BioGecko Vol 12 Issue 02 2023

ISSN NO: 2230-5807

Comparative Analysis of Different Hand Wash and Disinfectant on Bacterial Growth

Shahana Jabi
Assistant Professor,Department of Botany, School of Basic and Applied Sciences, Shri Guru Ram Rai University, Dehradun-248001

Preeti Juyal*
Assistant Professor,Department of Microbiology,School of Basic and Applied Sciences, Shri Guru Ram Rai University, Dehradun-248001
juyalpreeti@gmail.com

Manjusha Tyagi
Associate Professor,Department of Microbiology, School of Basic and Applied Sciences, Shri Guru Ram Rai University, Dehradun-248001

Rashi Bhargawa
Assistant Professor,Department of Maths, School of Basic and Applied Sciences, Shri Guru Ram Rai University, Dehradun-248001

Akanksha Raturi
Department of Botany,School of Basic and Applied Sciences, Shri Guru Ram Rai University,Dehradun-248001

Kanishka Thakur
Department of Botany,School of Basic and Applied Sciences, Shri Guru Ram Rai University,Dehradun-248001

Meenakshi Lodhi
Department of Botany,School of Basic and Applied Sciences, Shri Guru Ram Rai University,Dehradun-248001

**Corresponding author:* Preeti Juyal*
**Corresponding mail:juyalpreeti@gmail.com*

ABSTRACT

It is well known that hand hygiene is crucial to prevent and minimize healthcare-associated infections(Prat and Pellow *et al*,2001).Bacteria are very diverse and present every where such as in soil, water, sewage, standing water and even in human body.Transient bacteria are deposited on the skin surface from environmental sources and causes skin infections. Examples of such bacteria are *Pseudomonas aeruginosa* (Fluit and Schmitz *et al.*, 2001) and *Staphylococcus aureus* (Higaki and Kitagawa*et al.*, 2000).

Hand sanitizer is a liquid which is generally used to decrease infectious agentsfrom the hands. Formulations of the alcohol-based types are preferred to hand washingwith soap and water in most situations in the healthcare setting (Bolon, MK September2016). It is generally more effective at killing microorganisms and better tolerated than soap and water (Boyce and Pitter 25 October 2002).The general use of non-alcohol based versions has no recommendations (Bolon, MK September

2016). Outside the health care setting evidence to support the use of hand sanitizer over hand washing is poor (De Witt Hubert and Greenland et al., 1 July 2016, Meadows, Saux 1 November 2004).
Formalin is a 37% solution of formaldehyde gas in water. Diluted to 5% formaldehyde it is an effective disinfectant; at 0.2% - 0.4% it can inactivate bacteria and viruses. Unlike chlorine, formalin does not corrode stainless steel. It has a pungent, irritating odor; exposures must be limited due to its toxicity and carcinogenicity. A 100% formalin solution is equivalent to 37%–40% formaldehyde. In dialysis, a 4% formaldehyde (11% formalin) concentration is used.
Results can be concluded that Liquid Hand Wash and Formalin were found highly effective against all pathogens; Whereas Solid Hand Wash was found low effective against all pathogens. Results of this experiment also indicate that different pathogens acquired resistance to different hand wash and disinfectant. The antibacterial effect of hand wash and disinfectant are not only dependent on the type of hand wash and disinfectant but also on their concentration.
Key Words: Carcinogenicity, dialysis, Formalin, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, Transient bacteria.

INTRODUCTION

Hands are considered to be the primary route for transmitting microbes and infections to the individuals (Mondal, Kolhapure. 2004) Personal as well as hand hygiene is important to prevent many communicable diseases. The word “hygiene” is derived from the ancient Greek goddess “Hygeia” that means “goddess of healing.” Cleansing agents have been used around us for a long time and among them soap, liquid hand wash detergent etc. are not worthy. Antibacterial soaps have been used as personal hygiene for generations.
Bacteria are very sundry and diverse and can be found in water, soil, sewage, on human body and are of great importance with reference to health (Johnson and Goddard, *et al.,* 2002). In the year 1961, the U.S Public Health Service Recommendation mentioned that personnel clean and wash their hands with soap and water for one to two minutes before and after client contact (Osborne and Grube 1982).Hand wash are available as liquids, gels, and foams (Boyce and Pitter 25 October 2002).

Hand washing is very important and crucial when it is related to health care workers because of possible and probable cross contaminating of bacteria that may be pathogenic or opportunistic(Richards, Edwards *et al,*1999). Hygiene of hands and prevention of infection through the use of antibacterial liquid hand-wash has been well recognized. There are many and a large number of chemical compounds that have the potential to inhibit the growth, contamination and metabolism of microorganisms or kill them. The quantity and number of chemicals are vast and probably at least 10000 and among them 1000 chemicals are generally and commonly used in hospitals and homes. The important and significant groups of chemicals that help to destroy microorganisms are hydrogen, phenols, soaps, detergents, ammonia compounds, chlorine, alcohols, heavy metals, acids and certain compounds are available around us. Antisepsis, sanitization, disinfection, decontamination, sterilization and so on are a few terms that tell the process of cleaning by any cleansing agents. Various and several cleansing agents are available in the market that is found in various forms and in different formulations. Trichlorocarbanilide, triclosan and P-chloroin-xylenol (PCMX/ Chloroxylenol) are the mostly used antibacterial in medicated soaps. Actually, these are generally only contained at preservation level unless the product is properly marked as antibacterial, antiseptic or germicidal. Washing, scrubbing of hands with hand wash is the first of defense against bacteria and other pathogens that can affect us with flu, skin infection and even deadly communicable diseases (Kimel, 1996). Usually, most of the people believe that an antimicrobial portion of hand washes is effective at preventing communicable diseases. It is to be noted that now many researchers mention that high use of antimicrobial chemicals can have the reverse effect of spreading diseases and infections instead of preventing them(Poole, K 2002). Antimicrobial resistance and rendering individual more vulnerable to more microbial attacks like diseases or infections can result due to over utilization of antibacterial chemicals (White, and McDermolt, 2001). High use of these agents can

give rise to drug resident microorganisms in the future. Hospital and community-acquired infections are escalating and pose a serious public health problem worldwide (Hassan, Hassan Muhibi *et al.*)

Hand hygiene

Hands are considered to be the primary route for transmitting microbes and infections to the individuals (Mondal, Kolhapure. 2004) Personal as well as hand hygiene is important to prevent many communicable diseases. The word "hygiene" is derived from the ancient Greek goddess "Hygeia" that means "goddess of healing." The importance of hygiene is universally recognized and evidence-based. It is well known that hand hygiene is crucial to prevent and minimize healthcare-associated infections(Prat and Pellow *et al*,2001).Bacteria are very diverse and present every where such as in soil, water, sewage, standing water and even in human body. Bacteria's that attacks on human body is of great importance with reference to health(Johnson and Goodard*et al.*,2002). Examples of such bacteria are Pseudomonas aeruginosa (Fluit and Schmitz *et al.*, 2001) and *Staphylococcus aureus* (Higaki and Kitagawa *et al.*,2000). The importance of hand washing is more crucial when it is associated to health care workers because of possible cross contamination of bacteria that may be pathogenic or opportunistic(Richard and Edwards. 1999).Hand hygiene and prevention of infection has been well recognized(Hand-washing Liason Group. 1999). The importance of hand hygiene is also there for food handlers. Food handler includes those who deals with delivers and serve food (Horton, & Parker 2002).Germs are microorganisms such as bacteria and viruses that may lead to harmful diseases. They can enter intothe body through openings such as the nose, mouth and also through breaks in the skin. Today, hygiene is associated with disease prevention and health promotion, and the importance of hygiene is universally recognized and evidence based. Physical contact between people and objects is a key vehicle for the transmission of pathogens. Therefore, effective hand hygiene is a key intervention in disease prevention (Aiello and Coulborn *et al.*, 2008). In the community outside of the healthcare environment, studies have reported association between improvements in hand hygiene and reduction in rates of infectious disease. Pandemic and avian influenza are known to be transmitted via human hands (Pickering and Davis*et al*, 2011). Hands contamination also causes a number of episodes of illness for the majority of the registered symptoms with the strongest effects for common cold, coughing, fever, anddiarrhea (Hubner and Hubner*et al.*, 2010). Further, it is estimated that at any one time, more than 1.4 million people worldwide are suffering from infections acquired in hospitals. These nosocomial infections are also, in most cases, the result of poor hand hygiene. Thus, hand hygiene is a key component of good hygiene practices in the home and community and can produce significant benefits in terms of reducing the incidence of infection, most particularly gastrointestinal infections but also respiratory tract and skin infections (Bloomfield, 2007). It also prevents the transmission of pathogens to food. Decontamination of hands can be carried out by various means. This include either by washing hands with soap or by the use of various agents such as gloves, skin protectants and waterless hand sanitizers (HS), which reduce contamination on hands by removal or by killing the organisms in situ. Washing hands with soap is not feasible all times due to unavailability of resources. Thus hand sanitizer have gradually become the most effective means of preventing spread of diseases and were the subject of present study.

