



## Evaluation of the Comparative Activity of Alcohol-Based Hand Sanitizers and Toilet Soaps against some Bacterial Isolates

By Enwa Felix O, Anie Clement O, Oghenejobo Micheal & Ilaya Sonia A

*Delta State University, Nigeria*

**Abstract-** This study is aimed at comparing the activity of alcohol based hand sanitizers (Dettol<sup>®</sup> and Lovillea<sup>®</sup>) and toilet soaps (Lux<sup>®</sup> and Premier<sup>®</sup>) against bacterial isolates. The activity of Dettol<sup>®</sup> and Lovillea<sup>®</sup> was compared with the activity of Lux<sup>®</sup> and Premier<sup>®</sup> toilet soaps against the bacterial isolates (*Staphylococcus aureus*, *Staphylococcus epidermidis*, *Streptococcus specie* and *Shigella specie*) obtained from the palms of some individuals in Abraka, Delta state. Susceptibility of the bacterial isolates was evaluated using the agar well diffusion method by measuring the zones of inhibition observed after 24 hours of incubation. Their minimum inhibitory concentrations (MIC) were also determined using the agar dilution technique. Dettol<sup>®</sup> hand sanitizer had the highest zones of inhibition (5mm, 5mm and 3mm) against *Staph aureus*, *Staph epidermis* and *Shigella specie* respectively.

**Keywords:** sanitizers, toilet soaps, hand washing, bacterial.

**GJSFR-C Classification :** FOR Code: 069999



*Strictly as per the compliance and regulations of :*



# Evaluation of the Comparative Activity of Alcohol-Based Hand Sanitizers and Toilet Soaps against some Bacterial Isolates

Enwa Felix O<sup>α</sup>, Anie Clement O<sup>σ</sup>, Oghenejobo Micheal<sup>ρ</sup> & Ilaya Sonia A<sup>ω</sup>

**Abstract-** This study is aimed at comparing the activity of alcohol based hand sanitizers (Dettol<sup>®</sup> and Lovillea<sup>®</sup>) and toilet soaps (Lux<sup>®</sup> and Premier<sup>®</sup>) against bacterial isolates. The activity of Dettol<sup>®</sup> and Lovillea<sup>®</sup> was compared with the activity of Lux<sup>®</sup> and Premier<sup>®</sup> toilet soaps against the bacterial isolates (*Staphylococcus aureus*, *Staphylococcus epidermidis*, *Streptococcus specie* and *Shigella specie*) obtained from the palms of some individuals in Abraka, Delta state. Susceptibility of the bacterial isolates was evaluated using the agar well diffusion method by measuring the zones of inhibition observed after 24 hours of incubation. Their minimum inhibitory concentrations (MIC) were also determined using the agar dilution technique. Dettol<sup>®</sup> hand sanitizer had the highest zones of inhibition (5mm, 5mm and 3mm) against *Staph aureus*, *Staph epidermis* and *Shigella specie* respectively. Both soaps had no activity against Gram negative *Shigella specie*. Dettol<sup>®</sup> antiseptic was used as control and it gave zones of inhibition (36mm, 45mm, 35mm and 50mm) against *Shigella specie*, *Streptococcus specie*, *Staph aureus*, and *Staph epidermidis* respectively. For the MIC; the hand sanitizers inhibited all four organisms at 2ml, Premier<sup>®</sup> soap had MIC of 5mg/ml against *Staph epidermidis* while Lux<sup>®</sup> soap had MIC of 5mg/ml against *Staph epidermidis* and *Streptococcus specie*. The result revealed the efficacy of the antiseptic (control), hand sanitizers and soaps in descending order as follows; Antiseptic > Hand sanitizers > soaps. The use of soaps for hand hygiene appeared to be less efficacious against Gram negative organisms. However, both hand sanitizers and soaps can be used as effective measures to control the spread of diseases.

