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Potentiation of Disinfectant Efficiency of Different Dilutions of Ethanol, Bleach and Phenolics against *Pseudomonas Aeruginosa* and *Staphylococcus Aureus*

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ABSTRACT---- ***Ethanol, Bleach and Phenolics are three kinds of disinfectants which have been widely used in common laboratories. In this study, a compared experiment on these three disinfectants efficiency was conducted against Staphylococcus aureus and Pseudomonas aeruginosa using agar hole diffusion method. Different concentrations of bleach (1%, 2%, 3%, 4% and 5%) were used on both organisms. Also (50%, 60%, 70%, 85% and 95%) of ethanol as well as (5%, 10%, 20%, 25%, and 30%) Phenolics were used. Differences in concentrations tested was because, the original concentrations of the disinfectants differs. After 24 hours of incubation at 37^{0}C, the results showed that all the disinfectants inhibited the growth of the test organism in their concentrated forms. The diameter of zone of inhibitions were measured around each well by using a ruler in millimeters, using different concentrations, their efficacies varied. The results showed that 30% Phenolics had the best efficiency against both test organisms and 5% bleach had a better effect on Staphylococcus aureus than Pseudomonas aeruginosa, while ethanol showed least sensitivity. 70% concentration gave the highest effect on Staphylococcus aureus as compared with Pseudomonas aeruginosa.***

1. INTRODUCTION

In the mid 1800s, the Hungarian physician Ignaz Semmeliveis and English physician Joseph Lister used these thoughts to develop some of the first microbial control practice for medical procedures. These practices include hand washing with microbes killing chloride of lime and use of techniques of aseptic surgery to prevent microbial contamination of surgical wounds (Hamamah, 2004). Over the last century, scientists have continued to develop a variety of physical methods and chemical agents to control microbial growth. Control directed at destroying harmful microorganisms is called disinfection. It usually refers to the destruction of vegetative (non-endospore forming) pathogens example bacteria by using a disinfectant to treat an inert surface or substances (Bhatia and Icchpujani, 2008).

Bacteria are major causes of disease and even human death. A disinfectant is one of the diverse groups of chemicals which reduces the number of microorganisms present (normally on an inanimate object). There are various official definitions of the process of disinfection and disinfectants agents. It is defined as a chemical that inactivates vegetative microorganism but not necessarily high resistant spores (ISO, 2008). Cleaning and disinfection of surfaces are essential steps for maintaining the cleanliness of pharmaceutical industries, hospitals and environments (Rollins, 2000). Disinfectant as effective agents that kill or eliminates bacteria is widely used in various ways; especially in microbial laboratory. Disinfectant can be mainly divided into five agents; alkylating, sulfhydryl combining, oxidizing, dehydrating and permeable. The most commonly used disinfectants in laboratories are ethanol, bleach and Isol (Larson and Morton, 1991). Bleach also known as sodium hypochlorite is a broad spectrum disinfectant, non specific in their action, only action biological material that is present on any surface. They effects by oxidizing the cell of microorganism and attacking essential cell components including lipid, protein and DNA (Ho-Hyuk Jang *et al*, 2008). Ethanol, as a dehydrating agent, lies between the highly specific and broadly based categories. It is effective against actively growing bacteria and viruses with a lipid based outer surfaces, but are not effective against bacterial spores or viruses that prefer watery environment. They cause cell membrane damages, rapid denaturalization of proteins with subsequent metabolism interference an cell lyses (Larson and Morton, 1991). Another surface disinfectant is the compound that contain phenol group, a popular commercial brand of Isol, (a saponated brand of cresol) as a phenolics are intermediate level disinfectant derived from coal tar, that are effective on contaminated surfaces (Bittel and Hughes, 2003).

However, certain types of viruses and some bacteria are resistant to the killing action of Phenolics compound (ISO, 2008). Many studies have been done on comparison of disinfectant efficiency, and ethanol and bleach are believed to

have immediate effect against most organisms (Carly *et al*, 2006). For bacteria species, the effects of ethanol, bleach, phenol on *Pseudomonas aeruginosa* and *Staphylococus aureus* are the bedrock of this study.
Pseudomonas aeruginosa is a classical opportunities pathogen with innate resistance to many antibodies and disinfectants. It is invasive, toxigenic and produces infection in patients with abnormal host deficiencies (Stephen *et al*, 2004). *Staphylococus aureus* occur in 40 – 50% of humans. Hospitalized patients as well as medical and paramedical staff show higher incidence of carriage of it (Bhatia and Icchpujani, 2008) in this study, disinfectant experiment was conducted using different concentrations of laboratory ethanol as disinfectant A, household bleach (Jik) disinfectant B and saponated brand of cresol (Isol) disinfectant C against *Pseudomonas aeruginosa* and *Staphylococus aureus*
The over all aim is to find out the concentration of disinfectants that will be effective in eliminating Gram positive *Staphylococcus aureus* and Gram negative *Pseudomonas areuginosa*.

