American Journal of Nursing Research, 2014, Vol. 2, No. 3, 44-49
Available online at http://pubs.sciepub.com/ajnr/2/3/3

DOI:10.12691/ajnr-2-3-3

Isolation of Bacteria from Commonly Used Antiseptic and Disinfectant Solutions in Gondar University Hospital. North West Ethiopia

Teshiwal Deress, Mekonnen Girma, Wubet Birhan, Belete Biadgo, Teklay G/cherkos, Agersew Alemu*

School of Biomedical and Laboratory Sciences, College of Medicine and Health Sciences, University of Gondar, Gondar, Ethiopia
*Corresponding author: agersewalemu@yahoo.com

Received October 30, 2014; Revised November 07, 2014; Accepted November 09, 2014

Abstract **Background:** Antiseptics and disinfectants are chemical agents that inhibit or destroy microorganisms on living tissue (antiseptics) and on inanimate surfaces and objects (disinfectants). These chemical agents have been used extensively in hospitals and other health care settings for a variety of topical and hard surface applications. **Objective:** The aim this study was to determine bacterial contamination of commonly used antiseptic and disinfectant solutions and to perform antimicrobial susceptibility test of isolates in Gondar University Hospital, Northwest Ethiopia. **Methods:** In this cross sectional study a total of 86 diluted antiseptic and disinfectant samples were collected and assessed for possible bacterial contamination. Isolation of bacterial contaminants was done by culturing for aerobic bacteria on blood and Mac Conkey agar plates using surface plating method. Preliminary identification of bacteria was made based on gram reaction and colony characteristics. Further identification of bacteria was made using a series of biochemical tests then antibiotic susceptibility patterns of bacterial isolates were determined as per the standard procedures using 7 antimicrobial agents. Finally data were calculated manually using scientific calculator. **Results:** Among 86 samples studied, 3 (3.5%) of them were contaminated by aerobic bacteria. All the bacterial isolates were *Klepssilla* species (3*K. pneumonia* and 1 *K. ozanae).* These bacterial contaminants were isolated from 2 samples of 0.05% bleach and 1 sample of 70% alcohol. All the three isolates of *K. pneumonia* were multi-drug resistant, while *K. ozanae*was sensitive to all antimicrobial agents tested. **Conclusion and recommendation:** The contamination of diluted antiseptic and disinfectant solutions was as high 3(3.5%). All the bacterial contaminants of these antimicrobial substances were *Klebsiella*species of which all *K. pneumonia* species were multidrug resistant that poses a health risk to patients. Periodic assessment of antiseptic and disinfectant solutions for possible microbial contamination should be conducted.

Keywords: *antiseptic, disinfectant, bacterial contamination, drug resistance*

Cite This Article: Teshiwal Deress, Mekonnen Girma, Wubet Birhan, Belete Biadgo, Teklay G/cherkos, and Agersew Alemu, "Isolation of Bacteria from Commonly Used Antiseptic and Disinfectant Solutions in Gondar University Hospital. North West Ethiopia." *American Journal of Nursing Research*, vol. 2, no. 3 (2014): 44-49. doi: 10.12691/ajnr-2-3-3.

1. Back Ground

Antiseptic and disinfectants are chemical agents that inhibit or destroy microorganisms on living tissue (antiseptics) and inanimate surfaces and objects (disinfectants) [1]. These chemical agents are used extensively in hospitals and other health care settings for a variety of topical and hard surface applications. In particular, they are an essential part of infection control practices and aid in the prevention of nosocomial infections [2]. This wide spread use of antiseptic and disinfectant products has prompted some speculation on the development of microbial resistance, in particular, whether antibiotic resistance is induced by antiseptics or disinfectants [3].

A wide variety of active chemical agents (biocides) are found in these products, many of which have been used for hundreds of years for antisepsis and disinfection including; alcohols, phenols, iodine, and chlorine [4]. Most of these active agents demonstrate a broad spectrum of antimicrobial activity and they are used to reduce microbes on the living tissue or surface of medical equipments and other inanimate objects [1]. The mechanism of resistance to antiseptic and disinfectant solutions includes cellular impermeability, biofilm formation, efflux and mutations at the target site or over expression of a target site [5].