Hand sanitizer

Hand sanitizer is a liquid generally used to decrease infectious agents on the hands (Hand sanitizer-definition of hand sanitizer in English on 27 April 2018). Formulations of the alcohol-based type are preferable to hand washingwith soap and water in most situations in the healthcare setting (Bolon, MK September 2016). It is generally more effective at killing microorganisms and better tolerated than soap and water (Boyce and Pitter 25 October 2002). Hand washing should still be carried out if contamination can be seen or following the use of the toilet(WHO Expert Committee 2015). The general use of non-alcohol based versions has no recommendations (Bolon, MK September 2016). Outside the health care setting evidence to support the use of hand sanitizer over hand washing

is poor (De Witt Hubert and Greenland et al., 1 July 2016, Meadows, Saux 1 November 2004). They are available as liquids, gels, and foams (Boyce and Pitter 25 October 2002).
Alcohol-based versions typically contain some combination of isopropyl alcohol, ethanol (ethyl alcohol), or *n*-propanol (Boyce, and Pitter D; 25 October 2002). Versions that contain 60 to 95% alcohol are most effective (Boyce, and Pitter 25 October 2002). Care should be taken as they are flammable (Bolon, MK September 2016). Alcohol-based hand sanitizer works against a variety of microorganisms but not spores. Some versions contain compounds such as glycerol to prevent drying of the skin (Boyce, JM; Pitter D 25 October 2002). Non-alcohol based versions may contain benzalkonium chloride or triclosan (Long, Bruce et *al.*,2015, Block, *et al.*,2015).
Alcohol has been used as an antiseptic at least as early as 1363 with evidence to support its use becoming available in the late 1800s (Block, Seymour Stanton 2001). Alcohol-based hand sanitizer has been commonly used in Europe since at least the 1980s (Miller, Chris *et al.*, 2016). The alcohol-based version is on the World Health Organization's List of Essential Medicines, the most effective and safe medicines needed in a health system.

Disinfectant
Disinfection is a process in which chemical or physical means is used to control or destroy the microorganisms that are capable of causing diseases. There are three levels of disinfection1 (i.e. high, intermediate and low level) with respect to the effectiveness of the disinfection. Disinfecting agents are substances used to control or destroy harmful microorganisms such as bacteria, viruses, or fungi. Many disinfectants are non-specific in their action and will act against a spectrum of microorganisms. Chemical disinfectants can be grouped in accordance with their chemical properties. They work on various modes of action to destroy the microorganisms such as by rupturing the cell wall, denaturing proteins or lipids, oxidation, alkylation, etc. The efficacy of a disinfectant hinges on various factors including concentration, contact duration, temperature, pH, the presence of organic matters and metal ions. Choice of the disinfectant to be used depends on the particular situations. Some of the disinfectants are adopted because of the wide spectrum of destroying microorganisms in order to achieve effective disinfections. Others destroy a smaller range of disease-causing organisms but are preferred because the chemical disinfectants are less or non-toxic to human and the level of disinfections required is low. There are disinfectants which possess surfactant effect and are used to clean and disinfect in “one-step” process (Labour Department, Chemical Safety in the workplace. 2001).Disinfectants are antimicrobial agents that are applied to the surface of non-living objects to destroy microorganisms that are living on the objects (Division of Oral Health- Infection Control Glossary U.S. 30 April 2018).

A perfect disinfectant would also offer complete and full microbiological sterilization, without harming humans and useful form of life is inexpensive, and noncorrosive. However, most disinfectants are also, by nature, potentially harmful (even toxic) to humans or animals. Most modern household disinfectants contain Bitrex, an exceptionally bitter substance added to discourage ingestion, as a measure. Those that are used indoors should never be mixed with other cleaning products as chemical reactions can occur (Common cleaning Products 30 April 2018). The choice of disinfectant to be used depends on the particular situation. Some disinfectants have a wide spectrum (kill many different types of microorganisms), while others kill a smaller range of disease-causing organisms but are preferred for other properties (they may be non-corrosive, non-toxic, or inexpensive).(Hospital Disinfectants for is Disinfection of Environmental 30 April 2018).
Formalin as Disinfectant:
Formalin is a 37% solution of formaldehyde gas in water. Diluted to 5% formaldehyde it is an effective disinfectant; at 0.2% - 0.4% it can inactivate bacteria and viruses. Unlike chlorine, formalin does not corrode stainless steel. It has a pungent, irritating odor; exposures must be limited due to its toxicity and carcinogenicity. A 100% formalin solution is equivalent to 37%–40% formaldehyde. In dialysis, a 4% formaldehyde (11% formalin) concentration is used.

Formaldehyde is a cold sterilant that effectively kills all microorganisms, including spores and resistantViruses, when used in proper concentrations and given adequate contact time. There are several reports of serious to deadly septicemia, with inadequate formaldehyde concentrations being used to disinfect the fluid path of dialysis machinery. It is the most common periodic disinfectant used for fluid-delivery systems. It is an inexpensive and stable solution with a long shelf life. Formaldehyde Concentrations lower than 4% do not adequately kill *Mycobacterium chelonae* in water.

Ethanol as Disinfectant:

Journal of Media Virology 84(3), 543-547, 2012. Ethanol- containing hand rubs are used frequently as substitute for hand washing with water and soap. However, not all viruses are inactivated by a short-term rubbing with alcohol. The capacity of a single round of instructed and controlled hand cleaning with water and soap or ethanol-containing hand rub, respectively, was tested for removal of human rhinovirus administered onto the skin of healthy volunteers on the back of the hands. Hand washing with soap and water appeared to much more efficient for removing rhinovirus

Pathogens (Disease causing bacteria)

A pathogen in the oldest and broadest sense is anything that can produce disease. Typically the term is used to mean an infectious agent-a microorganism in the widest sense such as virus, bacterium prion, a plant, a fungus or even other microorganism. The ability of pathogen to cause is called pathogenicity.

Bacteria:

1. *Staphylococus aureus:*

Scientific Classification:-

Domain: Bacteria
Kingdom: Eubacteria
Phylum: Firmicutes
Class: Bacilli
Order: Bacillales
Family: Staphylococcus
Genus: Staphylococcus
Species: *S. aureus*

2. *Pseudomonas aeruginosa:*

Scientific classification:-

Kingdom: Bacteria
Phylum: Proteobacteria
Class: Gramma Proteobacteria
Order: Pseudomonadaceae
Family: Pseudomonadaceace
Genus: Pseudomonas
Species: *P.aeruginosa*

3. *Escherichia coli*:

Scientific Classification:-

Domain: Bacteria
Phylum: Proteobacteria
Class: Gammaproteobacteria
Order: Enterobacterials
Family: Enterobacteriaceae

Genus: Eschericha
Species: *E. coli*

MATERIAL AND METHOD

Collection of different hand washes and disinfectant: It was collected from local market of Dehradun, Uttarakhand, India.

Collection of pure culture: The bacterial cultures were collected from Pathology Lab of Mahant Indresh Hospital, Dehradun, India.

Chemicals and Media used:

Distilled water,Liquid and Solid hand wash, Formalin, Nutrient agar media and Nutrient broth

Preparation of Bacterial Inoculum

For Inoculum preparation nutrient broth was made according to manufacturer's instructions and 10ml of broth medium was dispensed in screw capped test tubes and sterilized by autoclaving at 121°C for 15 minutes. The test tubes were cooled and kept in an incubator for 24 hours at 35ºC to check sterility. The isolated strains were inoculated in the sterilized test tube containing the medium, and placed in an incubator overnight at 35°C. The presence of turbidity in broth culture was noted. Nutrient broth is used for generalcultivation of less fastidious microorganisms.

Composition of Nutrient Broth

13grams constituents were suspended in 1000ml distilled water and heated it if needed, to dissolve the medium completely then dispensed as desired and sterilized by autoclaving at 15 lbs pressure (121ºC) for 1 minute. A loop full of bacterial strain was inoculated in 10ml of Nutrient broth in 3 test tubes and incubated for 72 hrs to get active strains, and then stored in refrigerator for further use.

Composition of Nutrient Broth

Ingredient	Gms / Litre
Peptone	5.000
Sodium chloride (NaCl)	5.000
Beef extract	3.000
pH	7.4+0.2

Preparation Nutrient Agar Media(NAM)

28grams constituents were suspended in 1000ml distilled water and heated it if needed, to dissolve the medium completely. Dispensed as desired and sterilized by autoclaving at 15 lbs pressure (121ºC) for 15minutes and was mixed well before pouring.

Nutrient Agar Medium(NAM)

Ingredient	Gms/ Litre
Peptone	5.000
Sodium chloride(NaCl)	5.000
Agar	15.00
Beef extract	3.000
Final Ph(at 250C)	7.4+0.2

Preparation of Sterile discs

Whatman's No3 filter paper were made in 5mm discs and wrapped in aluminum foils andthen sterilized it in an oven for 30minutes.

METHODS

1. Disc Diffusion Method

For each test, 100ml nutrient broth was inoculated with few cells of pathogenic bacterium and incubated at 37ºC for 24 hrs in incubator. For each test, 15 petriplates were prepared for the growth of bacteria by pouring NAM in each petriplate and allowed them to solidify. After solidification, 1 mlof brothwas poured in each Petri plate with the help of micropipette. Broth culture was spread uniformly

on nutrient agar plate with sterile glass spreader. The plate was air dried for few minutes. Sterile filter paper disc were soaked with 100%, 80%, 60%, 40%, 20% concentration of different handwash and disinfectant. Then these discs were placed on inoculated nutrient agar plates. These plates were then incubated at 37ºC for 24hrs in incubator. After incubation, cleared zones were observed around the discs that indicates the inhibition of growth of the microorganisms.

