

ORIGINAL ARTICLE

ACETIC ACID VERSUS CHLORINE TABLET SOLUTION AS DISINFECTANT OF NON-CRITICAL ENVIRONMENTAL SURFACES

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ABSTRACT

Objectives: This study aims to determine the bactericidal activity of 4% acetic acid versus chlorine tablets against gram negative and gram-positive microorganisms based on percentage reduction of microorganisms in hospital surfaces and suggest that it may be an effective alternative disinfectant.

Methodology: This was an experimental study where microbiological sampling of hospital surfaces was used to determine bacterial growth. The study was conducted from November to December 2020 at National Children's Hospital, a 200 bed capacity tertiary government hospital catering to children 0 to less than 19 years old. Non-critical hospital surfaces such as beds, bed rails and bedside tables were swabbed before and after intervention cleaning with chlorine tablets or 4% acetic acid solution.

Result: Pre-swabbing, hospital surfaces showed the presence of *Bacillus* sp., *Klebsiella pneumoniae* and Coagulase Negative Staphylococcus (CONS). Post-application of 4% acetic acid solution resulted to 100% reduction of *Bacillus* sp., 70.8% reduction of CONS, and 19.5% reduction of *Klebsiella pneumoniae* while post-application of chlorine tablet solution showed 100% reduction of *Klebsiella pneumoniae* and CONS and 95.2% reduction of *Bacillus* species.

Conclusion: The use of 4% acetic acid solution significantly reduced more gram-positive than gram-negative organisms and is a highly effective disinfectant against *Bacillus* sp. but is not effective against gram-negative organisms as it does not fulfil the criteria of at least 90 percent reduction in bacterial growth. Chlorine tablet solution is a more effective disinfectant against gram-negative organisms than gram-positive organisms. Acetic acid 4% solution is not an effective alternative disinfectant to chlorine tablet solution, the currently used hospital disinfectant, but maybe used as an adjunct for better reduction of hospital environmental pathogens.

KEYWORDS: *Acetic Acid, Chlorine Tablet, Disinfectant, Healthcare Associated Infection, Bacteria*

INTRODUCTION

Healthcare Associated Infections (HAIs) or nosocomial infections are infections acquired during hospitalization that were not present during the time of admission.¹ It is a serious public health problem with significant consequences both individually and economically. It poses a great risk on the quality of care for patients and is responsible for high morbidity and mortality rates. A report released by the WHO last 2011 showed that HAIs occur in 7 and 10 out of every 100 hospitalized patients in high and low to middle-income countries respectively.² As a consequence, HAIs lead to prolonged hospital stay of patients.

The hospital environment is widely and naturally contaminated with microorganisms. These microorganisms dwell on inanimate surfaces surrounding a patient and pose a risk of transmitting these microorganisms to them. In a study done by Weber et al., he found that organisms such as Methicillin-resistant *Staphylococcus aureus* (MRSA), Vancomycin-resistant *Enterococcus* spp. (VRE), *Pseudomonas* spp., *Acinetobacter* spp., and Norovirus can survive for days to weeks on dry inanimate surfaces, while *C. difficile* spores survive for months on environmental surfaces.³ If the hospital environment is not properly cleaned and disinfected, these organisms pose a risk to newly admitted patients.

Noncritical environmental surfaces are objects which come in contact with intact skin, but not mucous membranes, and it includes bed rails, bedside tables and floors. A study done by Huslage et al. stated that the top five most touched surfaces in hospitals are bed rails, bed surfaces, supply carts, over-bed tables and intravenous pumps based on frequency of contact.⁴ Environmental surfaces frequently touched by hand could potentially contribute to secondary transmission by contaminating the hands of healthcare workers, thus disinfection of the patient's environment is critical to reduce the risk of healthcare associated infections.⁵

Healthcare Associated Infection Rate is an important measure to evaluate the quality of service delivery in health care facilities. Through the years, there has been several consensus to improve

cleaning and disinfection of environmental surfaces in healthcare facilities to decrease HAI rates.⁶ There are many disinfectants that are widely used to clean hospital surfaces such as glutaraldehyde, formaldehyde and chlorine compounds, but a study done by Cortesia et al. showed that vinegar has been used for thousands of years as a common disinfectant.⁷ Currently, the hospital uses chlorine tablet compound as the standard cleaning solution. This study aims to determine the bactericidal activity of 4% acetic acid versus chlorine tablets against gram-negative and gram-positive microorganisms based on percentage reduction of microorganisms in hospital surfaces and suggest that it may be an effective alternative disinfectant.