**Keywords:** sanitizers, toilet soaps, hand washing, bacterial.

## I. INTRODUCTION

Hand washing is one of the most important steps to avoid spreading germs. Germs are microorganisms such as bacteria and viruses that may lead to harmful diseases. They can live on the skin, mouth, intestines and breathing passages. They can enter the 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 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 *et al*, 2008). It is an integral procedure in the health care environment with healthcare workers receiving regular training about hand hygiene procedures (Hilburn *et al*; 2008, Johnson *et al* 2005, Harrington *et al* 2007). In the community outside of the healthcare environment, studies have reported association between improvements in hand hygiene and reduction in rates of infectious disease (Mello *et al*, 2007). It is estimated that simple hand washing could save one million lives a year (Curtis and Cairncross; 2003, WHO; 2000), many public health campaigns worldwide have addressed "hand hygiene" with varying success (Erasmus *et al* 2010, Pittet *et al*, 2009).

Bacteria are a tiny group of unicellular microorganisms. They can be classified into two groups, namely Gram Negative and Gram Positive organisms. An example of a Gram negative organism is *Escherichia Coli*; this type of bacteria is shaped like tiny pink rods and is found in raw meat or in the intestine of healthy humans and animals. *Staph. ylococcus* is an example of gram positive organisms; these are purple and clustered like Grapes. There are many types of *Staph. ylococcus* such as *Staph. aureus* and *Staph. epidermidis*. *Staph. aureus* colonizes mainly in the nasal passages while *Staph. epidermidis* is an occupant of the skin. Bacteria are found almost everywhere in environment such as air stool, water, sewage, human body, wounds, and other solid surfaces (Hurst *et al*, 2009). Although some are beneficial in the human body, others are not and may cause problems (Rolli and Jenner 1998). When pathogens or opportunistic microorganisms gain access into the body, they can cause infectious diseases, induce antigen-antibody reactions, mix with the normal flora and also may form bio-films (Macowiak, 1982).

Studies on the effectiveness of hand sanitizers have been somewhat conflicting. Some findings suggest that sanitizers are actually better than normal hand washing at killing micro-organisms while others have discovered that hand washing is still superior. The research indicates that there are many variable that would be causing these discrepancies. First of all, the concentration of alcohol – based sanitizers needs to be at least 60% to be effective. Alcohol based sanitizers at

Author <sup>α</sup> <sup>σ</sup> <sup>ρ</sup> <sup>ω</sup> : Department of Pharmaceutical Microbiology, Faculty of Pharmacy, Delta State University, Abraka, Nigeria.  
e-mail: felixenwa@yahoo.com

this concentration or higher are very effective at killing microbes but the alcohol evaporates quickly on the hands and may not be present on the skin long enough for adequate protection. As a result, unless the product can maintain high alcohol concentrations for a long period of time, it is probably not effective as regular hand washing hence, the center for disease control advises using warm water, working the soap into a lather and rubbing it for at least 20 seconds. However, hand washing with soap removes the body's own fatty acid from the skin, which may result in cracked skin that provides an entry portal for pathogens (Cagataz *et al*, 1998, Winnefield *et al* 2000). Also, high quality hand disinfectants contain additional skin care products like emollients. They also do not require the use of water which makes the application easy and uncomplicated.

## II. OBJECTIVES OF THE STUDY

- To isolate potential pathogenic bacteria such as *Staph.ylococcus aureus*, *Staph.ylococcus epidermidis*, *Streptococcus specie* and *Shigella specie* from the palms of individuals within Abraka, Delta state.
- To compare the activity of two different alcohol-based hand sanitizers (Dettol® and Lovillea®) and two different toilet soaps (Premier® and Lux®) commonly sold in Abraka, Delta state against the organisms isolated.

### a) Significance of the Study

This study serves to broaden the knowledge of the general public on the effect of hand sanitizers and toilet soaps against microorganisms for effective hand hygiene which is a key intervention in disease prevention.

Furthermore, the knowledge might encourage manufacturers of these products in ensuring better compliance to good manufacturing procedures.

### b) Scope of the study

The study covers comparison of the effects of two alcohol-based hand sanitizers and two toilet soaps commonly found in Abraka, Delta State.