As a therapeutic significance a number of bacterial contaminants isolated from antiseptics and disinfectants have exhibited resistance to commonly used antimicrobial agents. It has also been reported that contaminated antiseptics and disinfectants exhibit decreased efficacy and effectiveness [6]. The response of different types of microorganisms to antiseptics and disinfectants vary and result in micro-biostatic or microbiocidal effects [5]. Multiple nosocomial out breaks have resulted from a lack of intrinsic antimicrobial activity of antiseptics, resistant

pathogen, over dilution of the antiseptics, or the use of contaminated antiseptics [7].

Bacterial contamination of disinfectant solutions is common with preparation by unskilled personnel, use of unsterilized containers and prolonged use [1] with other contributing factors for the high levels of contamination (dilution of disinfectants with tape water, inadequate care of stock solution bottles and long storage of the diluted disinfectants in the wards) [8].

In adequate disinfection of medical devices or environmental surfaces may result from lack of intrinsic antimicrobial activity of the disinfectant, an incorrect choice of chemical disinfectant, a resistant pathogen, over dilution of the disinfectant, inadequate duration of disinfection, lack of contact between the disinfectant and the microbes, or the use of contaminated disinfectants [9]. With the emergence of pathogens such as methicillin–resistant *staphylococcus aureus (MRSA),* vancomycin resistant *entericocci (VRE*) and gram negative bacteria, for example, *Pseudomonas aeruginosa* and *acinetobacter s*pecies which are resistant to multiple antibiotics, there is an increased need for effective antisepsis and disinfection [10].

As with antibiotic resistance, resistance to these germicides may be an intrinsic property or may arise either by chromosomal gene mutation or by the acquisition of genetic materials [11]. As a result of this health care associated infections are important causes of morbidity and mortality all over the world [5]. The center for disease control and prevention (CDC) has estimated that health care associated infection accounts for an estimated 1.7 million infections, 99,000 deaths and $ 4.5 billion in excess health care costs annually [12]. Despite best efforts to eliminate these infectious microorganisms, they continue to emerge and re-emerge which contribute to human illness and death especially as a result of hospital acquired infections. The successful eradication of these pathogens with antiseptic/disinfectant solutions has been complicated by the development of highly resistant strains. As a result of extensive use of antiseptic/ disinfectant solutions, a significant proportion of the pathogens have not only developed resistance, but they also grow in the solution of these biocides [13]. The activity of biocides against microorganisms is not always consistent due to several basic methodological problems as well as high intrinsic resistance due to differences in membrane structure [14]. Persistent reports have shown that disinfectants designed for the control of infectious microorganisms are themselves subjected to microbial contamination [15]. It has become increasingly obvious that infections acquired in hospitals lead to increase morbidity and mortality which has also added noticeable to economic burden. As different researchers show that contaminated antiseptics or disinfectants pose a health risk to patients particularly in the pediatric and surgical wards [16]. Bacteria isolated from contaminated antiseptic/ disinfectant solutions exhibit increased resistance to commonly used antibiotics which contributes a serious public health problem, giving the fact that bacteria have the ability to share resistance markers, and once a resistance develops for one agent, a cross-resistance to other agents can occur [13]. Nosocomial infections associated with contaminated antiseptic products are difficult to assess. Several factors may limit the identification of infections related to antiseptics or disinfectant products [17], however; it is a recognized public health problem worldwide with the prevalence rate of 5-10% [18]. Generally these infections pose a problem of enormous magnitude globally by prolong hospitalization, increase cost of health care, and decrease the effectiveness of the treatment [19].

Our hospital had been using various antiseptics extensively, but there is no report on the microbial contamination of these biocides from any referral hospital from this part of the country. This study was aimed to determine the prevalence of bacterial contamination of commonly used antiseptic and disinfectant solutions, isolation of the bacteria and evaluation of sensitivity of the isolated bacteria to few of the antibiotics in routine patients' use at this hospital.