MATERIALS AND METHODS

This is an experimental study where microbiological sampling was used to determine the bacterial growth in selected non-critical hospital surfaces. The study was conducted from November 2020 to December 2020 at a 200 bed Tertiary Government Hospital, which caters to children 0 to less than 19 years old. The study was done in the General ward with the greatest number of healthcare associated infections for the month as validated by the Infection Prevention and Control Committee (IPCC). The study was done with routine terminal cleaning. A staff member from the janitorial service responsible for terminal cleaning was chosen and properly instructed by the trained and qualified supervisor on the correct process of disinfection. Return demonstration was done to ensure that the correct process was followed. Microbiological sampling through swabbing taken before and 1 hour post-application of 4% acetic acid or chlorine tablet solution was done to determine the number of microorganism found in selected non-critical hospital surfaces. The protocol was submitted and approved by the Institutional Review Board of the hospital.

Selection of Environmental Surface

A general ward was selected by the hospital IPCC for routine terminal cleaning. In the designated room, non-critical hospital surfaces which include 3 of each of the bed, bedrail, and bedside table were

randomly selected and underwent cleaning with a soap solution (200g detergent powder in 1 gallon of tap water). Note that in our hospital, monthly water analysis is done and water from the faucet was noted free from coliform bacteria. Cleaning was done by rubbing a damp cloth three times for at least 15 seconds over the surface and allowed to dry. Another clean damp cloth was used to remove the soap solution residue and allowed to dry. The clean cloth that was used was bought individually wrapped, then washed with a detergent powder, and hanged out to dry prior to use. Pre-intervention swab was done within 15 minutes after cleaning. Non-critical hospital surfaces with no bacterial growth in the culture prior to application of any solution were withdrawn from the study.

Application of Solution

Cleaning agents used were commercially available acetic acid (350 ml *Datu Puti*) with 4% acetic acid content, and chlorine tablet solution (*Biospot* effervescent chlorine tablets from the hospital Central Supply Unit) prepared as one 3.25 grams tablet dissolved in 1 liter of tap water. The non-critical hospital surfaces selected were then divided into two parts and assigned randomly for a particular solution. A clean damp cloth was soaked in solution (either 250 ml of 4% acetic acid solution or 250 ml of chlorine tablet solution) and was rubbed three times on the surface for at least 15 seconds and left for 1 hour. After an hour, another clean dry cloth was used to wipe any residue, and microbiological sampling using a swab was done in triplicate within 15 minutes.

Microbiological Sampling

Microbiological sampling and swabbing was done by a single hospital medical technologist who was blinded on the surface under study. Uniform swabbing of each non-critical hospital surface was done using horizontal strokes until all surfaces were covered. Each swab was then cultured in a trypticase soy broth and McConkey agar in the laboratory. Agar plates were incubated overnight and were examined the next day for any bacterial growth.

Statistical Analysis

Levene test for equality of variances was used to calculate if 4% acetic acid is equivalent to chlorine solution and can be an alternative disinfectant. Significance was defined as $p < 0.05$.

RESULTS

A representative from each of the non-critical hospital surfaces often touched by patients were included in the study, namely the bed, bed rail and bedside table. Swabbing was done post-cleaning with soap and water. Table 1 shows the microorganisms isolated and the bacterial load measured in colony forming units. Majority of bacteria seen were Coagulase Negative Staphylococcus, *Bacillus* species and *Klebsiella pneumoniae*. Coagulase Negative Staphylococcus species was seen on all non-critical surfaces in the study. *Klebsiella pneumoniae* had the most number of colonies seen on the bed rail with 9000 cfu after 24 hours of incubation. The bed and bed rail had the greatest number of bacteria seen.

Table 1: Bacterial growth pre-application of acetic acid or chlorine tablet solution

Surface	Bacteria	Colonies
Bed	<i>Bacillus</i> sp.	6000 cfu
	Coagulase Negative Staphylococcus sp.	3000 cfu
	<i>Klebsiella pneumoniae</i>	3000 cfu
Bed Rail	<i>Klebsiella pneumoniae</i>	9000 cfu
	Coagulase Negative Staphylococcus sp.	2000 cfu
	<i>Bacillus</i> sp.	1000 cfu
Bedside Table	Coagulase Negative Staphylococcus sp.	3000 cfu

Figure 1 shows the colonies of bacteria as seen on the agar plate after 24 hours of incubation pre-application of solution. Figure 1-A shows the colonies of *Klebsiella pneumoniae*, Coagulase Negative Staphylococcus sp., and Bacillus sp. seen on the bed rail. Figure 1-B shows the colonies of Bacillus sp., Coagulase Negative Staphylococcus sp., and *Klebsiella pneumoniae* seen on the bed. Figure 1-C shows the colonies of Coagulase Negative Staphylococcus sp. seen on the bedside table.