2. MATERIAL AND METHOD

SAMPLES
Disinfectants were bought from main market Enugu (Ethanol disinfectant A, Jik disinfectant B, and Isol disinfectant C). The samples were aseptically collected using sterile swab stick and brought to the laboratory.

ISOLATION OF BACTERIA
Bacteria were Isolated from clinical sample and Bathroom.
- *Staphylococcus aureus* from wound pus swabs
- *Pseudomonas aeruginosa* from female bathrooms in caritas university Enugu.

Culture media for the organisms (MacConkey Agar & Blood Agar) were prepared according to the manufactorers instructions; samples was aseptically inoculated on the media and Incubated at 37^{o}C for 24hours. As described by Uzoechi *et al.*,2016 and cheesbrough,2000

PURIFICATION
After 24hours of incubation, the colonies that appeared to be similar were picked using a sterile wire loop and sub cultured on a nutrient agar in order to get pure colonies of the isolated organisms (*Staphylococcus aureus and Pseudomonas aeruginosa*).

IDENTIFICATION OF ISOLATES
Pure colonies of all the Isolates were identified using standard procedures described by Johnson and Case1995 and modified by Uzoechi *et al*.,2016 and Corper 2000

PREPARATION OF DISINFECTANTS
The method of Committee on Research Standard (CRS), 2005 And DHOP 2009 was adopted as shown below

➔ Original concentration in (%)	Disinfectant	Disinfectant	Disinfectant
Percent	95%	5%	30%

Various concentration of disinfectant A were prepared thus: 95% 85% 70% 60% and 50%

Using this formular $\frac{RV}{O}$ where R = Required concentration
V = Required volume of water
O = Original concentration.

If R = 85%, V = 10ml, O = 95%
➔ $\frac{85\% \times 10ml}{95\%} = \frac{850ml}{95} = 8.95ml$
:. 8.95ml of original concentration + 10 – 8.95ml = 1.05ml of water. For 85% concentration of disinfectant A.
Other disinfectants were diluted in the same way:
For each disinfectant, five different disposable tubes were used with disinfectant name, tube number and concentration and labeled thus:

Tube	**Concentration (%)**
1	95%
2	85%
3	70%
4	60%
5	50%

ANTIMICROBIAL SUSCEPTIBILITY TESTING (USING KIRBY BAUER DIFFUSION ASSAY WELL METHOD)

Procedure

Obtained twelve sterile disposable Petri dishes and labeled two each for one bacterial and disinfectant.

A permanent marker was used to divide each plates into six equal parts and numbered the bottom of the plates according to the concentration for each disinfectant, by writing the names of the disinfectant on the bottom of the plate. The sixth sector was written water for control. This was done for all the original plate and the replicates.

- The prepared 25ml nutrient agar media was poured into each of the plates.
- A loopful of the isolates was inoculate uniformly on each of the plates and this was done in all the plates with the test organisms
- The plates were allowed to dry for few minutes.
- For the test plates, a sterile cap borer was use to bore well in the six sectors labeled on the plates.
- 1ml each of different concentrations of the three disinfectants was pipette inside the well. But the centre sector for control 1ml of sterile water was pipette.
- The plates were allowed to stayed for 30minutes before incubation
- All the plates were incubated overnight at 37oC for 24 hours. After over night incubation, the plates were examined for zone of inhibitions and was result recorded. Method was described by (Rollins and Joseph, 2000 and Lages *et al.*,2008)

3. RESULTS

RESULTS OF THE TEST

i. ***CONTROLS:*** The control sectors showed uniform colonies around the well. No clear zone of inhibition.