2. Well Diffusion Method

For each test, 5 plates were prepared for the growth of bacteria by pouring NAM in each Petri plate and allow them to solidify. After solidify, pour 1 ml of incubated broth in each Petri plate with help of micropipette. Broth culture was spread uniformly on nutrient agar plate with sterile glass spreader. The plate was air dried for few minutes. The wells are prepare with well puncture than dip in agar plate and wells in loaded 100%, 80%, 60%, 40%, 20% concentration of commercial from of different hand wash and disinfectant. These plates were then incubated at 37ºC for 24 hrs in incubator. After incubation, clear zones were observed around the discs that indicates the inhibition of growth of the microorganisms.

RESULTS AND DISCUSSION

The resultsobtained from this experiment shows that different types of microorganisms vary in their response toward different liquid handwash and disinfectants.

In liquid hand wash was found to be effective against *Pseudomonas sp.* were the zones of inhibition ranged from 4.8 mm maximum to 0.7 mm minimum whereas in case of disinfectant it ranged from 6.0 mm. and 4.0mm.

1. Results for Liquid Hand Wash

The effect of Dettol on *Pseudomonas*, *Staphylococcus* and *E. coli* is shown in the table no. 1(a –f) and Fig. (a -f) respectively and the comparison between the zone of inhibition of the three pathogens is shown in the bar graph 1 and 2 which shows that the Dettol was more effective against*Pseudomonas*andleast effective against *E. coli*.

Table 1.a- Zone of inhibition (mm)for *Pseudomonas sp.* (Disc Method)

Hand wash	Concentration (%)	Plate 1	Plate 2	Plate 3	Mean
Liquid Hand Wash	100	2.2	2.3	0.9	4.8
	80	0.9	1.7	1.5	3.1
	60	1.6	1.1	1.0	3.0
	40	0.3	0.3	0.3	0.3
	20	0.4	0.3	0.1	0.7

Table 1.b- Zone of inhibition (mm) for *Pseudomonas sp* (Well Method)

Hand wash	Concentration (%)	Plates 1	Plates 2	Plates 3	Mean
Liquid Hand Wash	100	2.2	2.1	1	4.6
	80	2.2	1	1.9	3.8
	60	1	1.4	1.2	2.8
	40	1	1.4	0.6	2.6
	20	0.4	0.3	0.3	0.8

Table 1.c-Zone of inhibition (mm) for *Staphylococcus sp.* (Disc Method)

Hand wash	Concentration (%)	Plate 1	Plate 2	Plate 3	Mean
Liquid Hand Wash	100	3.3	3	3.5	3.2
	80	2.1	2	2.4	2.1
	60	2.0	1.9	1.7	1.8
	40	1.6	1.9	2	1.8

	20	2.4	1.2	1.4	1.6

Table 1.d- Zone of inhibition (mm) for *Staphylococcus sp.* (Well Method)

Hand wash	Concentration (%)	Plate 1	Plate 2	Plate3	Mean
Liquid Hand Wash	100	2.7	1.9	3.1	2.5
	80	2.1	2.6	2.6	2.4
	60	2.5	2.1	2.2	2.2
	40	2.3	1.9	1.5	1.9
	20	1.6	1.7	1.2	1.5

Table 1.e- Zone of inhibition (mm) for *E. coli* (Disc Method)

Hand wash	Concentration (%)	Plate 1	Plate 2	Plate 3	Mean
Liquid Hand Wash	100	2	2.1	1.8	1.9
	80	1.5	1.5	1.4	1.4
	60	1.3	1.2	1.4	1.3
	40	0.6	0.4	0.5	0.5
	20	0.4	0.4	0.3	0.3

Table 1.f- Zone of inhibition (mm) for *E. coli* (Well Method)

Hand wash	Concentration (%)	Plate 1	Plate 2	Plate 3	Mean
Liquid Hand Wash	100	0.4	1.9	4	2.1
	80	0.2	2.1	1.7	1.3
	60	2.1	No Zone	1.7	1.2
	40	0.2	0.5	0.3	0.3
	20	No Zone	No Zone	No Zone	_

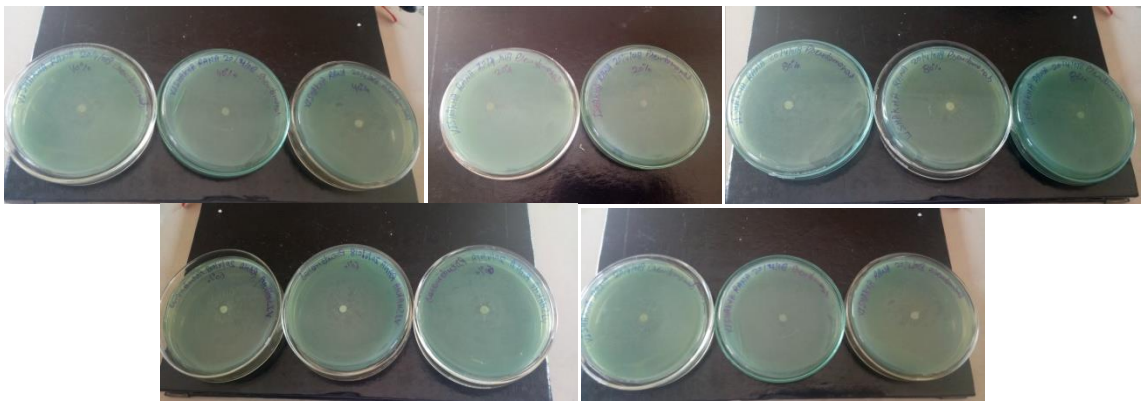


Fig. a- Liquid Hand Wash showing the Antibacterial Effect on *Pseudomonas sp (Disc method)*

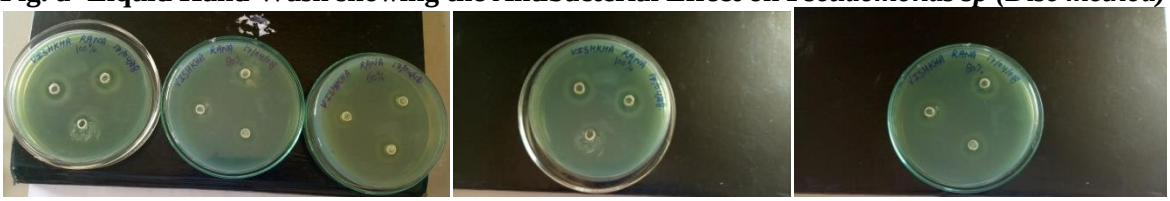


Fig. b- Liquid Hand Washshowing the Antibacterial Effect on *Pseudomonas sp. (Well method)*

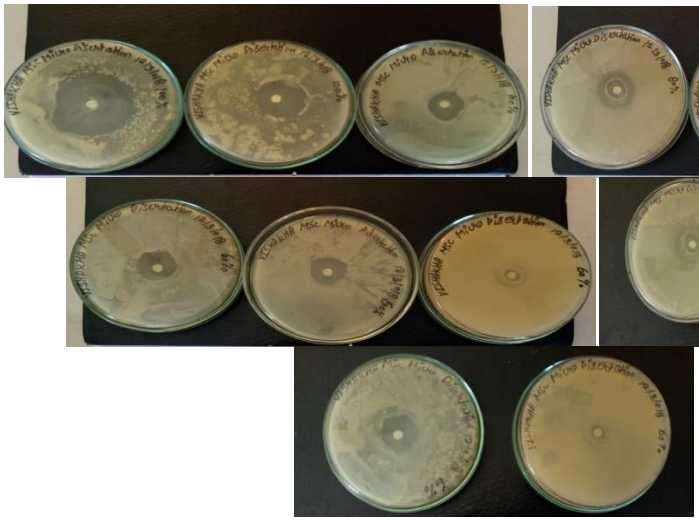


Fig. c- Liquid Hand Wash showing the Antibacterial Effect on *Staphylococcus sp (Disc method)*

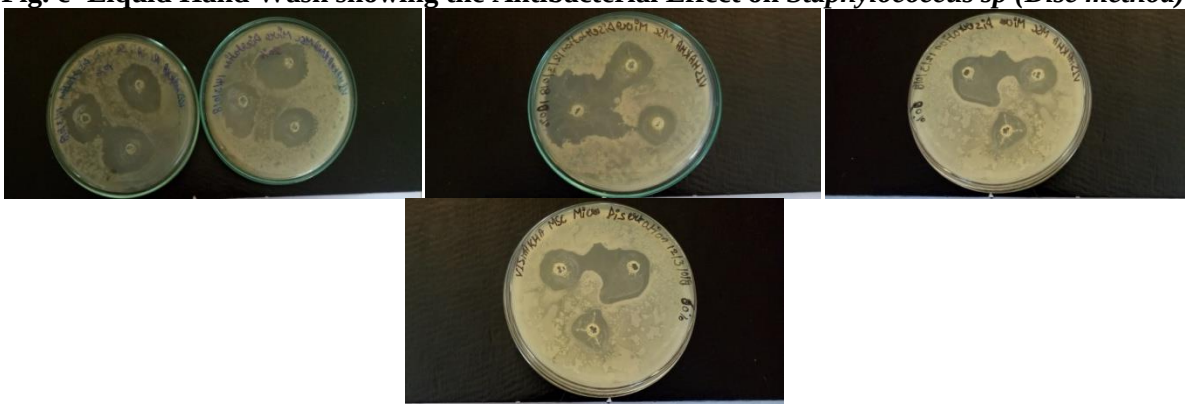


Fig. d- Liquid Hand Wash showing the Antibacterial Effect on *Staphylococcus sp (Well method)*