### c) Limitation of the Study

This study was targeted on comparing the effects of some selected hand sanitizers and soaps against isolated bacteria species from the palms of individuals. The toxic effect of these products on the human skin was not determined.

### d) Objectives of the Study

- To isolate potential pathogenic bacteria such as *Staph.ylococcus aureus*, *Staph.ylococcus epidermidis*, *Streptococcus specie* and *Shigella specie* from the palms of individuals within Abraka, Delta state.

- To compare the activity of two different alcohol-based hand sanitizers (Dettol® and Lovillea®) and two different toilet soaps (Premier® and Lux®) commonly sold in Abraka, Delta state against the organisms isolated.

### e) Significance of the Study

This study serves to broaden the knowledge of the general public on the effect of hand sanitizers and toilet soaps against microorganisms for effective hand hygiene which is a key intervention in disease prevention.

Furthermore, the knowledge might encourage manufacturers of these products in ensuring better compliance to good manufacturing procedures.

### f) Scope of the study

The study covers comparison of the effects of two alcohol-based hand sanitizers and two toilet soaps commonly found in Abraka, Delta State.

### g) Limitation of the Study

This study was targeted on comparing the effects of some selected hand sanitizers and soaps against isolated bacteria species from the palms of individuals. The toxic effect of these products on the human skin was not determined.

## III. MATERIALS AND METHODS

### a) Collection of the Different Alcohol-Based hand Sanitizers and Toilet Soaps

Two commonly used alcohol-based hand sanitizers commercially sold were purchased. One of the sanitizers was purchased from a pharmacy while the other was purchased from a super market, both in Abraka, Delta state Nigeria. The toilet soaps used were also purchased from a supermarket in Abraka. The alcohol-based hand sanitizers bought are; Dettol® and Lovillea® alcohol-based hand sanitizer while the toilet soaps bought are; Premier® soap and Lux® soap.

### b) Culture media

The culture media used in the study include: Nutrient agar (Fluka, Germany), Macconkey Agar (Himedia India), Peptone water (Himedia India), Nutrient broth (San. Diego USA), Mueller Hinton Agar (Titan Biotech, India), Mannitol salt Agar (Titan Boitech, India).

### c) Specimen

Swab from palms of both hands.

### d) Reagents and chemicals used

Ethanol (BOH, India), crystal violet (Avishkar, Germany), Safranin (Avis, Germany), Iugol's Iodine (Brema, Nigeria), Hydrogen peroxide, sugars (Glucose, Lactose and Sucrose).

### e) Alcohol – based hand sanitizers

- Dettol® (Reckitt Benckiser)
- Lovillea® (PT Mandon, Indonesia)

f) Toilet Soaps

- Premier® (Pz cussons Nigeria Plc. Nigeria )
- Lux® (Unilever Nigeria Plc, ogun state)

g) Microbial Cultures

*Staph.ylococcus aureus*, *Staph.ylococcus epidermidis*, *Streptococcus specie*, *Shigella specie*.

h) Sample collection of bacterial isolate

Samples were obtained from individuals within Abraka, Delta state. The samples were obtained from the palms of both hands of the individuals using a sterile cotton swab. The swab specimens collected were then transported immediately to the laboratory for handling and analysis. The swab sticks containing the samples were aseptically streaked in different Nutrient Agar plates, after which the plates were incubated at 37°C for 24hours. After incubation, distinct colonies were observed on fifty eight Nutrient agar plates. The distinct colony found on each plate was then inoculated in separate nutrient agar slants prepared in McCartney bottles and incubated at 37°C for 24hours. Thereafter, growth was observed on all the fifty eight nutrient agar slants and the slants were properly stored for subsequent studies.

i) Characterization Based on Colony Morphology and physiology

i. Macroscopic Identification of Colonies

The organism identified in the course of this study were *Staph.ylococcus aureus*, *Staph.ylococcus epidermidis*, *Streptococcus species* and *Shigella specie*.

j) Biochemical Tests and Other Identification Tests

The following biochemical tests were carried out to confirm the identified organisms. The test includes;

- Catalase test.
- Coagulase test
- Indole test
- Fermentation test

k) Bacterial susceptibility to alcohol- based hand sanitizers and soaps

Muller-Hinton Agar was prepared according to manufacturer's specifications, sterilized, cooled, 20mls each poured into eight sterile petri dishes and kept for 45 minutes in order to allow it solidify. Thereafter, the test organisms were aseptically inoculated into four different properly labelled petri dishes containing already solidified Muller Hinton agar by using different sterile swab sticks to pick the organisms from prepared overnight broth and streaking the organisms all over the petri dishes. This procedure was carried out using another four properly labeled petri dishes which served as duplicate for the experiment.