ii. ***TEST SECTORS:*** The efficacies of the different disinfectants varied on dilutions. The result showed that all the disinfections inhibited the growth of the test organisms in their concentrated forms by showing different diameters of zone of 12inhibitions around each well, which was measured using meter rule in millimeter as shown in tables below:

Table 1: Results of diameter of zone of inhibition of ethanol, phenolics and bleach for *Staphylococcus aureus:*

Disinfectants	Concentrations (%)	Diameter of zone of inhibitions (mm)
A	95	2
	85	7
	70	20
	60	16
	50	14
B	5	24
	4	17
	3	14
	2	10
	1	0
C	30	38
	25	29
	20	44
	10	21
	5	20

Table 2:
Results of Diameter of zone Inhibitions of ethanol, phenolics, bleach for *Pseudomonas aeruginosa*

Disinfectants	Concentrations (%)	Diameter of zone of inhibitions (mm)
A	95	0
	85	8
	70	16
	60	15
	50	9
B	5	18
	4	16
	3	11
	2	7
	1	2
C	30	17
	25	16
	20	13
	10	10
	5	7

The tables below evaluated the test organisms response to each compound based on their different concentrations.

Table 3:
***Pseudomonas aeruginosa* response to Ethanol, Phenolics and Bleach**

Disinfectants	Concentrations (%)	Diameter of zone of inhibitions (mm)	Response
A	95	0	Resistant
	85	8	Resistant
	70	16	Susceptible
	60	15	Intermediate
	50	9	Resistant
B	5	18	Susceptible
	4	16	Susceptible
	3	11	Intermediate
	2	7	Resistant
	1	2	Resistant
C	30	17	Susceptible
	25	16	Susceptible
	20	13	Intermediate
	10	10	Resistant
	5	7	Resistant

Table 4:
***Staphylococcus aureus* response to ethanol, phenolics and bleach**

Disinfectants	Concentrations (%)	Diameter of zone of inhibitions (mm)	Response
A	95	2	Resistant
	85	7	Resistant
	70	20	Susceptible
	60	16	Susceptible
	50	14	Intermediate
B	5	24	Susceptible
	4	17	Susceptible
	3	14	Intermediate
	2	10	Resistant
	1	0	Resistant
C	30	38	Susceptible
	25	29	Susceptible
	20	44	Susceptible
	10	21	Susceptible
	5	20	Susceptible

N.B; Method Source (Johnson and Case, 1995) using this value below as standard:

	Diameter of zone of Inhibition (mm)
Resistant	10 or less
Intermediate	11 - 15
Susceptible	16 or more