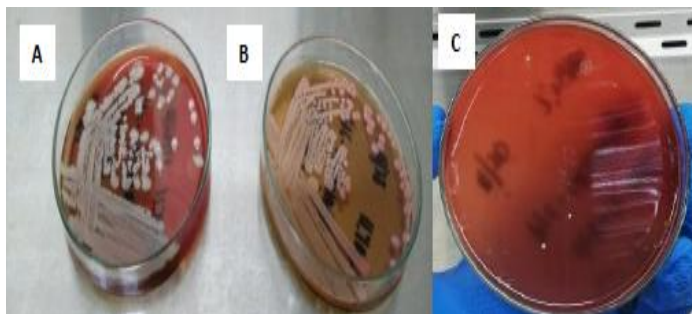


Figure 1: Bacterial colony pre-application of solution. 1-A Colony growth on bed rail. 1-B Colony growth on bed. 1-C Colony growth on bedside table.

The mean colony count on all surfaces contaminated with a specific bacterium and the percent reduction after application of 4% acetic acid solution is shown in Table 2. There was 100% reduction of Bacillus species, 70.8% reduction of Coagulase Negative Staphylococcus species, and 19.5% reduction of *Klebsiella pneumoniae* after application of 4% acetic acid solution.

Table 2: Bacterial reduction post-application of 4% acetic acid solution

Bacteria	Pre-Application (mean colony count) cfu	Post-Application (mean colony count) cfu	Percent Reduction (%)
Bacillus sp.	3500	0	100
Coagulase Negative Staphylococcus sp.	2667	778	70.8
<i>Klebsiella pneumoniae</i>	6000	4833	19.5

Table 3 shows the mean bacterial load measured in colony forming units and the percent reduction after application of chlorine tablet solution.

Table 3: Percent bacterial reduction post-application of chlorine tablet solution

Bacteria	Pre-Application (mean colony count) cfu	Post-Application (mean colony count) cfu	Percent Reduction (%)
<i>Klebsiella pneumoniae</i>	6000	0	100
Coagulase Negative Staphylococcus sp.	2667	0	100
Bacillus sp.	3500	167	95.2

Table 4 shows the percent reduction in bacterial load post-application of the two solutions. Acetic acid 4% solution was better in reducing Bacillus sp. compared to chlorine tablet solution. However, chlorine tablet solution was far more superior in reducing *Klebsiella pneumoniae*.

Table 4: Comparison of percent reduction in bacterial load with 4% acetic acid solution vs chlorine tablet solution

Bacteria	Percent Reduction in Bacterial Load with 4% Acetic Acid Solution (%)	Percent Reduction in Bacterial Load with Chlorine Tablet Solution (%)
Bacillus sp.	100	95.2
Coagulase Negative Staphylococcus sp.	70.8	100
<i>Klebsiella pneumoniae</i>	19.5	100

Table 5 shows the Levene Test for Equality of Variances. All p-values obtained were greater than 0.05.

Table 5: Levene Test for Equality of Variances

Bacteria	Solution	Percentage Reduction	p-Value
Bacillus sp.	4% Acetic Acid	100	.363
	Chlorine Tablet	95.2	
Coagulase Negative Staphylococcus sp.	4% Acetic Acid	70.8	.084
	Chlorine Tablet	100	
<i>Klebsiella pneumoniae</i>	4% Acetic Acid	19.5	.064
	Chlorine Tablet	100	

DISCUSSION

Healthcare Associated Infections (HAIs) are one of the major causes of morbidity and mortality among hospitalized patients. In this tertiary Government Hospital where the study was conducted, HAI is one of the main causes of prolonged hospital stay. To address the burden of HAIs, the hospital Infection Control Committee emphasizes strict hand washing, environmental cleaning, disinfection, and patient monitoring & surveillance.

A study done by Boyce on environmental cleaning found that there was lack of time spent in cleaning the hospital environment. This suggests that suboptimal cleaning and disinfection practices cause persistent contamination and increases risk for resistant pathogens.⁶

The Centers for Disease Control and Prevention (CDC) stresses the importance of the environment in transmitting microorganisms and recommends that surfaces near patients be properly disinfected.⁸ Many studies showed that hospital acquired pathogens often contaminate non-porous surfaces such as beds, bedside tables, rails and medical equipments.⁹ This was consistent with our study where both gram-negative and gram-positive bacteria were isolated on the beds and bed rails. The beds and bed rails are often touched by both the patient and healthcare provider, thus cross-contamination is a possibility especially when hand hygiene practices are not done properly.