A 5mm cork borer was used to bore holes in the solidified agar on each petri dish. Using a 2ml syringe,

few drops each of the hand sanitizers, 10mg/ml solution of the soaps, and Dettol® antiseptic (used as control) was added to their respective holes in the petri dish. After 5minutes, all the petri dishes were carefully packed with a masking tape and transferred into the incubator for 24hours at 37°C. Zones of inhibition were observed and recorded after 24hours.

l) Determination of minimum inhibitory Concentration

The determination of the minimum inhibitory concentration of the soaps was carried out to determine the lowest concentration of the soaps that can inhibit the visible growth of the test organisms (*Staph.ylococcus aureus* *Staph.ylococcus epidermidis*, *Streptococcus specie* and *Shigella specie*) after 24 hours of incubation. The agar dilution technique was used and it involves the following process. Firstly, 19ml of sterilized Muller Hinton agar was poured with 1ml of each dilutions of the soaps (Lux® and Premier® soaps) into eight different petri-dishes and allowed to solidify. Four of the petri-dishes contained 1ml each of the several dilutions (10mg/ml, 5mg/ml, 2.5mg/ml and 1.25mg/ml) of one soap (Premier®) plus 19ml each of the sterilized Muller Hinton agar while the other four petri-dishes contained 1ml each of several dilutions (10mg/ml, 5mg/ml, 2.5mg/ml and 1.25mg/ml) of the other soap (Lux®) plus 19mls each of the sterilized Muller Hinton agar. Thereafter, the test organism were streaked onto the different properly labelled plates seeded with the soap solutions using a flamed wire loop. The plates were packed with a Masking tape and incubated at a temperature of 37°C for 24 hours. After 24hours incubation, the least concentrations of each of the soaps that inhibited the test organisms were taken as the minimum inhibitory concentration.

For the alcohol-based hand sanitizers, different volumes of the hand sanitizers were tested to know if increased volumes of hand sanitizers enhance their effectiveness. Firstly, 19.8ml, 19.5ml, 19ml, 18.5ml and 18ml of sterilized Muller Hinton agar was poured with 0.2ml, 0.5ml, 1ml, 1.5ml and 2ml respectively of the two different alcohol-based hand sanitizers being tested into ten different petri- dishes. Five of the petri-dishes contained different volumes (0.2ml, 0.5ml, 1ml, 1.5ml and 2ml) of one alcohol based hand sanitizer (Dettol®) plus corresponding volumes of sterilized Muller Hinton agar while the other five petri dishes contained different volumes (0.2ml, 0.5ml, 1ml, 1.5ml and 2ml) of the other hand sanitizer (Lovillea®) plus corresponding volumes of the sterilized Muller Hinton agar. The mixture on each of the petri dishes was swirled gently and allowed to solidify. Thereafter, the test organisms were aseptically streaked onto the different prepared plates seeded with the alcohol based hand sanitizers using a flamed wire loop and then incubated at 37°C for 24 hours. After 24 hours of incubation, the least volume the hand sanitizers that inhibited the growth of the test organisms was observed and tabulated.