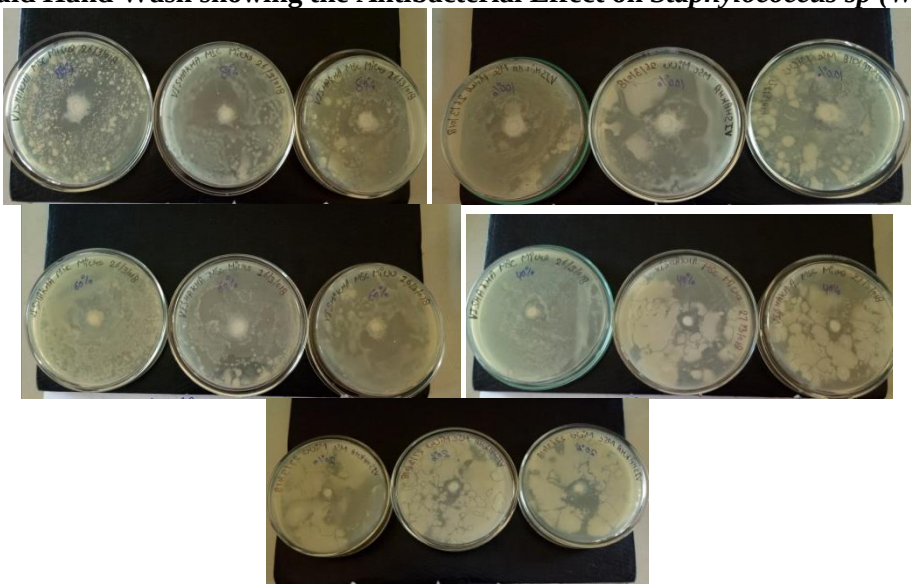


Fig. e- Liquid Hand Washshowing the Antibacterial Effect on *E. coli* (Disc method)

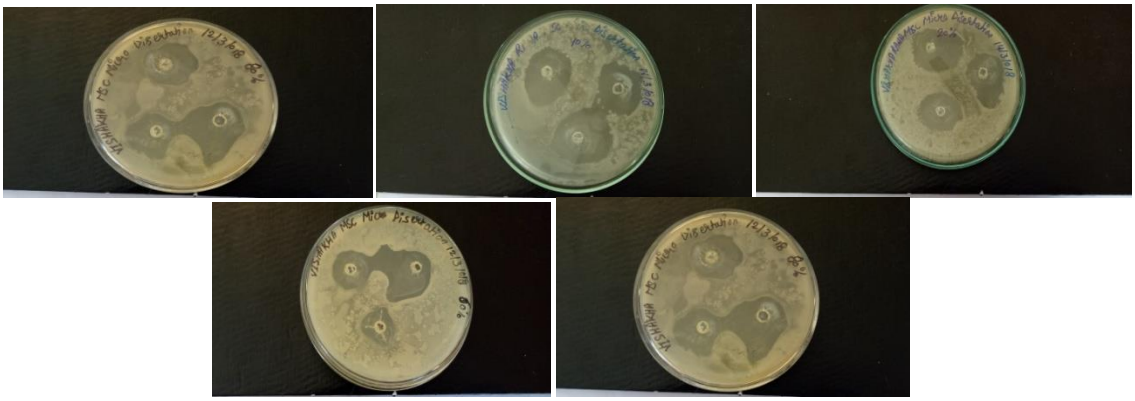
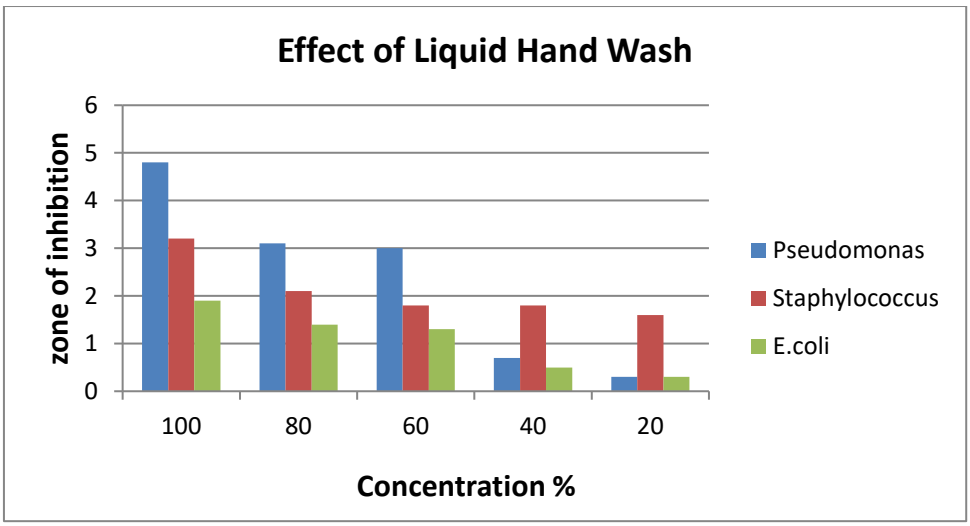
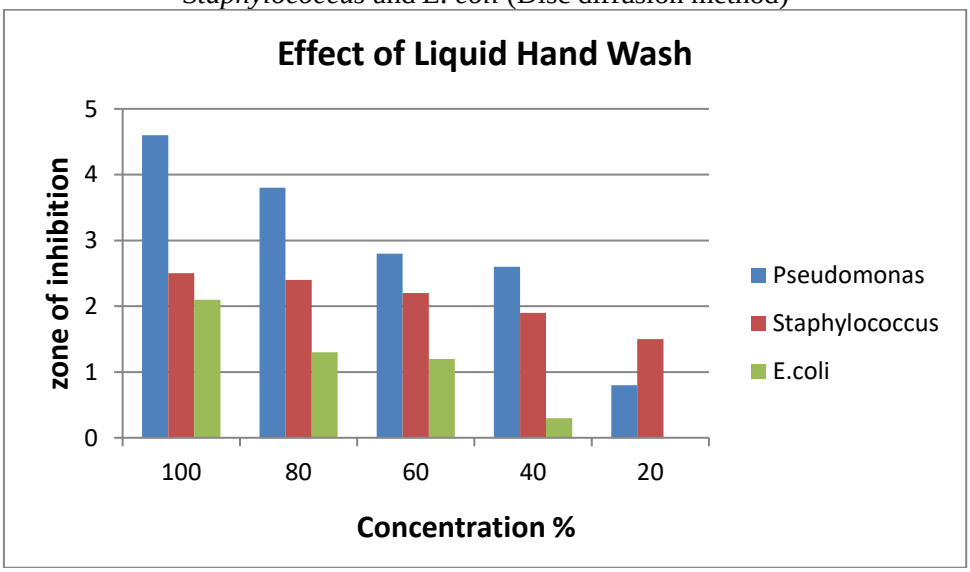


Fig. f- Liquid Hand Washshowing the Antibacterial Effect on *E. coil. (Well method)*



Graph 1. Depicts antibacterial effect of Liquid Hand Wash on growth of *Pseudomonas, Staphylococcus* and *E. coli* (Disc diffusion method)



Graph 2. Depicts the antibacterial effect of Liquid Hand Wash on the growth of *Pseudomonas, Staphylococcus* and *E. coli (*Well Diffusion Method)

2. Result for Solid Hand Wash

The effect of Solid Hand Wash on *Pseudomonas*, *Staphylococcus*, and *E. coli* is shown in the table 2(a-f)and Fig. (g- l) respectively and the comparison between the zones of inhibition of the three pathogens shown in the graph which show that the Solid Hand Wash was more effective against *E.coli* and was least effective against *Pseudomonas sp.*

Table 2.a-Zone of inhibition (mm) of Solid Hand Washfor *Pseudomonas sp* (Disc Method)

Hand wash	Concentration (%)	Plate 1	Plate 2	Plate3	Mean
Solid Hand Wash	100	1.3	1.9	1.7	1.6
	80	1.6	1.6	1.2	1.4
	60	1.3	1.3	1.3	1.3
	40	1.4	1.4	1.2	1.3
	20	1.1	0.7	0.2	0.6

Table 2.b. Zone of inhibition (mm) of Solid Hand Wash for *Pseudomonas sp* (Well Method)

Hand wash	Concentration (%)	Plate 1	Plate 2	Plate 3	Mean
Solid Hand Wash	100	1.6	1.6	1.6	1.6
	80	1.3	1.6	1.3	1.4
	60	1.2	1.5	1.7	1.4
	40	1.5	1.3	1.5	1.4
	20	1.2	1.2	1.2	1.2

Table 2.c Zone of inhibition (mm) of Solid Hand Wash for *Staphylococcus sp (*Disc Method)

Hand wash	Concentration (%)	Plate1	Plate 2	Plate3	Mean
Solid Hand Wash	100	1	1.3	1	1.1
	80	1.3	2	0.3	1.2
	60	0.6	0.9	0.9	0.8
	40	0.1	0.1	0.4	0.2
	20	0.2	0.1	0.1	0.1

Table 2.d Zone of inhibition (mm) of Solid Hand Wash for *Staphylococcus sp.* (Well Method)

Hand wash	Concentration (%)	Plate 1	Plate 2	Plate 3	Mean
Solid Hand Wash	100	1.4	1.8	1.7	1.6
	80	1.5	1.3	1.5	1.5
	60	1.5	1.4	1.3	1.4
	40	1.4	1.5	1.2	1.3
	20	1.4	1.1	1.2	1.2