PATTERNS OF THE ANTIMICROBIAL EFFICACIES OF VARYING CONCENTRATIONS OF THE DISINFECTANT ON THE TEST ORGANISMS

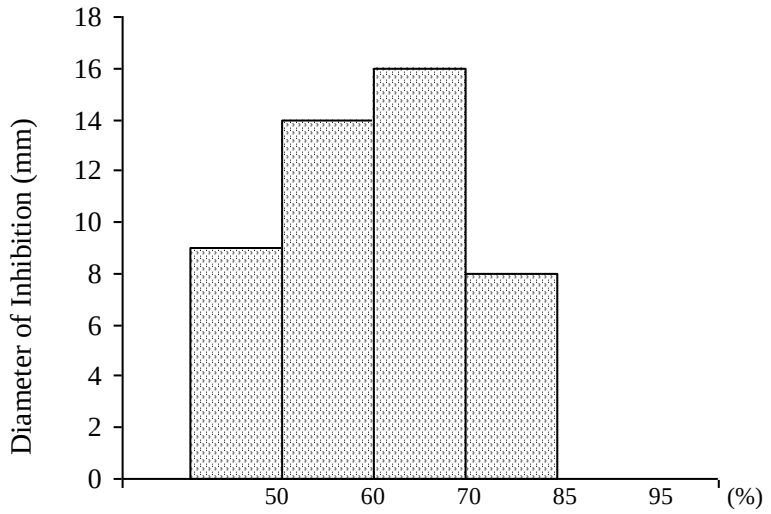
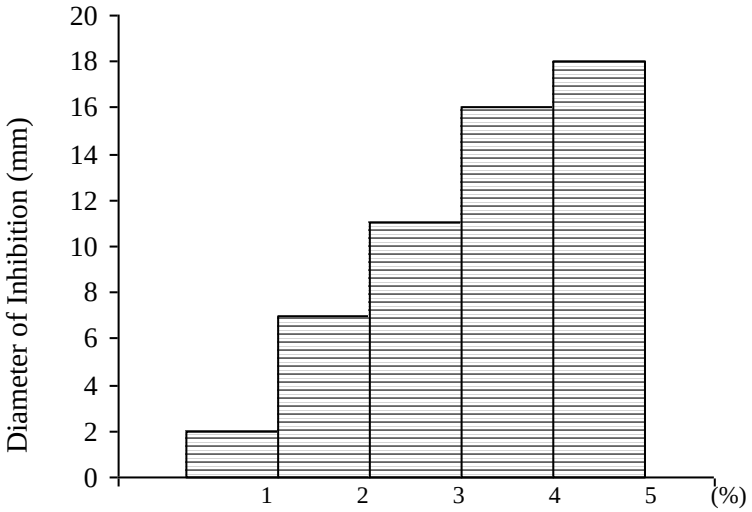
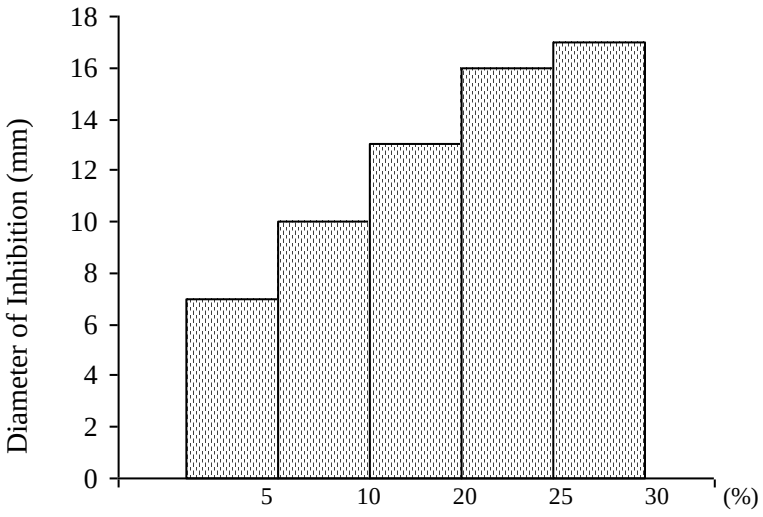