## IV. RESULT AND DISCUSSION

Table 3 : Effect of hand sanitizers and soaps against bacterial isolates

Test organism	Hand Sanitizers		Toilet soaps		Control
	Dettol®	Lovillea®	Lux® 10mg/ml	Premier® 10mg/ml	Dettol® antiseptic
<i>Staph. aureus</i>	5	3	2	2	35
<i>Staph. Epidemidis</i>	5	4	5	2	50
<i>Streptococcus spp</i>	2	5	6	5	45
<i>Shigella specie</i>	3	2	-	-	36

**Key:**

Zone of inhibition in millimeter (mm)

(-) sign represents no visible zone of inhibition

Table 4 : Minimum inhibitory concentrations (MIC) of the hand sanitizers and soaps

Table 4a : MIC of Dettol® hand sanitizer

Test organisms	Dettol®				
	0.2ml	0.5ml	1ml	1.5ml	2ml
<i>Staph. aureus</i>	+	+	+	+	-
<i>Staph.epidermidis</i>	+	+	+	+	-
<i>Streptococcus spp.</i>	+	+	+	+	-
<i>Shigella specie</i>	+	+	+	+	-

Table 4b : MIC of Lovillea® Hand Sanitizer

Test organisms	Lovillea® Hand Sanitizer				
	0.2ml	0.5ml	1ml	1.5ml	2ml
<i>Staph. aureus</i>	+	+	+	+	-
<i>Staph. epidermidis</i>	+	+	-	-	-
<i>Streptococcus spp.</i>	+	+	+	+	-
<i>Shigella specie</i>	+	+	+	+	-

Table 4c : MIC of Lux® Soap

Test organisms	Lux® Soap			
	10mg/ml	5mg/ml	2.5mg/ml	1.25mg/ml
<i>Staph. aureus</i>	+	+	+	+
<i>Staph. epidermidis</i>	-	-	+	+
<i>Streptococcus spp.</i>	-	-	+	+
<i>Shigella specie</i>	+	+	+	+

Table 4d : MIC of Premier® Soap

Test organisms	Premier® Soap			
	10mg/ml	5mg/ml	2.5mg/ml	1.25mg/ml
<i>Staph. aureus</i>	+	+	+	+
<i>Staph. epidermidis</i>	-	-	+	+
<i>Streptococcus spp.</i>	+	+	+	+
<i>Shigella specie</i>	+	+	+	+

**Key:**

(-) sign indicates no growth (inhibition)

(+) sign indicates growth (no inhibition)



## V. DISCUSSION

Two alcohol based hand sanitizers (Dettol® and Lovillea®), and two toilet soaps (Lux® and Premier® at 10mg/ml) were tested against four isolated bacterial species. Based on the data obtained from table 3, Dettol® had the highest zones of inhibition i.e. 5mm, 5mm, and 3mm against *Staph.ylococcus aureus*, *Staph.ylococcus epidermidis* and *Shigella specie* respectively, with a zone of inhibition (2mm) against *Streptococcus specie*. Lovillea® hand sanitizer had zones of inhibition (3mm, 4mm, 5mm and 2mm) against *Staph. aureus*, *Staph. epidermidis*, *Streptococcus specie* and *Shigella specie* respectively. For the soaps, Lux® soap had the highest zone of inhibition (6mm) against *Streptococcus specie*, with zones of inhibition; 2mm and 5mm against *Staph. aureus* and *Staph. epidermidis* respectively but there was no zone of inhibition for *Shigella specie*. Premier® soap had the lowest zone of inhibition i.e. 2mm against *Staph. aureus* and *Staph. epidermidis*, with a zone of 5mm against *Streptococcus specie* and no zone of inhibition against *Shigella specie*. Comparing the activity of the hand sanitizers and toilet soaps, three bacterial isolates (*Staph. aureus*, *Staph. epidermidis* and *Shigella specie*) were more susceptible to Dettol® hand sanitizer while *Streptococcus specie* was more susceptible to Lux® soap and least susceptible to Dettol®. Furthermore, Premier® soap had the least activity against *Staph. aureus* and *Staph. epidermidis*. The soaps had no effect against gram negative *Shigella specie* which indicates that the hand sanitizers were more effective than the toilet soaps against gram negative *Shigella specie*. The result also shows that *Shigella specie* is the most resistant amongst the isolated bacterial species, this is because it displayed the least zones of inhibition to the hand sanitizers and it was the only organism that displayed resistance to the soaps tested. *Streptococcus specie* and *Staph.ylococcus epidermidis* displayed the highest margin of susceptibility.