Table 2.e Zone of inhibition (mm) of Solid Hand Wash for *E. coli* (Disc Method)

Hand wash	Concentration (%)	Plate 1	Plate 2	Plate 3	Mean
Solid Hand Wash	100	1.6	1.8	1.4	1.9
	80	1.7	1.7	2	1.8
	60	1.6	1.2	1.2	1.5
	40	0.4	1.3	1	0.9
	20	0.2	0.5	0.9	0.4

Table 2.f Zone of inhibition (mm) of Solid Hand Wash for *E. coli.*(Well Method)

Hand wash	Concentration (%)	Plate 1	Plate 2	Plate3	Mean

Solid Hand Wash	100	4	2.5	2	2.8
	80	3	2.6	2.7	2.7
	60	2.4	2.4	2.5	2.4
	40	2	2.3	2.1	2.1
	20	1.9	1.9	2.1	1.9

Fig. g- Solid Hand Wash showing the Antibacterial Effect on *Pseudomonas sp.(Disc method)*

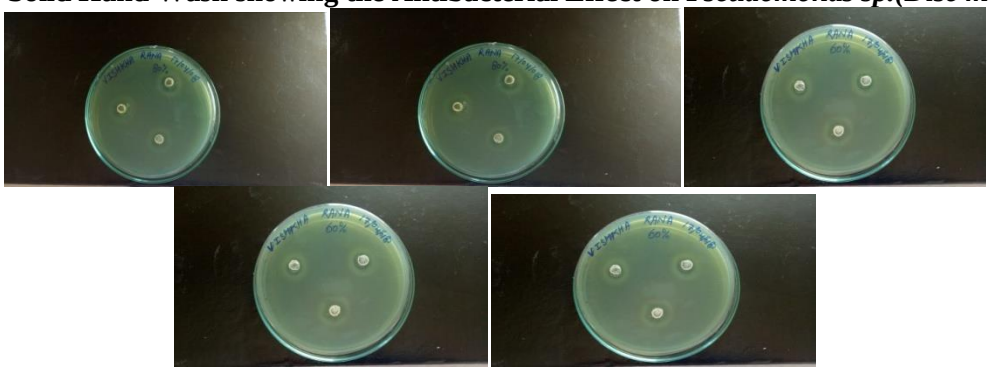


Fig. h- Solid Hand Wash showing the Antibacterial Effect on *Pseudomonas sp. (Well method)*

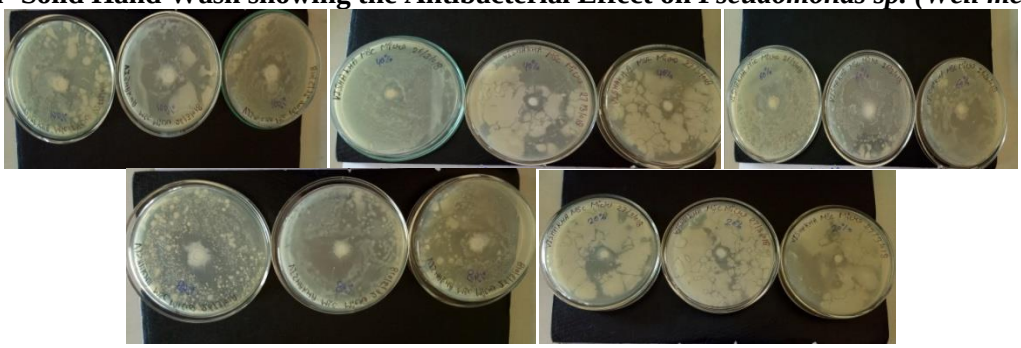


Fig. i- Solid Hand Wash showing the Antibacterial Effect on *Staphylococcus sp. (Disc method)*

Fig. j- Solid Hand Wash the Antibacterial Effect on *Staphylococcus sp. (well method)*

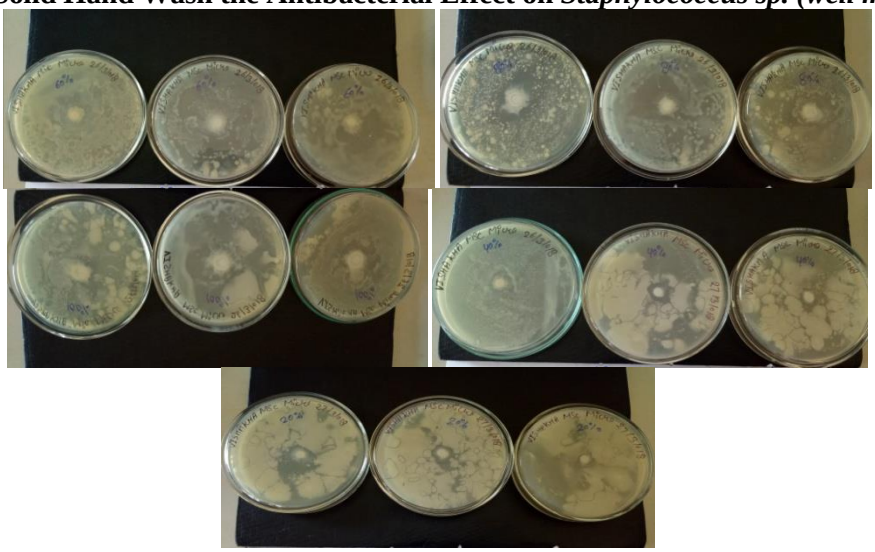


Fig. k- Solid Hand Wash showing the Antibacterial Effect on *E. coli*. (Disc method)

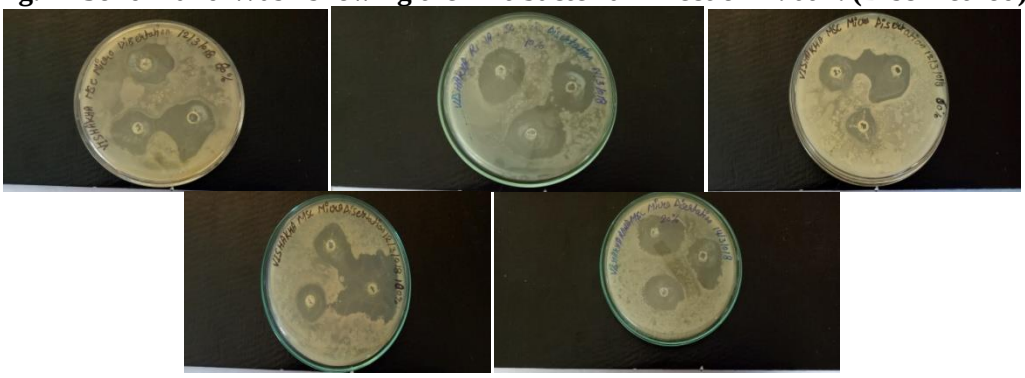
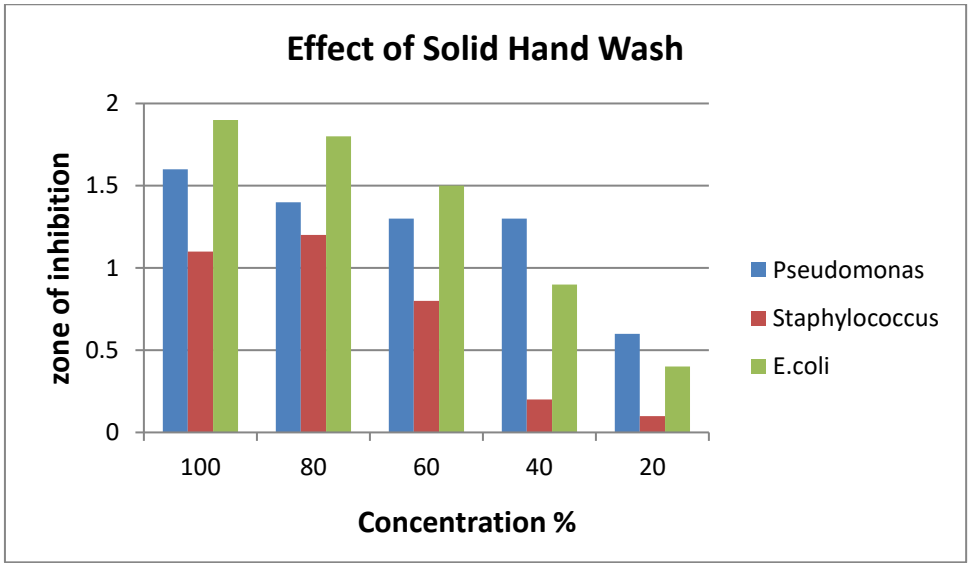
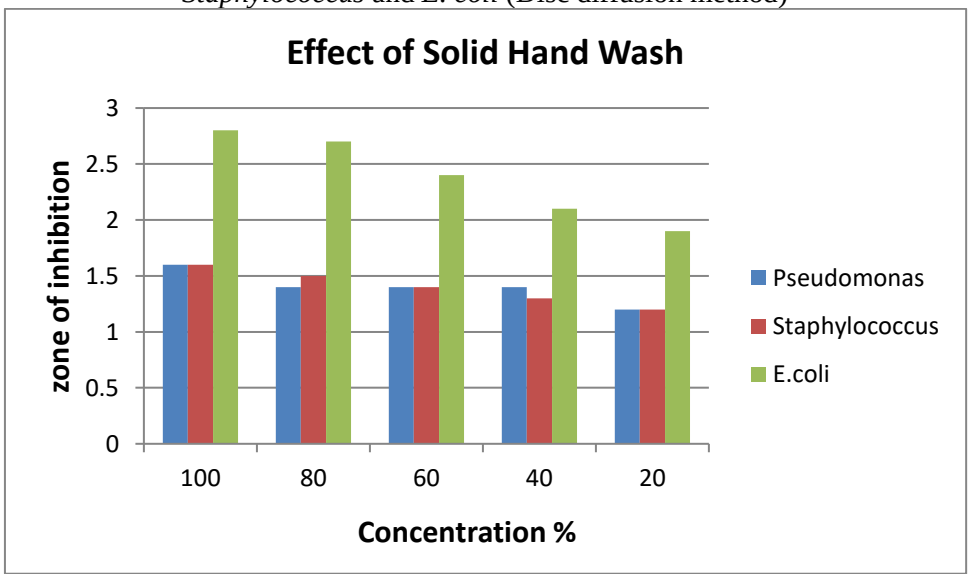