2. Materials and Method

2.1. Study Area and Period

The study was conducted at Gondar University teaching Hospital which is located in Gondar town in Amhara regional state. Gondar is 739 km far from Addis Ababa to the northwest of Ethiopia. By the time being the University is working and striving for providing different services for the community including conducting different under graduate and post graduate programs and problem solving research activities. This teaching hospital is one of the biggest tertiary level referral and teaching hospitals in the region. A large number of people from the surrounding areas and nearby regions visit the hospital both for inpatient and as an outpatient treatment. This teaching hospital consists of an operating room, intensive care unit (ICU), 13wards with 327 beds, outpatient department, and one main laboratory. The study was carried out from March - June, 2013.

2.2. Study Design

A hospital based cross sectional study was conducted. Information and antiseptic and disinfectant samples which were relevant to this study were collected from wards, laboratory rooms, intensive care unit (ICU), and inpatient department.

2.3. Sample Size Determination

During the study period there were a total of 4 types of antiseptic solutions (ethanol alcohol, tincture iodine, savlon, and hydrogen peroxide) and 1 disinfectant (bleach) which were in-use in wards, laboratory rooms, intensive care unit (ICU), and inpatient department were included in this study.

2.4. Sample Collection, Isolation and Identification

2.4.1. Sample Collection

For the sake of consistency of the data obtained, samples were collected two times from each site with two weeks interval. After mixing about 10ml diluted (working) solutions of antiseptics and disinfectants were collected aseptically with sterile test tubes (with 15 ml capacity). Then the collected samples were immediately labeled and transported to the microbiology laboratory.

2.4.2. Isolation and Identification of Bacteria

Isolation of bacteria was made by diluting samples 1 to 9 with Tryptone soya broth and incubating at 37°C for 24 hours. After 24 hours of incubation they were sub cultured on blood agar and Mac Conkey agar plates and incubated at 37°C for 24-48 hours before reporting negative. Preliminary identification of bacteria was made based on gram reaction, colony characteristics, and changes in physical appearance in differential media. Based on the gram reactions obtained further identification of bacteria was made by a series of biochemical tests (triple sugar iron agar, indole, Simon's citrate agar, lysine iron agar, urea, mannitol, and motility) (26).

2.4.3. Antimicrobial Susceptibility Test of Isolates

The antimicrobial susceptibility test of the isolates was performed according to the national committee for clinical laboratory standards (NCCLS) method usingKibry-Bauer disk diffusion test on Mulle-Hinton agar. In short the isolated bacterium was suspended in a nutrient broth and incubated for 30min to make it comparable with 0.5% McFarland standard. After incubation a sterile cotton swab was dipped in to the suspension and bacteria were inoculated on to the Muller-Hinton agar. A total of 7 antimicrobial agents were used at the following concentration: Sulfamethoxazol/trimethoprim 25µg; Chloramphinecol 30µg; Nitrofurantoin 100µg; Ampicillin 10µg; Amoxicillin 30µg; Ciprofloxacin 10µg, and Gentamycin 10µg. These antibiotic discs were placed by using disc dispenser and the plates were incubated for 24 hrs at 37 OC. Finally results were interpreted as 'Resistant', 'Intermediate' or 'Susceptible'after measuring the zone of inhibition and being compared with the standards. *Escherichia coli ATCC 25922* and *S. aureus* ATCC25923 were employed as strain of quality control for the antimicrobial susceptibility test [27].

2.5. Data Analysis and Interpretation

The data obtained were calculated manually using scientific calculator and presented in the form of texts and statistical summary tables.