Fig 1: *Pseudomonas aeruginosa d*isinfectants A test result



Concentration of disinfectant B

Fig 2: *Pseudomonas aeruginosa* disinfectants B test result



Concentration of disinfectant C

Fig 3: *Pseudomonas aeruginosa* disinfectants C test result

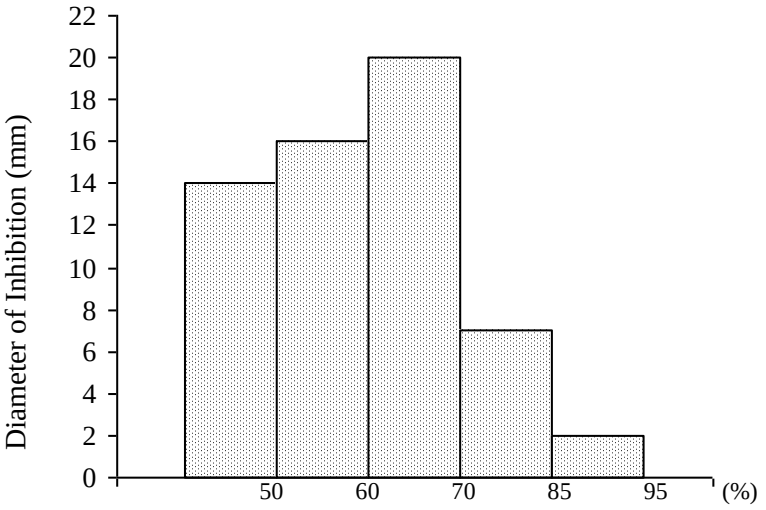


Fig 4*:* *Staphylococcus aureus* disinfectants A test result

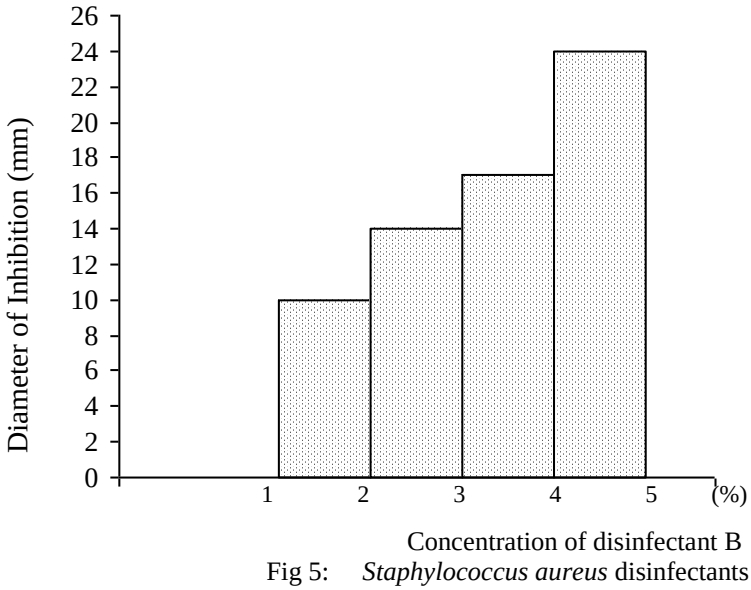


Fig 5: *Staphylococcus aureus* disinfectants B test result

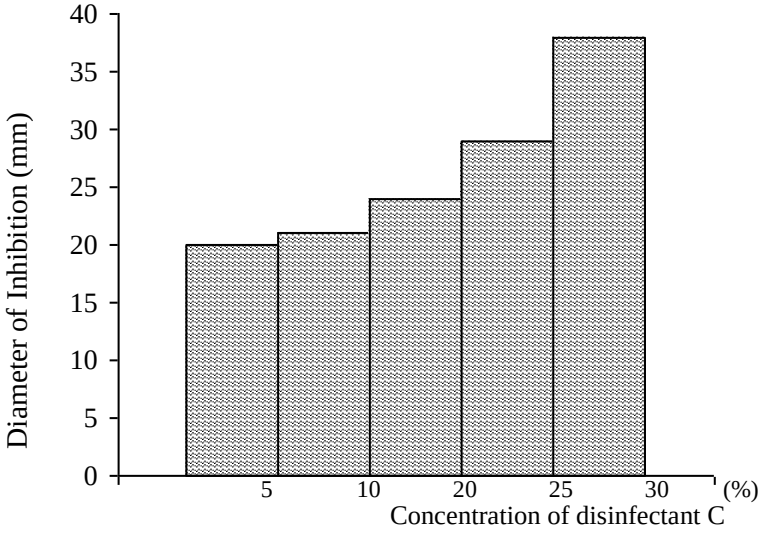


Fig 6: *Staphylococcus aureus* disinfectants C test result

From the figures 2, 3, 5, 6, it was shown that the diameters of the zones of inhibition decreased as the concentration of disinfectant decreased except in figure 1 and 4 where the higher the concentration, the lower the diameter of zone of inhibition.

4. DISCUSSION

From the different diameters of zones of inhibition of the three disinfectants under study, it was discovered that all the disinfectants inhibited the growth of the test organisms in their concentrated forms. On dilutions, their activities varied. Disinfectant C at 30% concentration showed the highest activity on *Staphylococcus aureus*, whereas Disinfectant. B and A showed the least. The distribution of the activities in decreasing order is as shown phenolics > bleach > ethanol.

Disinfectants B and C showed the highest activities at the concentrations of 5% 30% on *Pseudomonas aeruginosa*, whereas disinfectant A showed the least on the same organism. The distribution of their activities in decreasing order is as shown, bleach > phenolics > ethanol.

However, on the contrary, disinfectant A has the lowest antimicrobial effect as compared to others on both organisms. From table 6, disinfectant C had the highest inhibitory activity and can be deduced to be highly bactericidal on both organisms. According to Weber *et al*, 1999, phenolics which is active ingredient for disinfectant C are active against bacteria (especially gram positive bacteria). This tallies with my findings, a phenolics p[roves highest inhibition against *Staphylococcus aureus*. Owing to their high activity level, disinfectants C maintain their activities in the presence of organic material (milk) as they last long on surfaces unlike ethanol which evaporates easily (Weber *et al*, 1999 and Critin et al; 2005). Also since the mode of action of phenols in mainly by protein penetration and cell disruption, this extrapolates the bactericidal action of phenols (McDonell and Russel 2001).