Fig. l- Solid Hand Wash showing the Antibacterial Effect on *E. coli (Well method)*



Graph 3. Depicts antibacterial effect of Solid Hand Wash on growth of *Pseudomonas*, *Staphylococcus* and *E. coli* (Disc diffusion method)



Graph 4. Depicts antibacterial effect of Solid Hand Wash on growth of *Pseudomonas*, *Staphylococcus* and *E. coli* (Well diffusion method)

3. Result for Formalin

The effect of Formalin on *Pseudomonas, Staphylococcus* and *E. coli* is shown in the table 3(a-d) and Fig. (m- p) respectively and the comparison between the zones of inhibition of the three pathogens is shown in the bar graph which shows that the Formalin was more effective against pathogens.

Table 3.a- Zone of inhibition (mm) of Formalin for *Pseudomonas sp.*(Disc Method)

Disinfectant	Concentration (%)	Plate 1	Plate 2	Plate 3	Mean
Formalin	100	4.9	4.3	5.3	4.8
	80	3.8	4.4	5.2	4.4
	60	4.1	4.1	4.1	4.1
	40	3.1	2.4	2.1	2.5
	20	2.3	2.4	2.5	2.4

Table 3.b- Zone of inhibition (mm) of Formalin for *Pseudomonas sp.* (Well Method)

Disinfectant	Concentration (%)	Plate 1	Plate 2	Plate 3	Mean
Formalin	100	5	5.2	5	5.2
	80	5.1	5.3	5	5.1
	60	5	5.1	5	5
	40	6.1	4.3	4.5	4.9
	20	4	4	4.1	4.1

Table 3.c-Zone of inhibition (mm) of Formalin for *Staphylococcus sp.*(Disc Method)

Disinfectant	Concentration (%)	Plate 1	Plate 2	Plate 3	Mean
Formalin	100	6.6	8.5	5.6	6.9
	80	6.6	5.4	6.1	6
	60	5.2	5.3	6	5.5
	40	4.5	4.4	4	4.4
	20	2.5	2.4	2.4	2.5

Table 3.d- Zone 0f inhibition (mm) of Formalin for*Staphylococcus sp.*(Well Method)

Disinfectant	Concentration (%)	Plate 1	Plate 2	Plate 3	Mean
Formalin	100	6	6.1	6	6
	80	5	6	6	5.6
	60	5.2	5.4	5	5.2
	40	5	4	4.3	4.4
	20	4	4.1	4.3	4.1

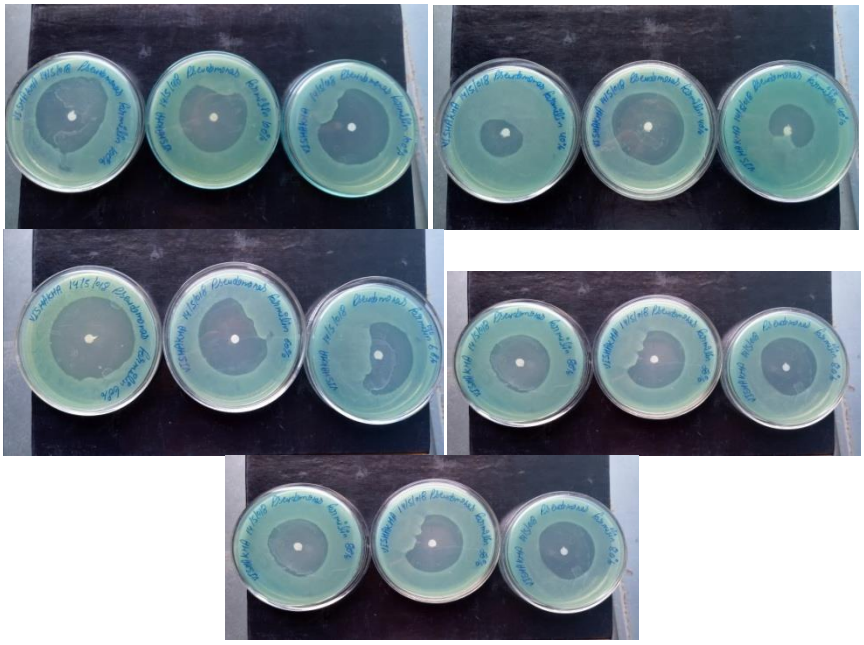


Fig. m- Formalin showing the Antibacterial Effect on *Pseudomonas sp. (Disc method)*

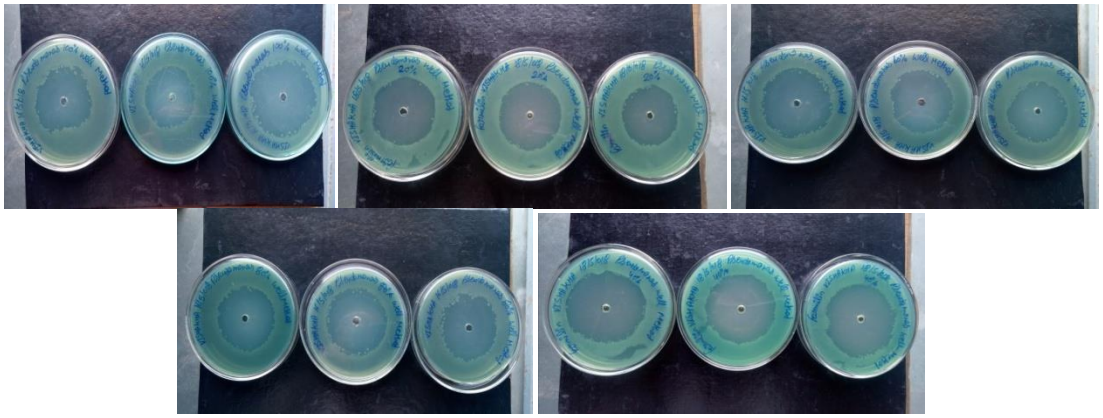


Fig. n- Formalin showing the Antibacterial Effect on *Pseudomonas sp. (Well method)*

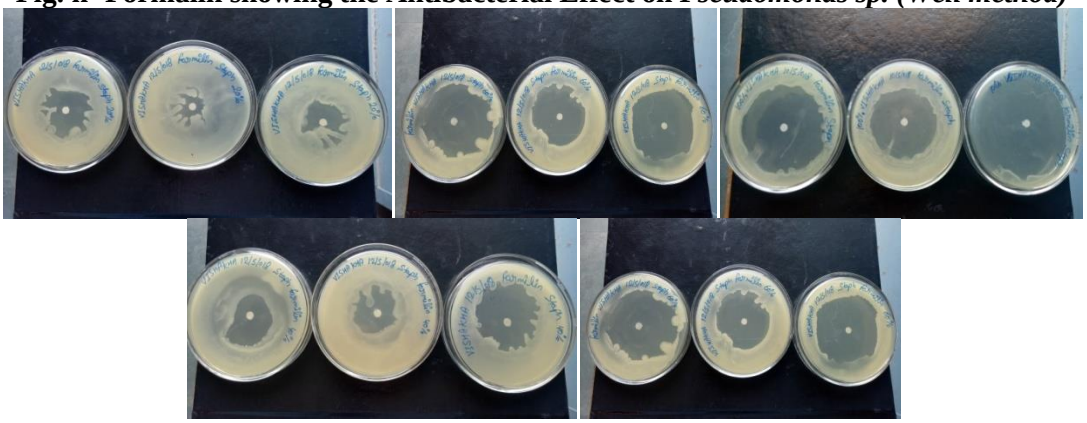


Fig. O- Formalin showing the Antibacterial Effect on *Staphylococcus sp. (Disc method)*