2.5.1. Quality Control

Quality of the data obtained was insured by following standard procedures of each step. The functionality of instruments was checked regularly before starting the procedure. Wire loops were sterilized with flame. Sterility of the culture media plates were checked by incubating 15% of prepared plates at 37°C for 24 hours and assessed for possible contamination. Bacterial suspensions of isolates were compared with 0.5% McFarland standard and sterile cotton swabs were used to inoculate bacterial suspension on Mueller Hinton medium. *Escherichia coli ATCC 25922* and *S. aureus*ATCC25923 were employed as strain of quality control for the antimicrobial susceptibility test [27]. Inoculated plates were incubated at 37°C for 24 hours and zones of inhibition were measured and interpreted using interpretative Chart.

2.5.2. Ethical Clearance

The study was approved by the ethical review committee of the School of Biomedical and Laboratory Sciences, University of Gondar before the actual data collection. Then the director of the hospital communicated with the staffs who were working at the sample collection sites for giving permission for us. Then hospital health care service room heads were informed about the purpose of the study.

3. Result

A total of 86 samples of antiseptic and disinfectant solutions were collected from wards, inpatient department, ICU, and laboratory rooms yielded bacterial growth with prevalence of 3/86 (3.5%). Two out of twenty one samples of 0.05% bleach and one out of twenty five samples of 70% alcohol were contaminated by aerobic bacteria. All of the isolates of bacterial contaminants were *Klebsiella Spp.* (3 *K.Pneumoniae* and1 *K. Ozaenae*). *K. pneumoniae* was isolated from two samples of 0.05% bleach and one sample of 70% alcohol while *K. ozaenae*was isolated from one sample of bleach together with *K. pneumoniae* (Table 1).

Table 1. Bacterial contaminants of antiseptic and disinfectant solutions and their percentage of contamination in Gondar University Hospital, Ethiopia, 2013

Antiseptic/disinfectant solution	Number of sample tested	Number of contaminated sample	Percentage of contamination (%)	Isolated bacteria
70% Alcohol	25	1	4	1 *K. pneumoniae*
2% savlon	14	0	0	
2% Tincture iodine	14	0	0	
3% H2O2	12	0	0	
0.05% of Bleach	21	2	9.25	2 K. *pneumoniae and 1 K.ozaenae*
Total	86	3	3.5	

Keys: K. pneumoniae= Klebsiellapneumoniae, K. ozaenae= Klebsiellaozaenae

Except laboratory rooms all stock antiseptic solutions were diluted in the pharmacy and distributed to Wards, inpatient department and ICU, but in laboratory sections concentrated alcohol was diluted into 70% with distilled water by the laboratory personnel themselves. From the result of our interview in most health care service rooms under study bleach was diluted with tape water. In all cases the containers were recycled bottles that originally contained the concentrated antiseptics/disinfectants, and in other instances they were glass bottles bought for this purpose. All antiseptic and disinfectant solutions investigated were found to be before their expired date. After and before working hours all in-used antiseptic solutions were stored in closed condition with their lids in shelves. Among different health care service rooms under study bacterial contamination of antiseptics and disinfectant solutions were observed in Orthopedics Ward, medical Ward and General Ward. Bacterial contaminants

isolated from 0.05% of bleach solution were obtained from medical and Orthopedics ward whereas; in general ward the isolate was obtained from 70% alcohol (Table 2). (Table 3).

3.1. Antimicrobial Susceptibility Test

Drug susceptibility patterns of bacterial isolates were determined using disk diffusion techniques and the result showed that all the *K. pneumoniae* isolates were resistant to most antimicrobials tested. This organism was sensitive only for Ciprofloxacin and Nitrofurantoin whereas *K. ozaenae*was sensitive for all antimicrobial agents (Table 3). (Table 2).

