petri plates were flame sterilised.
- To study the effect of the sanitizers, four different petri plates punched with three holes in each were used as shown in the fig 5.

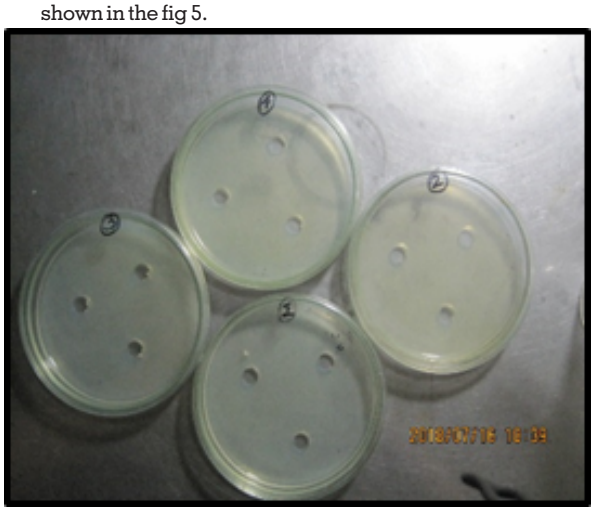


Figure 5: Nutrient Agar plates with 3 wells

- In the wells of the respective plates 20 μl of the sanitizers was added to study their effect on *Staphylococcus aureus*.
- Plates were initially incubated at 37°C for 36 hours.
- After 36 hours of incubation, the zone of inhibition (clear zone around the well) was observed whose diameter was measured using a regular scale.
- Diameter was measured at four different orientations giving the labelling for each diameter as d1, d2, d3, d4 to give four different readings for all the three wells and then the average of them was calculated to give the exact zone of inhibition.
- The results in terms of zone of inhibition were calculated for the four sanitizers mentioned for comparative analysis of their efficacy.
- After a critical analysis, sanitizer showing the greatest zone of inhibition was titled as the most effective sanitizer that exhibited maximum inhibition on the growth of *Staphylococcus aureus*.

3. Results:

- **Images:** The four different sterilizers (Figure 1: Sterillium, Godrej Protekt, Tree wear and Clear Handz) used in the experiment showed different zones of inhibitions as shown in the figures 6, 7, 8 and 9.

Figure 6: Godrej Protekt – Positive

Figure 7: Sterillium – Positive

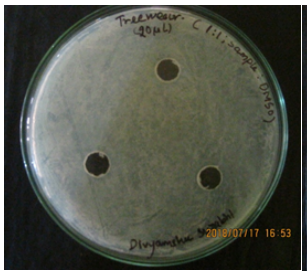


Figure 8: Tree Wear – Negative

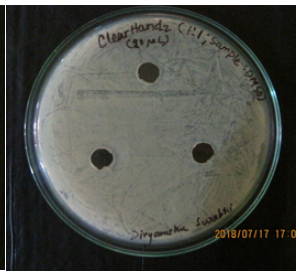


Figure 9: Clear Handz – Negative

Inhibited zone circles were calculated for the positive result sanitizers as they showed inhibitory zones while the other sanitizers displayed negative results which indicates that they are inefficient in controlling the proliferation of the target pathogen Tables 3 & 4).

Table 3: Calculating Zone of Inhibition for Sterillium

Sterilium (in mm)									
	Well 1			Well 2			Well 3		
	Total diameter	Well diameter	Zone of inhibition	Total diameter	Well diameter	Zone of inhibition	Total diameter	Well diameter	Zone of inhibition
d_1	19mm	8mm	19-8=11mm	16mm	8mm	16-8=8mm	17mm	8mm	17-8=9mm
d_2	19mm	8mm	19-8=11mm	17mm	8mm	17-8=9mm	17mm	8mm	17-8=9mm
d_3	18mm	8mm	18-8=10mm	17mm	8mm	17-8=9mm	18mm	8mm	18-8=10mm
d_4	18mm	8mm	18-8=10mm	16mm	8mm	16-8=8mm	17mm	8mm	17-8=9mm
∑ Zone of Inhibition ÷ 4			42÷4= 10.5mm	∑ Zone of Inhibition ÷ 4			∑ Zone of Inhibition ÷ 4		37÷4= 9.25mm