The CDC states that there are many factors that can affect the efficacy of disinfection, such as type and level of microbial contamination, concentration of the disinfectant, and exposure time of microorganisms to the disinfectant.¹⁰ In this study, the concentration used was 4% acetic acid since a study done in a hospital in Tuguegarao City, Philippines found that acetic acid concentration of 4-7% is effective as a disinfectant in non-critical hospital surfaces and is commercially available in the market.^{11,12} A study from the CDC showed that low chlorine concentrations of <5ppm and 25ppm would have biocidal effect on vegetative bacteria and *Mycoplasma* sp. respectively in the setting of an absent organic load, and a higher concentration of

chlorine at 1000ppm could kill *M. Tuberculosis*.¹³ The CDC requires that the concentration of chlorine to be effective for infection control be either 5000-6000ppm or 500-600ppm depending on its intended use.¹⁴ For this study a 1000ppm chlorine concentration was used for general disinfection of non-critical hospital surfaces.

The contact time of microorganism and disinfectant that was used in the study was 1 hour. This gave a longer contact time for the disinfectant and the surface under study. A study done by Abreu et al. showed that for a disinfectant to be effective, a contact exposure of at least 5-10 minutes is needed.¹⁵ A case study done in St. Paul Hospital, Tuguegarao City showed that vinegar inhibits growth of microorganisms after at least 30 minutes to 2 hours of exposure to disinfectant.¹¹

In this study, pre-intervention swabbing was done to document the organisms found on hospital surfaces and its microbial load. Three microorganisms were isolated namely *Bacillus* species and Coagulase Negative *Staphylococcus* species, both gram-positive organisms, and *Klebsiella pneumoniae*, a gram-negative organism. Findings in our study differ from the study done by Zubair et al. at a hospital in Pakistan which showed that most organisms seen in hospital surfaces pre-disinfection were mainly gram-negative bacteria (56.7%). In our study, the most frequent organism isolated was Coagulase Negative *Staphylococcus* species comprising 42.9% of isolates. This is consistent with the study done at a hospital in Nigeria and Pakistan where CONS was frequently seen in 28.3% and 22.3% of isolates, respectively. In our study, gram-negative organisms were seen on bed surfaces, consistent with the study of Zubair.¹⁶

For the year 2019, the top causes of Healthcare Associated Infections in our institution were Coagulase Negative *Staphylococcus* and *Klebsiella pneumoniae*, similar to the pathogens isolated in our study. *Klebsiella pneumoniae* is one of the most common resistant organisms based on data collected by the Infection Prevention and Control Committee of the hospital for the year 2019. Weber et al. showed that some organisms can survive on surfaces for weeks and spores survive for months,

thus the importance of proper disinfection of patient's surroundings coupled with hand hygiene for all persons in charge of the patient to prevent cross-contamination and infection with multidrug resistant organisms.³

Log reduction was used to measure the effectiveness of a disinfectant. Effectiveness of a disinfectant starts with a 1 log reduction which is equivalent to 90 percent reduction of microorganisms. The effectiveness of a disinfectant goes high as the log reduction increases. In our study, chlorine tablet solution was highly effective against gram-negative and gram-positive organisms. This is consistent with a study done by Rweyendela et al. which showed that chlorine tablet solution resulted to a 99.99% reduction in *Pseudomonas aeruginosa*, as well as *Candida albicans*, *Staphylococcus aureus*, *Streptococcus mutans* and *Bacillus subtilis* spores.¹⁷ In our study, 4% acetic acid solution was effective against gram-positive organisms consistent with the case study done at St. Paul Hospital, Tuguegarao City.¹¹ The present study also proved that 4% acetic acid solution is a more effective disinfectant compared to chlorine tablet solution against *Bacillus* species, which was seen in both the bed and bed rail surfaces. This means that 4% acetic acid solution is clinically significant in reducing gram-positive microorganisms more than gram-negative bacteria.

The efficacy of a disinfectant is affected by the type and level of microbial contamination and its exposure time. However, this study had a limited range of microorganisms that grew on environmental surfaces and only a single exposure time-point was used to test the effectiveness of the solutions.

Levene Test for Equality of Variances was used to identify if there is a significant difference in terms of percentage reduction in bacterial growth between surfaces where the two solutions were applied. All p-values were noted to fall above 0.05, thus there is no statically significant difference between 4% acetic acid and chlorine tablet solution in terms of percentage reduction of microorganisms.