Table 2. Prevalence of bacterial contaminants of disinfectants and antiseptics solutions by source in Gondar University teaching Hospital, Ethiopia, 2013

Sample	Wards No. of samples			Lab. Rooms No. of samples			Impatient department No. of samples			ICU No. of samples			Total
	Tested	positive		Tested	positive		Tested	positive		Tested	positive		
		No			No	%		No	%		No	%	
savlon	11	0	0	0	0	0	2	0	0	1	0	0	14
70% alcohol	13	1	7.7	9	0	0	2	0	0	1	0	0	25
3%H2O2	10	0	0	0	0	0	1	0	0	1	0	0	12
2% Tincture iodine	12	0	0	0	0	0	1	0	0	1	0	0	14
0.05% bleach	11	2	18	8	0	0	1	0	0	1	0	0	21
Total	57	3	0	17	0	0	7	0	0	5	0	0	86

Table 3. Bacterial contaminants of antiseptic and disinfectant solutions and their drug susceptibility patterns in Gondar University Hospital, Ethiopia, 2013

No	Antiseptic and disinfectant solutions	Bacterial isolates	Drug sensitivity pattern of isolated bacteria						
			Antibiotic discs						
			SXT	CHL	F	AMP	AMC	CIP	GEN
1	Bleach	*K.pneumoniae*	I	R	S	R	R	S	R
2	Bleach	*K.pneumoniae*	I	R	S	R	R	S	R
3	Bleach	*K.ozaenae*	S	S	S	S	S	S	S
4	Alcohol	*K.pneumoniae*	I	R	S	R	R	S	R

Key: I=Intermediate, R=Resistant, S=Sensitive, AMC=Amoxicillin, AMP=Ampicillin, CHL= Chloramphenicol, CIP=Ciprofloxacin, F= Nitrofurantoin, GEN= Gentamycin, SXT= Sulfamethoxazol/Trimethoprim.

4. Discussion

Samples of antiseptic and disinfectant solutions obtained from Wards, inpatient department, ICU, and laboratory rooms yielded bacterial growth with prevalence of 3.5% which obviously pose a health risk to patients in sensitive areas of the hospital such as pediatric/neonatal wards, ICU, and surgical ward. This result is slightly higher than studies conducted in Trinidad 2.8% and Thailand 1.8%, 2.1% conducted at different periods [1, 20]. The most feasible explanation for this high prevalence of contamination may be due to the fact that studies conducted in these countries include different antiseptic and disinfectant solutions in their study than our samples which might have higher strength of bactericidal activity resulting low prevalence. In fact our result is favorably comparable with the 3% prevalence found in Danish hospitals [24].

Considerably higher levels of prevalence have been reported from other countries including 7.9 % in Malaysia [22], 15.14% in India [23], 34.4 % and 63.1% in Nigeria [8,25] and 43 % in Japan [21]. With regard to these higher levels of prevalence, the active ingredients of those antiseptic and disinfectant solutions in these studies might be weak to kill those bacterial contaminants rather they might contribute for genetic modification of bacteria resulting occurrence of new resistant strains. In addition to this some of these studies included leftover diluted antiseptic and disinfectant solutions in their study which might weak bactericidal activity resulting highly vulnerable for bacterial contamination.

In this study all the bacterial isolates were *Klebsiella species* which is supported with the study conducted in India of which 22.72% of the bacterial isolates were *Klebsiella spp.* Among the five antiseptic and disinfectant solutions investigated, bleach was highly contaminated with 9.5% samples of bleach being contaminated with *Klebsiella* spp. Similarly in Thailand 12.5% samples of bleach solution were contaminated [20]. This reflecting that with the extensive use of bleach as a medical equipment and general disinfectant in this Hospital there might be emergence of resistant *Klebsiella* strains those might adapt the active ingredients of this solution, nevertheless; when we compare we should have to consider the types and concentrations of different antiseptic/disinfectants solutions.

All the samples of savlon, tincture iodine, and hydrogen peroxide weren't showing any visible growth for aerobic bacteria. This suggests that resistant strains for the active ingredients of these solutions might not be emerged indicating these chemical substances are still active to all aerobic bacteria, but studies conducted in Nigeria indicate that 1% savlon was the most contaminated, with 15% of the samples being positive for aerobic bacteria [6]. In this study all the isolated bacterial contaminants were *Klebsiella* spp. Whereas, all the aerobic bacteria isolated

from disinfectant samples in four hospitals of Trinidad were *Pseudomonas* spp. [6] and also in Nigeria *Pseudomonas* spp. Found to be the most bacterial contaminants of antiseptic and disinfectant solutions accounting 67.2% [8]. This might be due to different antiseptic and disinfectant solutions might have selective antimicrobial activity as a result of difference in bacterial cell wall permeability for different antiseptic/disinfectant solutions.