Table 4: Calculating Zone of Inhibition for Godrej Protekt

Godrej Protekt (in mm)									
	Well 1			Well 2			Well 3		
	Total diameter	Well diameter	Zone of inhibition	Total diameter	Well diameter	Zone of inhibition	Total diameter	Well diameter	Zone of inhibition
d1	21mm	8mm	21-8=13mm	20mm	8mm	20-8=12mm	19mm	8mm	19-8=11mm
d2	20mm	8mm	20-8=12mm	20mm	8mm	20-8=12mm	20mm	8mm	20-8=12mm
d3	20mm	8mm	20-8=10mm	19mm	8mm	19-8=11mm	20mm	8mm	20-8=12mm
d4	19mm	8mm	19-8=11mm	19mm	8mm	19-8=11mm	19mm	8mm	19-8=11mm
∑ Zone of Inhibition ÷ 4			48÷4= 12mm	∑ Zone of Inhibition ÷ 4		46÷4= 12.5mm	∑ Zone of Inhibition ÷ 4		46÷4= 12.5mm
Average zone of inhibition ± Standard deviation (± 0.2)			(12+12.5+12.5) ÷ 3 = (37) ÷ 3 = 12.33mm = 12.33 ± 0.2 mm						

1. DISCUSSION:

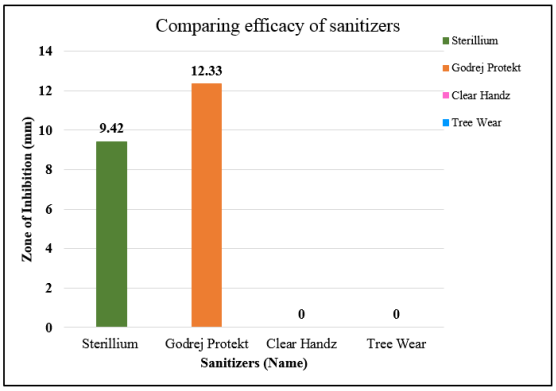
Present invitro study was conducted to test the efficacy of the locally marketed hand sanitizers (Figure 1: Sterillium, Godrej Protekt, Tree wear and Clear Handz) and to answer our research question "*Which available commercial sanitizers is most effective in controlling the growth of Staphylococcus aureus?*". Staph is a very common pathogenic bacterium; which spreads very quickly from person to person in any atmosphere. Being a highly pathogenic bacterium, it can cause a range of illnesses from minor skin infections: pimples, boils, cellulitis and abscesses to major diseases: pneumonia, meningitis, osteomyelitis and sepsis [Mayo Clinic]. It often spreads due to improper maintenance of the local residing area and hospitals. The best way to control the spread of this pathogen is by proper sanitation of the locality, health centre and personal hygiene.

Hand sanitizers are utilized for sanitizing and taking care of these pesky microbes [WHO – Infection Prevention and Control]. Four locally available hand sanitizers were specifically selected (Figure 1: Sterillium, Godrej Protekt, Tree wear and Clear Handz) as they are in routine use in personal hygiene by people of all age groups in various conditions. And are recommended to be used in addition to regular hand washing with soap and water for overcoming the negative impact of microbial contamination directing to health risks [WHO – Infection Prevention and Control]. This study reflects upon one of the most leading issues in the world i.e. 'the importance of sanitizers being efficient'. As staph's presence is all over the globe and so is the usage of sanitizers. So, the probability of them coming into contact is high, stating that sanitizers utilized need to efficient enough to attack against staph.

For evaluating the efficiency of the chosen hand sanitizers (Figure 1: Sterillium, Godrej Protekt, Tree wear and Clear Handz). Agar diffusion method was conducted which provides with a clear zone of inhibition in co-ordinance with the effect of four selected sanitizers on the growth of *Staphylococcus aureus*. The experiments were conducted in a sterile environment to restrict any foreign pathogens/microbes from intervening with the present research as it could highly alter the objective/aim and of course the results and conclusions drawn.

ANALYSIS:

A set of clear results were observed at the end of the experiment that displayed strong correlation with hypothesis: " *Staphylococcus aureus* is less susceptible to alcohol sanitizers". The present hypothesis's stance can be supported with the comparison of the zones of inhibition calculated (Table 3 & 4). Godrej Protekt showed to be more effective against *Staphylococcus aureus* over Sterillium as their zones of inhibition were distinctive and have a difference of '2.91mm'. With Godrej Protekt having the upper hand. Godrej Protekt:12.33 > 9.42: Sterillium Graph 1.