From the data obtained from the determination of the minimum inhibitory concentration as shown in table 4 above, *Shigella specie* was the most resistant bacteria because it was not inhibited by any of the soaps and was only inhibited by the hand sanitizers at the highest volume of 2ml. Lux® soap had MIC value at 5mg/ml against *Staph. epidermidis* and *Streptococcus specie* while Premier® soap had MIC value at 5mg/ml against *Staph. epidermidis* only. Lovillea® hand sanitizer inhibited *Staph.ylococcus epidermidis* only at a lower volume of 1ml while all four organisms were inhibited by the two alcohol based hand sanitizers (Lovillea® and Dettol® ) at a volume of 2ml. The most susceptible organism inhibited by the hand sanitizers and soaps (at lower concentrations) was *Staph.ylococcus epidermidis*.

## VI. CONCLUSION

Hand washing is one of the most important steps to avoid spreading germs. Germs can live on the skin, mouth, intestines etc. and can enter the 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. Therefore, effective hand hygiene is a key intervention in disease prevention (Aiello *et al*, 2008). The study revealed that *Staph.ylococcus aureus*, *Staph.ylococcus epidermidis*, *Streptococcus specie* and *Shigella specie* are present on the hands of humans. The result also revealed better efficacy of the alcohol based hand sanitizers in comparison to the toilet soaps because all four bacterial isolates were susceptible to them; with Dettol hand sanitizer having better activity against more bacterial isolates. Also, the soaps had no effect against Gram negative *Shigella specie* which makes them less efficacious. However, the activity of the hand sanitizers against the bacterial isolates was poor compared to that of the antiseptic which was used as control. Therefore, there is need to confirm the concentration of alcohol in hand sanitizers sold in order to verify the 99.9% germ killing ability of these products as claimed by the manufacturers.

The efficacy of alcohol based hand sanitizer is affected by several factors such as the type, concentration and volume of alcohol used, the contact time, (CDC, 2002), the test method (invivo and invitro), target organisms and matrix. (Liu *et al*, 2010.)

## VII. RECOMMENDATION

The outbreak of epidemic infections such as Ebola hemorrhagic fever (caused by the Ebola virus), HIV, Diarrhea, etc., some of which are highly infectious and can be easily transmitted through infected hands calls for the need to evaluate the effect of antimicrobials such as hand sanitizers as well as soaps commonly used for hand washing. The use soap and water only for hand hygiene can be effective if there is availability of clean water but if not, hand sanitizers are preferable because they are rinse free and as a result, do not require water. However, when hands are visibly dirty, hand sanitizers are not as effective as soap for cleansing. Hence, if hands are visibly dirty, effective hand hygiene can be achieved by first washing hands with soap and water after which hand sanitizers can be used but if not, hand sanitizers are preferably used alone. Furthermore, manufacturers and regulatory authorities should enforce stringent quality control measures during production and routine inspection to ensure the efficacy of these products. Finally, sanitation as a means of proper hygiene is essential for good health benefits for social and economic developmental purposes.