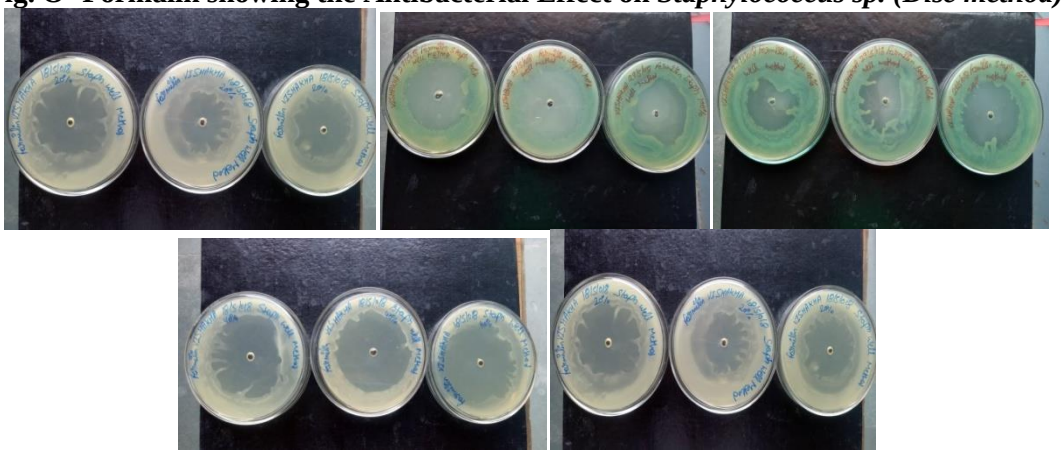
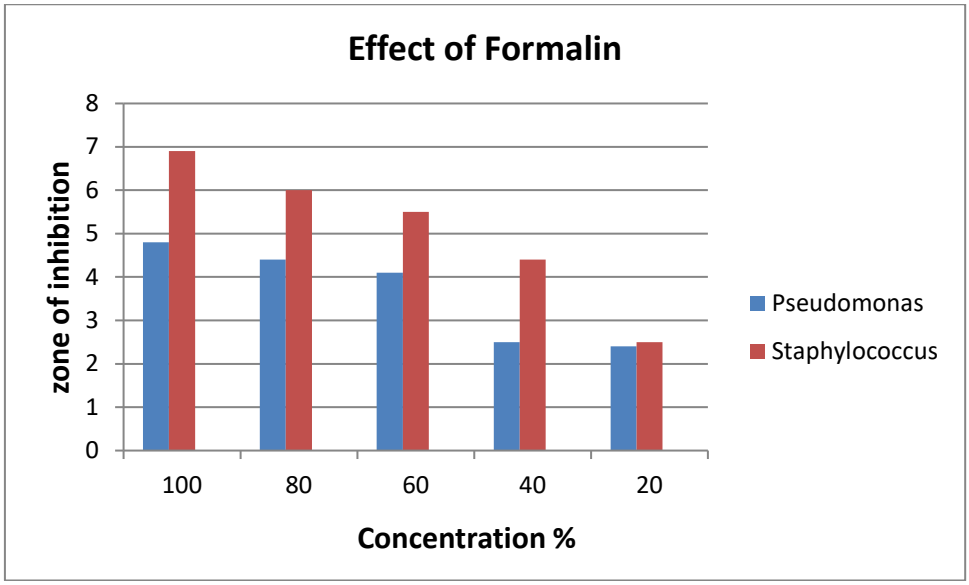
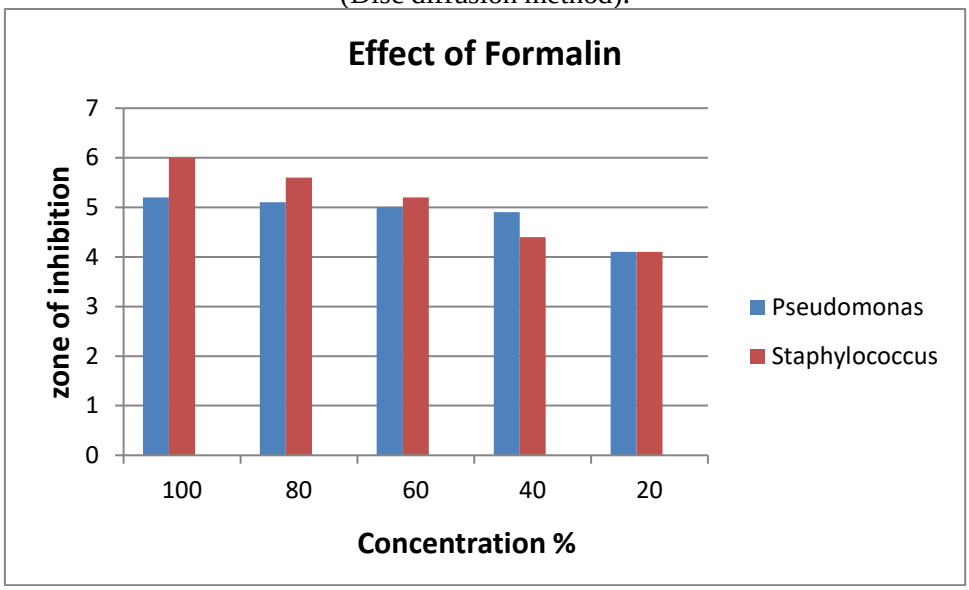


Fig. p- Formalin showing the Antibacterial Effect on *Staphylococcus sp. (Well method)*



Graph5- Depicts antibacterial effect of Formalin on growth of *Pseudomonas*, and*Staphylococcus* (Disc diffusion method).



Graph. 6-Depicts antibacterial effect of Formalin on growth of *Pseudomonas*,and*Staphylococcus* **(**Well Diffusion method)

DISCUSSION

Result of this experiment illustrated that different pathogens acquire resistance to different hand wash and disinfectant. The results also suggests that the antibacterial effects of liquid hand wash and disinfectant are not only dependent on the type of hand wash and disinfectant but also on their concentrations.

Similar results were found by Vaishali et al., 2011, they demonstrated that it is practically impossible to keep our surrounding free from microorganisms because they can grow in the presence of moisture. Sinha et al, 2009, have isolated and identified the most frequently occurring bacterial species as *E. coli*, *Micrococcus luteus* (Nonpathogenic) and *Serratia marcescens*, *Stahylococcus aureus, Pseudomonas aeruginosa and Bacillus cereus* (Pathogenic). They have used the disinfectant that are sold maximum and tried to compare their efficacy using in vitro analysis. People are happy with what

they use and only a few are open to try new product launched. Instead of the composition and inhibition factor, to select a specific disinfectant, odour and cost are major criteria. With respect to the above stated information obtained by survey analysis and confirm the earlier work done in laboratory, commonly used disinfectant like Formalin.

The present study also revealed that Liquid Hand Wash is effective antiseptic against *Pseudomonas, Staphylococcus and E. coli* and the zone of inhibition was maximum for *Pseudomonas* and minimum for *E.coli.* Nobody knows the significance of liquid hand washes even these are much better than plain soaps due to their ingredient and effectiveness on our skin of hands and as well as suitable for all type of skin might be that was most sensitive. Mostly hand washes protect us from many daily encountered bacteria.

In February (*Sajed et al., 2014)*showed that liquid soap significantly depressed the bacterial population than plain soaps. Similar result havebeen deducedby Toshima in 2001 (*Shahida., 2009).* Actually the soap hold ingredient (Iodophor and Triclosan). Triclosan is a bactericidial and appears to act upon several non specific targets. Plain soap is less effective than liquid soap; this was also confirmed by Connie and George from London in Text Book diagnostic Microbiology that liquid soap has a greater effect on inhibition and removal of bacteria population than bars (*Kaiser.2006).*

In 2014 Padma singh, Anchal Raniand Shampa Pal isolated bacteria as lab contaminated were *Bacillus, S. aureus, Pseudomonas, Micrococcus.* All these bacterial were identified on the basis of biochemical test and Bergey's mannul. To check the efficacy of chemical sterilization against the isolated bacteria by disc diffusion test first the zone of inhibition of different disinfectant on lab contaminant were observed by Kirby Bauer method (1966). In 2009 saba Riaz, Adeel Ahmad Shasida Hasnain illustrated that the plain soap also possessed antibacterial activity although lesser then that of antibacterial soaps. It was seen clearly that Gram positive bacteria were killed at low concentration of soap than Gram negative bacteria. The best of all soap used is lifebuoy white (antibacterial) because the calculation of the efficiency then the others used. The most resistant bacterium of all the soaps is *K. pneumonia* following *P. aeruginosa.* It is proved experimentally that antibacterial soaps kill the bacterial at a specific concentration; they also have bacteriostatic activity and can inhibit the growth of bacteria. Beauty soaps contain some natural and plant extracted ingredients in their composition which have the ability to inhibit or kill the bacteria so they also gave some bactericidal activity.

CONCLUSION

From the above results it can be concluded that Liquid Hand Wash and Formalinwere found highly effective against pathogens, Whereas Solid Hand Washwas foundlow effected against pathogens.

Results of this experiment also indicate that different pathogens acquired resistance to different liquid hand wash and disinfectant. The result also suggests that the antibacterial effect of hand wash and disinfectant are not only dependent on the type of hand wash and disinfectant but also on their concentration.

REFERENCES

1. Hassan AO, Hassan RO, Muhibi MA, Adebimpe WO. A survey of *Enterobacteriaceae*in hospital and community acquired infections among adults in a tertiary health institution in Southwestern Nigeria.

2. Mondal S, Kolhapure SA. Evaluation of the antimicrobial efficacy and safety of pure hands herbal hand sanitizer in hand hygiene and on inanimate objects. Antiseptic. 2004;101:55–7.

3. Pratt RJ, Pellowe C, et al. The epic project: Developing national evidence-based guidelines for preventing healthcare associated infections. Phase 1: Guidelines for preventing hospital-acquired infections. Department of Health (England) J Hosp Infect. 2001;47(Suppl 1):S3–82.