Graph 1: Graphical representation

The other two sanitizers (Tree Wear and Clear Handz) turned out be a couple of negative results. Probably due to their incapability in penetrating into the thick peptidoglycan cell wall of *Staphylococcus aureus*. Or their efficiency could have been poor due to their composition (i.e. the ingredients used) or because of their dense fluidity. However, an attempt to reduce their fluidity was done without effecting their efficiency; by mixing them with a solvent: DMSO – Dimethyl Sulfoxide for liquefying them [Wadhwani, T. K Desai, D Patel, D Lawani, P Bahaley, P Joshi, V Kothari].

Previous studies aimed at evaluating the efficiency of a variety of hand sanitizers over a range of microbes but with different approaches towards the idea of testing the efficacy of sanitization. Nonetheless, few similarities can be drawn in co-ordinance to the present investigations. One such study "Comparative assessment of antimicrobial efficacy of different hand sanitizers: An *in vitro* study" conducted by Jain VM et. al (2016) presented that Sterillium usually displayed a greater zone of inhibition when compared to the rest of the sanitizers and managed to inhibit both gram-negative and positive organisms to a satisfactory extent. The reason for this could be because of the nature of Sterillium being a combo of both disinfectant and sanitizer. Disinfectants are regarded as anti-microbial agents that are bactericidal meaning that they destroy the micro-organisms to such an extent mandated for hygienic and surgical indications. Whereas, sanitizers are bacteriostatic with an immediate arrest in the metabolic activity of the microbes making them lose their proliferation and pathogenicity properties[Bactericidal Vs Bacteriostatic, & Jain VM, Karibasappa GN, Dodamani AS, Prashanth VK, Mali GV]. Thus, reducing the number of microbes to a level i.e. safe public health requirement.

5. CONCLUSION:

it was observed that among the four sanitizing products; Godrej Protekt (12.33±0.2 mm) held maximum microbial efficacy against the gram-positive bacterium; *Staphylococcus aureus*. Followed by Sterillium (9.42±0.8 mm). Tree Wear and Clear Handz, displayed negative results with zero zone of inhibition.

Evaluation:

Unfortunately, as "Nothing is ever certain" quoted by Alice Sebold. This current investigation possesses its own limitations such as – the unravelled reason behind the negative results obtained.

The herbal sanitizer: Tree Wear's anti-microbial activity might have been restricted due to its dense fluidity although an attempt was made to liquify the sample by mixing it with DMSO (Dimethyl Sulfoxide). However, diluting the sample did not have any changes on its inability to show effect on the microbial population. This may be attributed to the ingredients with which the sanitizer is formulated. In the study carried out by Jain VM et. al (2016) reasoned that the herbal components tend to have lower anti-microbial activity when compared to the products containing alcohol (propanol). Tree Wear utilized different ingredients such as : Coconut oil, Aloe Vera gel, Bergamot Oil, Juniper Berry Oil along with Wheat germ/Vitamin E oil. That might have resulted in its non-observable inhibition [Jain VM, Karibasappa GN, Dodamani AS, Prashanth VK, Mali GV]

The other sanitizer: Clear Handz showcased negative result which could have been due to the following: First, the fluidity of the sanitizer; this might have been the cause as gel-based sanitizers tend to slide off the surface of our hands/bacteria whereas sanitizers like Sterillium are soaked into the hands/bacteria showing greater after-effects. Thus, the gel property of Clear handz was reduced with help of a diluent DMSO (Dimethyl Sulfoxide). However, no inhibition was seen.

So, the other reason could have been influenced by Staph's unique trait of gaining resistance to anti-microbial agents in short, a period [Foster, Timothy J]. But no investigation was conducted in the present research for cross-checking this claim as this current research did not focus on 'anti-microbial resistance of Staph'.

In the end, the primary focus of this study was not to prove that the most effective sanitizer (Godrej Protekt) is recommended over the other sanitizers (Sterillium, Tree wear and Clear Handz). But to verify our hypothesis: : " *Staphylococcus aureus* is less susceptible to alcohol sanitizers" and to emphasize that alcohol sanitizers may not be effective on a gram-positive bacterium: *Staphylococcus aureus*.

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