Moreover, from the results, it indicated that bleach had an ideal bactericidal effect against both *Pseudomonas aeruginosa and Staphylococcus aureus* at 55 and 5% Concentrations as seen in tables 3 and 4. According to Barindra *et al* 2006, former study, it found that oxidation reactions will occur when bleach is dissolved in water, which can destroy organisms fold structure leading to sterilization. Another study also found similar result that bleach is rapidly bactericidal achieving a 5log10 kill of *Pseudomonas aeruginosa* and other vegetative organisms in one minute (Fraise, 1999).

The data's in figures 2, 3, 4 and 5 generally showed that diameters of zone of inhibition decreases as the concentrations of disinfectant decreases, but the observation was stable in disinfectant A. from the results in figures 1 and 4, it was shown that as the concentration of ethanol increased, the diameter decreased. Ethanol are rapidly bactericidal rather than bacteriostatic against vegetative forms of bacteria (gram $_{+}$ve and gram-ve), but their cidal activities drop sharply when diluted below 60% concentration and optimum bactericidal concentration in the range of 60% - 90% solution in water, volume/volume (Moorer, 2003). The result showed that 70% ethanol gave better effect on both test organisms than other ethanol concentrations. According to Moorer 2009, 70% ethanol had been found to be most effective to denature protein thereby killing bacteria, because of its diffusion rate and transportation into the cells organism. It evaporates at a slow rate and less harmful to the hand, this is the reason why it's been used in the laboratories for disinfection. Below 70% does not denature protein, while 85%-absolute ethanol evaporates fast and leave the protein untouched. They leave traces

on the applied surfaces thus, adding unwanted reagents. Also, they are harmful to the skin thereby making it dry and may not be effective.

From this study, it confirmed Carly *et al* 2006, study which showed similar result that higher concentrations are less effective as the action of denaturing proteins is inhibited without the presence of water. They also evaporate rapidly which makes extended exposure time difficult to achieve unless items are immersed in the ethanol (Carly *et al*, 2006).

According to Yi Hsing *et al*, 2002 researches, it also found that some kinds of bacteria cannot be billed easily and have some characteristics of resistance on ethanol. Its sterilization in mainly due to dehydration of protein enzyme deactivation and prevent bacteria growth. Different proteins have different biological characters which cause selectivity in ethanol deactivation of organisms. However, this conforms with Yi Hsing *et al*, 2002, as *Pseudomonas aeruginosa* are more resistant to disinfectant A.

In addition, disinfectant C and B are both effective disinfectants for sterilization against *pseudomonas* aerations and *Staphylococcus aureus* but disinfection C has the highest inhibitory effect.

5. CONCLUSION

The main goal of this study is to compare the efficiency of three disinfectants at five different concentrations. Conclusively, among the three common disinfectants tested in this project, disinfectant C in all its concentration had this best efficiency against both *Pseudomonas aeruginosa and Staphylococcus aureus.*

When these antimicrobial agents are used to disinfect sites suspected to be contaminated with gram positive bacteria, they should be used in their concentrated forms. Any dilution above this will only succeed in providing the user with a false sense of security