4. Johnson, S.A., Goddard, P.A., Iliffe, C., Timmins, B., Rickard, A.H., Robson, G. & Handley, P.S. (2002). *Comparative susceptibility of resident and transient hand bacteria to para-chloro- meta xylenol and triclosan.* J. Appl. Microbiol. 93: 336-344

5. Fluit, A.C., Schmitz, F.J. &Verhoef, J. (2001). *Frequency and isolation of pathogens from Blood stream, nosocomial pneumonia, skin and soft tissue, and urinary tract infections occurring in European patients.* Eur. J. Clin. Microbiol. Infect. 20: 188 - 191.

6. Higaki, S., Kitagawa, T., Kagoura, M., Morohashi, M. & Yamagishi, T. (2000). *Predominant Staphylococcus aureus isolated from various skin diseases.* J. Int. Med. Res. 28: 87-190.
7. Richards, M.J., Edwards, J.R., Culver, D.H. &Gaynes, R.P. (1999). *Nosocomial infections in medical intensive care units in the United States. National Nosocomial Infections Surveillance System.* Crit. Care Med. 27: 887-892.
8. Hand-washing Liason Group. 1999. *Hand washing. A modest measure – with big effects*. Br. Med. J. 318-686.
9. Horton, R. & Parker, L. (2002). *Informed Infection Control Practice*. Churchill Livingstone, Second Edition. London.
10. Johnson, S.A., Goddard, P.A., Ilife,C..Timmens, B., Richard., A.H., Robson, G and Handley, P.S (2002). *Comparative susceptibility of resident and transient hand bacteria to para-chlorometa-xylenol and triclosan.* Journal of Applied Microbiology. 93: 336-344
11. . Osborne, R.C and Grube, J (1982). *Hand disinfection in dental practice,* Journal of Clinical Preview. Dent 4:11-15.
13. Richards, M.J, Edwards,J.E., Culver, D.H and Gaynes, R.P (1999*). Nosocomial infections in medical intensive care units in the United States. National Nosocomial infections surveillance system*. Critical Care Medicine journal 27. 887-892.
14. Larson, E,. McGinley, K., Grove, G.L., Leyden. J.J and Talbot, G.H (1989). *Physiologic, microbiologic and seasonal effects of hand washing on the skin of health care personnel*. American Journal of Infection control 14: 5-90.
15. Kimel, L.S (1996). *Hand washing education can decrease illness absenteeism.* Journal of School Nursery. 12: 14-18.
16. Poole, K (2002). *Mechanisms of bacterial bioade and antibiotic resistance.* Journal of Applied Microbiology. 92: 555-564.
17. White, D.G and McDermolt, P.F ((2001). *Biocides, drug resistance and microbial evolution;* Current Opinion in Microbiology; 4: 313-317.
18. Aiello AE, Coulborn RM, Perez V, Larson EL (2008*): Effect of hand hygiene on infectious disease risk in the community setting: a meta analysis.* Am J Public Health 98: 1372-1382.
19. Johson PD, martin R, burrelllJ, grabsch EA, kirsa SW, et al. (2005). Effeciency of an alcohol/chlorhexidine hand hygiene program in a hospital with high rate of nosocomial mechicilinresistantStaph.ylococcus aureus (MRSA) injection. Med J Aust 183:509-514.
20. Harrington G, Watsn K, Barley M, L and G, burrel 5 et al (2007*). Reduction in hospital wide incidence of infection or colonization with molecular resistance Staph.ylococcus aureus with use of antimicrobial hand hygiene gel and stastitical process control chart infect control hosp Epidermal* 28:837-844.
21. Hilburn, J., Hammond, B. S., Fendler, E. J., &Groziak, P. A. (2003). *Use of alcohol hand sanitizer as an infection control strategy in an acute care facility*. *American journal of infection control*, *31*(2), 109-116.
22.Enwa Felix, O., Anie Clement, O., &OghenejoboMicheal&Ilaya Sonia, A. (2015). *Evaluation of the Comparative Activity of Alcohol-Based Hand Sanitizers and Toilet Soaps against some Bacterial Isolates.*
23. Pickering A J, Davis J and Boehm A B (2011), *"Efficacy of Alcohol-Based Hand Sanitizer on Hands Soiled with Dirt and Cooking Oil",J Water Health, 9*(3) 429-433.
24. Hübner N O, Hübner C, WodnyM ,Kampf G and Kramer A (2010), *"Effectiveness of Alcohol-based Hand Disinfectants in a Public Administration: Impact on Health and Work Performance Related to Acute Respiratory Symptoms and Diarrhea"*, http:// www.biomed central.com/1471-2334/10/250
25.Hand sanitizer - definition of hand sanitizer in English | Oxford Dictionaries". Oxford Dictionaries | English. Retrieved on 27April, 2018.
26. Bolon, MK (September 2016). "Hand Hygiene: An Update". *Infectious disease clinics of North America*. **30** (3): 591–607.

27. Boyce, JM; Pittet, D; Healthcare Infection Control Practices Advisory, Committee.; HICPAC/SHEA/APIC/IDSA Hand Hygiene Task, Force. (25 October 2002). "Guideline for Hand Hygiene in Health-Care Settings. Recommendations of the Healthcare Infection Control Practices Advisory Committee and the HICPAC/SHEA/APIC/IDSA Hand Hygiene Task Force. Society for Healthcare Epidemiology of America/Association for Professionals in Infection Control/Infectious Diseases Society of America". *MMWR. Recommendations and reports : Morbidity and Mortality Weekly Report. Recommendations and reports*. **51** (RR-16): 1–45, quiz CE1–4.
28. *The selection and use of essential medicines: Twentieth report of the WHO Expert Committee 2015 (including 19th WHO Model List of Essential Medicines and 5th WHO Model List of Essential Medicines for Children)* (PDF). WHO. 2015. p. 323
29. *De Witt Huberts, J; Greenland, K; Schmidt, WP; Curtis, V (1 July 2016). "Exploring the potential of antimicrobial hand hygiene products in reducing the infectious burden in low-income countries: An integrative review". American journal of infection control*. ***44*** *(7): 764–71*
30. Meadows, E; Le Saux, N (1 November 2004). "A systematic review of the effectiveness of antimicrobial rinse-free hand sanitizers for prevention of illness-related absenteeism in elementary school children". *BMC Public Health. **4**: 50.*
31. *Long, Bruce W.; Rollins, Jeannean Hall; Smith, Barbara J. (2015). Merrill's Atlas of Radiographic Positioning and Procedures (13 ed.). Elsevier Health Sciences. p. 16.*
32. Baki, Gabriella; Alexander, Kenneth S. (2015). Introduction to Cosmetic Formulation and Technology. John Wiley & Sons. p. 173.
33. Block, Seymour Stanton (2001). *Disinfection, Sterilization, and Preservation*. Lippincott Williams & Wilkins. p. 14.
34. Miller, Chris H.; Palenik, Charles John (2016). *Infection Control and Management of Hazardous Materials for the Dental Team* (5 ed.). Elsevier Health Sciences. p. 269
35. Division of Oral Health - Infection Control Glossary". U.S. Centers for Disease Control and Prevention. Retrieved 30 April 2018.
36. Division of Oral Health - Infection Control Glossary". U.S. Centers for Disease Control and Prevention. Retrieved 30 April 2018.
37. "Common Cleaning Products May Be Dangerous When Mixed" (PDF). New Jersey Department of Health and Senior Services. Retrieved 30 April 2018.
38. "Hospital Disinfectants for General Disinfection of Environmental Surfaces" *(PDF). New York State Department of Health*. Retrieved 30 April 2018.
39. Labour Department, Chemical Safety in the Workplace – Guidance Notes on Risk Assessment and Fundamentals of Establishing Safety Measures, 1st edition, 2001, HKSAR Government.
40. Savlon in medicines.org.uk\
41. Lucet, J. C., Rigaud, M. P., Mentre, F., Kassis, N., Deblangy, C., Andremont, A., & Bouvet, E. (2002). Hand contamination before and after different hand hygiene techniques: a randomized clinical trial. *Journal of hospital Infection*, *50*(4), 276-280.
42. Lowbury, E. J. L., & Lilly, H. A. (1973). Use of 4% chlorhexidine detergent solution (Hibiscrub) and other methods of skin disinfection. *Br Med J*, *1*(5852), 510-515.
43. Marchetti, M. G., Kampf, G., Finzi, G., &Salvatorelli, G. (2003). Evaluation of the bactericidal effect of five products for surgical hand disinfection according to prEN 12054 and prEN 12791. *Journal of Hospital Infection*, *54*(1), 63-67.
44. Nishimura, C. (2006). Comparison of the antimicrobial efficacy of povidone-iodine, povidone-iodine-ethanol and chlorhexidine gluconate-ethanol surgical scrubs. *Dermatology*, *212*(Suppl. 1), 21-25.
45. Luby, S. P., Halder, A. K., Huda, T., Unicomb, L., & Johnston, R. B. (2011). The effect of handwashing at recommended times with water alone and with soap on child diarrhea in rural Bangladesh: an observational study. *PLoS medicine*, *8*(6), e1001052.
46. CDC,(2000) center for disease control.retrived august (2016)
47. CDC,(2015) ,Landro,L.(2009)

48. Guinan,M.(2002). The effective of acomprehensive handwashing program on absenteeism in element schools. American journal of infection control.retrived (2016)
49. Rolfe, T.(2016) Tourchscreens: The mosquito of the digital age. Infection control. tips.(2016).
50. Scott, E (2010) prevention of the spread of infection. American journal of infection control (2016).