## REFERENCES RÉFÉRENCES REFERENCIAS

1. Akimitsu, N, Hamamoto. H., Inoue, R.I; Shoji, M; Akamine, A; Takemori, K.I; Hamasaki, N; Sekimigu K (1999) "increase in resistance of Methicillin-Resistant *Staph.ylococcus aureus* to B-lactams caused by mutations conferring resistance to benzalkonium chloride, a disinfectant widely used in Hospitals"" Antimicrobial agents and chemotherapy (American society for Microbiology) 43(12): 3040-3043.
2. Andrew, J.M. (2001) "Determination of minimum inhibitory concentration "Journal of antimicrobial chemotherapy. 48 (supplementary1): 5-16.
3. 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.
4. Boyce John M, Pittet Didier (October 25, 2002) "Guideline for hand hygiene in healthcare settings, Recommendations of the healthcare infection control practices advisory committee and the
5. Cutis V, Cairncross S (2003) Effect of washing hands with soap on diarrhea risk in the community: A systematic a review. Lancet infect Dis 3:275-81.
6. Davison H.C, Low J.C and Wool House M.E (2000). "What is antibiotic resistance and how can we measure it "Trends in microbiology 8(12):554-9.
7. Dyer D.L, Gerenriach K.B, Wadhams P.S, (1998). "Testing a new alcohol-free hand sanitizer to combat infection" AORN J 68 (2): 239 – 41, 234 – 44, 247 – 51.
8. Erasmus v, kuperus MN, Richards JH, vos Mc, oenema A, *et al* (2010). Improving hand hygiene behavior of nurses using action planning. a pilot study in the intensive care unit and surgical ward . j Hosp infect 76:161-4
9. Evans CA, *et al* (1950). Bacterial flora of the Normal human skin. Journal of investigative Dermatology, 15:305-324.
10. Fish D.N (2002) "Optimal antimicrobial therapy for sepsis" Am J Health Syst pharm. 59 suppl 1: 513 – 9.
11. Fredrickson J.K, Zachara J.M, Balkwill D.L, Kennedy D, Li S.M, Kostand Ariches h.m, Daly M.S, Romie M.F, Brockman F.J (2004). Geomicrobiology of high level nuclear waste. Contaminated vadose sediments at the Hanford sites Washington states. Applied and environmental microbiology 70 (7): 4330 – 41.
12. Geo FB (2004) "microbiological approach to clinical medicine" Journal of clinical pathological 45: 493-496.
13. Gibson L.L, Rose J.B, Haas C.N, Gerba C.P and Rusin P.A (2002). Quantitative assessment of risk reduction from hand washing with antibacterial soaps. Journal of Applied Microbiology 92: 136 – 143.
14. 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 stastitital process control chart infect control hosp Epidermal 28:837-844.
15. Hay R.J. Fungi and fungal infections of the skin, In: Noble WC, editor: The skin microflora and microbial skin disease. Cambridge UK: Cambridge University press; 1993. Pp. 232 – 263.
16. Heise E.R (1982) "Diseases associated with immune suppression" Environmental health perspective 43; 9 – 19.
17. Hibbard, John S. (2005) "Analysis comparing the antimicrobial activity and safety of current antiseptic agent" J: Infusion Nursing (Infusion Nurses Society) 28 (3): 194 – 207.
18. Hilburn. J, Hammond B5, fendler EJ, graziak PA (2003). Use of alcohol hand sanitizer as an injection control strategy in an acute care facility.
19. Ishiga T, Honda K, Shimuzu S (2005) "Whole organism biocatalysis" Current opinion in chemical biology 9(2):174-80.
20. Jimenez L, Chaing M (2006). Virucidal activity of a quaternary ammonium compound: a surrogate for norovirus. Am J Infect control 34(5):269 – 73.
21. Johson PD, martin R, burrell IJ, grabsch EA, kirsas SW, *et al.* (2005). Efficiency of an alcohol/chlorhexidine hand hygiene program in a hospital with high rate of nosocomial mecillin-resistant *Staph.ylococcus aureus* (MRSA) injection. Med J Aust 183:509-514.
22. Kampf G, Kramer A, (2004) Epidemiologic background of hand hygiene and evaluation of the most important agents for scrubs and rubs clinical microbiology Review 17: 863 – 893.
23. Katherine Harmon (2009). "Bugs inside: what happens when the microbes keep us healthy Disappear? Scientific American.
24. Kramer, Axel, Peter Rudolph, Gouter Kampf, Didier Pittet (2002)"Limited efficacy of alcohol based hand gels" lancet 359(April 37) 1489-1490.
25. Lark R.L, (2001). An outbreak of coagulase negative *Staphylococcus* surgical site infections following aortic valve replacement infection control and Hospital epidemiology. 22; 618 – 623.
26. Larson EL, early E, cloonon P, sugrue S, parades M (2000). An organization chymate intervention associated with increased handwashing and decreased nosocomia injection. Behav Med 26: 14-22.
27. Lee YL, *et al* (1994). Colonization by *Staphylococcus* species resistant to Methicillin or quinolone on hands of medical personal in skilled nursing facility. American Journal of Infection control, 22, 346-351.