6. REFERENCES

- Barindra. S. Ghosh. D, Saha. M, Mukherjee .J. (2006). Purification and Characterization of a Salt, Solvent, Detergent And Bleach Tolerant Protease From A New Gamma-Proteobacterium Isolated from the Marine Environment of the sundarbans. *Process Biochemistry*, **41**(1);208-215.
- Bittel, K. and Hughes, R. (2003). Superfluous and Antibiotic Resistance Pulse, University of Arizona.Retrieved from *http://Pulse_ Pharmacy.ariozna.edu/Disease_Epidemics/science.html.* Accessed on May 27, 2010.
- Bhatia .R. and Ichhpujani. R.(2008). *Essentials of Medical Microbiology*,4th edition. New Delhi India, Jaypee Brothers Medical Publishers Limited.Pp:54-55,141,259.
- Cheesbrough. M. (2000). *District Laboratory Practice, Tropical Countries*; part 2. United Kingdom, Cambridge university press Edinburgh.Pp:64-66,70
- Cooper , M.S.,(2000). Biocides, disinfectants and preservative. *The Microbiological Update*: **18** (3); 1-4.
- CRS, (2005). Progress in the control of airborne infections. *American Journal of Public Health and the Nation's Health*. **40**(1): 82 – 88. (Committee on Research Standards).
- Carly, N. J, DiCristina, J.A, and Lindsay. D. S,(2006). Activity of bleach, ethanol and two commercial disinfectants against spores of *Encephalitozoon cuniculi*. *Veterinary Parasitology*, **136**(1);3-4,**31**(1);343-346.
- Cristina,V. D ., Farias, L. M, Diniz, C.G, Alvarez-Leite, M .E and Camargo ,E R,(2005). New Methods in the evaluation of chemical disinfectants used in health care services. American Journal of Infection Control, **33**(3);162-169.
- DHQP, (2009). Cleaning and Disinfecting, Retrieved from *www.cdc.gov /oralhealth/infectioncontrol/glossary.htm*. Accessed on July 6, 2010 (Division of Healthcare Quality Promotion).
- Fraise, A.P.(1999). Choosing disinfectants. *Journal of Hospital Infection*,**43**(4);255-264.
- Gaonkar, T.A, Geraldo.I., Shintre.M. and Modak, S.M(2006). In vivo efficacy of an alcohol-based surgical hand disinfectant containing a synergistic combination of ethylhexylglycerin and preservatives. *Journal of Hospital Infection*,**63**(12)147-155.
- Hamamah, A.A. (2004). Do Different Dilutions of Disinfectants Affect the Development of Bacterial Resistance California State Science Fair Abstract. Retrieved from *http://www.usc.edu/cssf/history/2004/ projects.* Accessed on May 27, 2010.
- Ho-Hyuk .J, Sung-Ho.A, and Kim, M. D,(2008). Use of hydrogen peroxide as an effective disinfectant to *Actinobacillus ureae*. Process Biochemistry,**43**(4);225-228.
- Johnson, T., and Case, C., (1995). Chemical Methods of Control. Adapted from *Laboratory Experiments in Microbiology*. 4th edition. Benjamin/Cummings Publishing Company, Redwood City From National Health Museum, *http://www.accessexcellence.org/ activity_exchange.*
- Lages, S.L., Ramakrishnan, M.A. and Goyal, S.M. (2008). In-vivo Efficacy of Hand Sanitisers Against Feline Calicivirus: a Surrogate for Norovirus. *The Journal of Hospital Infection*; **68**(2): 159 – 163.
- Larson. E.L, and Morton, H .E,(1991). Disinfection, Sterilization and Preservation,4th edition. Pp11-15.

- Moorer., W.R. (2009). Effectiveness of 70% Ethanol.Retrieved from *http/www.wikianswers/ethanol.com*. Accessed on 23/08/2010.
- McDonell, G. and Russel A. (2001). Antiseptic, disinfectant activity, action and resistance . *Clinical Microbiology Review*. **14**(12): 227 – 247.
- Moorer, W.R., (2003). Antiviral activity of alcohol for surface disinfection. *International Journal of Dental Hygiene*, **1**(3): 138
- Rollins, D.M., and Joseph S.W., (2000). Antibiotoic Disk Susceptibilities Department of Cell Biology and Molecular Genetics, University of Maryland. *http://www.life.edu/classroom/bsci424/labmaterials method/antibioticdisk.htm*.
- Stephen, B., Qiang, Z. Fan, R. Kametia, M. and Schwab, C. (2004).Ethanol suppresses cytokine responses induced through Toll-like receptors as well as innate resistance to *Eschericha coli* in mouse model for bridge drinking. *Alcohol*, **33**(2): 147 – 155.
- Uzoechi A. U. **,** Dike S.K., Nwanekwu K.E.N (2016) Anti-microbial Evaluation of SomeAnti-Diarrhoreal Plants Used by indigenous Abajah and Amaigbo people, Imo State *Nigerian journal of Microbiology* 2016 Vol 30
- Weber, D.J., Barbee S.L., Sobsey, M.D. and Rutala W.A., (2000). The Effect of Blood On The Antiviral Activity of Sodium Hypochlorite, A Phenolic and a Quaternary Ammonium Compound. *Infection Control and Hospital Epidemiology* **20**(12): 821 – 827.
- Yi Hsing L., Miyamoto C., and Meighen E.A., (2002). Cloning Sequencing and Functional Studies of the Posgene From Vibrio Larve. *Biochemical and Biophysical Research Communications*, **293**(1): 26, 456 – 462.