For the therapeutic relevance, all the 3 isolates of *K. pneumoniae* were resistant to most of the antimicrobials tested, whereas; *K. ozaenae*was sensitive to all antimicrobial agents. Similarly most *Pseudomonas spp.* isolates from antiseptic and disinfectant solutions elsewhere were multi drug resistant [6,8]. This may be due to cross resistance to antibiotics which is obviously stated that bacteria isolated from contaminated antiseptic/ disinfectant solutions exhibit increased resistance to commonly used antibiotics, giving the fact that bacteria have the ability to share resistance markers, and once a resistance develops for one agent, a cross-resistance to other agents can occur [13]. In this study anaerobic bacteria were not investigated due to limited laboratory facilities.

5. Conclusion

In the current study, the overall contamination rate of bacterial contaminants of antiseptic and disinfectant solutions was 3.5%. All the bacterial contaminants of antiseptic and disinfectant solutions were *Klebsiella* species. Multiple antibiotics resistance to most of the antibiotics tested were extremely high which should be given great emphasis on the therapeutic regimens for these bacterial species. This contamination rate is obviously unacceptable because of the health risk to patients in high-risk areas of the hospital such as pediatric/neonatal wards, ICU, and surgical ward. Further study should be conducted with comprehensive way and large sample size and there should be periodic assessment of antiseptic and disinfectant solutions for possible microbial contamination.

Abbreviation

AMC: Amoxicillin, AMP: Ampicillin, CDC: Center for Disease Control and Prevention, CHL: Chloramphenicol, CIP: Ciprofloxacin, F: Nitrofurantoin, GEN: Gentamycin: Intermediate, *K. ozaenae: Klebsiellaozaenae, K. pneumoniae: Klebsiellapneumoniae*, MRSA: Methicillin Resistant *Staphylococcus Aureus*, R: Resistant, S: Sensitive, SPPs: Species, SXT: Sulfamethoxazol/ Trimethoprim, VRE: Vancomycin Resistant *Enterococci.*

Competing Interests

The authors declare that they have no competing interests.

Authors' Contributions

TD and MG were the primary researcher, conceived the study, designed, participated in data collection; conducted data analysis drafted and finalized the manuscript for publication.

AA and WB, BB, TG assisted in data collection and reviewed the initial and final drafts of the Manuscript TD, MG and WB interpreted the results, and reviewed the initial and final drafts of the manuscript. All authors read and approved the final manuscript.

Acknowledgements

We would like to acknowledge Gondar University Teaching Hospital laboratory staff for their cooperation in sample collection.

References

[1] Danchivijtr S., Dhirapputra C., Rongrungruang Y., Nitay S., Varagon V., Microbial contamination of antiseptics and disinfectant. *Journal of Medical Association of Thailand*. 88(10).133-9. 2005.

[2] Rutala W., APIC guidelines for selection and use of disinfectants. *American journal of infectioncontrol.* 23.313-42. 1995.

[3] Black S.S., Disinfection, sterilization and preservation 4th ed. *Philadelphia. LeandFebier*. 3-17. 1991.

[4] Gerland M., Denver R., Antiseptic and disinfectant activity, action and resistance. *Clinical Microbial Review*. 12(1): 147-9. 1991.

[5] Alp S., Bacterial resistances to antiseptics and disinfectants. *Mikrobiyol Bulletin*. 41(1). 155-61. 2007.

[6] Tswana G., Alica L., Patrica S., Abiodun A., Microbial contamination of disinfectants and antiseptics. *Pan American Journal of public health.* 14(3):193-9. 2003.