28. Liu, P., Yuen Y., Hsiao, H.M., Jaykus., L.A., and Moe C., (2010). Effectiveness of liquid soaps and hand sanitizer against Norwalk virus on contaminated hands. *Appl. Environ. Microbial.* 76(2): 394-399.
29. Löffler, Harald, Gunter Kampf (2008) "Hand disinfection: How irritant are alcohol? *Journal of Hospital Infection* 70:44 – 8.
30. Mackintosh C.A, Hoffman P.N (1984) "An extended model for Transfer of Micro-organisms via the hands, Differences between organism and the effect of alcohol disinfection" *Journal of hygiene* 92: 345-55.
31. Macowiak, (1982). Textbook of Medical Microbiology, Medicine, Microbiology and immunology, Mc Graw-Hill companies Inc. 23<sup>rd</sup> Edition, 550P.
32. Marples R.R, Towers A.G. (1979) A laboratory model for the investigation of contact transfer of micro-organisms. *Journal of Hygiene (London)*; 82; 237 – 248.
33. Montes LF, Wilborn WH (1969); location of skin flora. *British journal of Dermatology*, 23-26.
34. Patrick D.R, Findon G, Miller T.E. (1997) Residual moisture determines the level of touch-contact-associated bacterial transfer following hand washing. *Epidemiology and infection.* 119; 391 – 325.
35. Pederson L.K, E. Held, J.D. Johansen and T. Agner (2005). "Less skin irritation from alcohol based disinfectants than from detergent used for hand disinfection". *British Journal Dermatology* 152 (6): 1142.
36. Pietsch, Hanns. (2001). "Hand antiseptic: Rubs Versus Scrubs, alcoholic solutions versus alcohol gels" *J. Hospital Infection (Hospital infection society)* 48 (suppl A): 533-536.
37. Pittet D, Allegranzi B, Byce J (2009). The world health organization guidelines on hand hygiene in health care their consensus recommendations, *Infect Control Hosp Epidemiol*30: 611-22.
38. Prescott L.M, Harely and Klein, A.D. (2009). *Microbiology* (6<sup>th</sup> Edition). Tata Mc Graw- Hill Publishing Company Limited, New Delhi. 912p.
39. Price PB, (1938). The bacteriology of normal skin: a new quantitative test applied to a study of the bacterial flora and the disinfectant action of mechanical cleansing. *Journal of infectious Disease.* 63:301-318.
40. Rayan GM, Flournoy DJ (1987), Microbiologic flora of human fingernails. *Journal of hand surgery. American*; 12:605-607.
41. Rolter M. (1999). "Hand washing and hand disinfection" *Hospital epidemiology and infection control.*
42. Saiman L (2004) "Microbiology of early CF lung disease" *pediatric respiratory reviews* 5 supply A: 5367 – 9.
43. Sandora, Thomas J (2008) "Reducing absenteeism from gastrointestinal and respiratory illness elementary school: a randomized, controlled trial of an infection-control Intervention *Journal: Pediatrics* (Evanston) ISSN; 0031 – 4005.
44. Sears CL (2005). A dynamic partnership: Celebrating our gut flora "Anaerobic"11(5): 247-51.
45. Son c, chuk t, childers T, usick S, Dowching M, *et al* (2011) practically speaking rethinking hand hygiene improvement programs in health care setting. *Am J infect control* 39:716-24.
46. Turnidge J.D, Ferraro M.J, Jorgensen J.H, Murray P.R, Baron E.J, Pfaller M.A, and Tenover F.C, (2003) *Susceptibility test methods: General considerations. Manual of clinical microbiology.* 8<sup>th</sup> ed. American society of clinical Microbiology. p1103.
47. University of Virginia cavalier daily. March 26, Research shows hand sanitizers more effected against cold.
48. WHO report 2000, General world health organization 164.
49. Wilson M. (2005) *Microbial inhabitants of humans: their ecology and role in health and disease.* New York, Ny: Cambridge university press.



This page is intentionally left blank