[7] Dixon R.E., Kaslow R.A., Mackel D.C., Fulkerson C., Mallison G.F., Aqueous quaternary amonium antiseptics and disinfectants. *JAMA.* 236:2415-17. 1976.

[8] Ogunsola F.T., Orji B., Oduyebo O.O., Contamination level of in-use disinfectants. *African Journal of Medicine and Medical Science*. 3(2):111-4. 2002.

[9] Rutala W.A., Cole E.C., Antiseptic and disinfectant activity. *Infection control*. 5: 215-18. 1984.

[10] Rusell A.D., Hugo W.B., Aylffe G.A., Principles and practice of disinfection, preservation and sterilization 3rd ed. *Oxford. Blackwell Science.* 250-67.

[11] Center for disease control and prevention. 2007; Health care associated infections. Center for disease control and prevention. *Atlanta G A.*

[12] David J, William A, Emily E. Out breaks associated with contaminated antiseptics and disinfectants. *Antimicrobial Agents and Chemotherapy*. 51(12): 4217-24. 2007.

[13] EI-Mohamood A.M., Doughari J.H., Bacterial examination of some diluted disinfectants routinely used in the specialist hospital Yola, Nigeria. *African Journal of pharmacy and pharmacology*. 3(5): 185-90. 2009.

[14] Keah K.C., Jegathesan M., Tan S.C., Chan S.H., Chen O.M., Cheong Y.M., Disinfection: gaps between recommended and actual practice. *Southeast Asian Journal of Tropical Medicine and Public Health*. 26: 795-8. 1995.

[15] Otokunefor T.V., Usoh C.U., Microbial contamination of in-use disinfectants in small private medical facilities. *Scientia Africana*. 8(2): 17-25. 2009

[16] Gaidhal T., Lara A., Sealy P., Adesiyun A.A. Microbial contamination of antiseptics and disinfectants. *Pan American Journal of public health.* 14(3): 193- 200. 2003.

[17] Chang C.Y., Furlong L.A., Microbial Stowaways in Topical Antiseptic Products. *The New England Journal of Medicine*. 367:2170-3. 2012.

[18] Tulu O., Setting up an infection control program in the hospital. *Journal of the Nigerian Infection control Association.* 2(1):4-8. 1999.

[19] Robert A.W., Controlling antimicrobial resistance in hospitals: Infection control and use of antibiotics. *Emerging Infectious Diseases*. 7(2): 188-192. 2001.

[20] Sangkasuwan S., Yospol P., *Efficacy* and contami- nation of in-*use disinfectants* in Rajvithi General. Hospital. *Journal of Medical Association of Thailand. 78*(*1*): *36-9. 1995.*

[21] Oie S., Kamiya A., Microbial contamination of antiseptics and disinfectants. *American Journal of Infecttion control*. 24(5): 389-95. 1996.

[22] Keal K.C., Jesathesaw M., Chaw S.H., Chee O.M., Cheong Y.M., Soleiman A.B., Bacterial contamination of hospital disinfectants. *Medical Journal of Malaysia*. 50(4): 193-9. 1995.

[23] Argunwadkar V.P., Bal A.M., Joshi S.A., Kagal A.S., Bharadwaj R.S., Contaminated antiseptics- an unecessary hospital hazared. *Indian Journal of Medical Science*. 55(7):393-8. 2002.

[24] Christensen E.A., Jepsen O.B., Kristensen H., Steen G., In-use tests of disinfectants. *ActaPatholMicrobiolImmunolScand [B]*. 90: 95-100. 1982.

[25] Olayemi A.B., Obayan Y., Contaminated disinfectants in health clinics in Ilorin, Nigeria. *Infection Control of Hospital Epidemiology*. 15: 581-2. 1994.

[26] Chesbrougn M. *Manual of MedicalMicrobiology*, Oxford Press, Britain, UK, 2000.

[27] Bauer A.W., Kirby W.M., Sherris J.C., Turck M. "Antibiotic susceptibility testing by a standardized single disk method," The American Journal of Clinical Pathology. 5(4), 493-496. 1966.