CONCLUSION AND RECOMMENDATIONS

In conclusion, our study showed that there is a significant reduction in the growth of gram-positive bacteria compared to gram-negative bacteria post-application of 4% acetic acid solution, which makes it an effective disinfectant against *Bacillus* sp. It is not an effective disinfectant for gram-negative bacteria since it does not fulfil the criteria of at least 90 percent reduction in bacterial growth to be qualified as an effective disinfectant. On the other hand, chlorine tablet solution is a more effective disinfectant against gram-negative organisms compared to 4% acetic acid. Acetic acid 4% solution cannot be used as an alternative disinfectant to chlorine tablet solution, the currently used hospital disinfectant, but maybe used as an adjunct for better reduction of hospital environmental pathogens.

REFERENCES

1. World Health Organization. The Burden of Healthcare Associated Infection Worldwide [Internet]. Geneva: WHO [updated May 1, 2017; cited 2020 November 18]. Available from https://www.who.int/gpsc/country_work/burden_hcai/en/
2. World Health Organization. Report on the burden of endemic health care-associated infection worldwide [Internet]. Geneva: WHO; 2011 [cited 2021 Jun 15]. Available from http://apps.who.int/iris/bitstream/handle/10665/80135/9789241501507_eng.pdf?sequence=1
3. Weber D, Rutala W, Miller M, Huslage K, Sickbert-Bennett E. Role of hospital surfaces in the transmission of emerging health care-associated pathogens: Norovirus, Clostridium difficile, and Acinetobacter species. *Am J Infect Control* [Internet]. 2010 June [cited 2021 Jun 15];38(5Suppl1):S25-33. Available from: [https://linkinghub.elsevier.com/retrieve/pii/S0196-6553\(10\)00408-6](https://linkinghub.elsevier.com/retrieve/pii/S0196-6553(10)00408-6) DOI: 10.1016/j.ajic.2010.04.196
4. Huslage K, Rutala W, Sickbert-Bennett E, Weber DJ. A quantitative approach to defining "high-touch" surfaces in hospitals. *Infect Control Hosp Epidemiol* [Internet]. 2010 Aug [cited 2021 Jun 15];31(8):850-3. Available from: http://www.diverseyvericlean.com/images/pdf/whitepapers/additional/Huslage_Rutala_2010_High_Touch_Surfaces.pdf DOI: 10.1086/655016 PMID: 20569115
5. Kaur K, Arora P, Biswal M. Hospital Surface Disinfection: Need, Gaps, Challenges and Management for "Basin and Mop" Method. *J Hosp Med Manage* [Internet]. 2018 Dec [cited 2021 Jun 15];4(3):10. Available from <https://hospital-medical-management.imedpub.com/hospital-surface-disinfection-need-gapschallenges-and-management-for-basin-andmop-method.php?aid=23929>
6. Boyce J. Modern technologies for improving cleaning and disinfection of environmental surfaces in hospitals. *Antimicrob Resist Infect Control* [Internet]. 2016 Apr [cited 2021 Jun 15];5:10. Available from <https://aricjournal.biomedcentral.com/articles/10.1186/s13756-016-0111-x#citeas> DOI:10.1186/s13756-016-0111-x
7. Cortesia C, Vilchèze C, Bernut A, Contreras W, Gómez K, de Waard J, et al. Acetic Acid, the active component of vinegar, is an effective tuberculocidal disinfectant. *mBio* [Internet]. 2014 Feb [cited 2021 Jun 15];25;5(2):e00013-14. Available from: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3940030/> DOI: 10.1128/mBio.00013-14
8. Centers for Disease Control and Prevention (US). Healthcare Associated Infections [Internet]. USA: CDC. Updated 2020 Apr 21 [cited 2021 Jun 15]. Available from <https://www.cdc.gov/hai/prevent/resource-limited/cleaning-procedures.html>
9. Han JH, Sullivan N, Leas BF, Pegues DA, Kaczmarek JL, Umscheid CA. Cleaning Hospital Room Surfaces to Prevent Health Care-Associated Infections: A Technical Brief. *Ann Intern Med* [Internet]. 2015 Oct 20 [cited 2021 Jun 15];163(8):598-607. Available from: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4812669/> DOI: 10.7326/M15-1192
10. Rutala W, Weber DJ. Healthcare Infection Control Practices Advisory Committee. Guideline for Disinfection and Sterilization in Healthcare Facilities, 2008 Update: May 2019 [Internet]. Chapel Hill, NC; 2019. [cited 2021 Jun 15]. Available from: <https://www.cdc.gov/infectioncontrol/pdf/guidelines/disinfection-guidelines-H.pdf>
11. Global Green and Healthy Hospitals. Acetic Acid: A Safer Alternative as Surface Disinfectant [Internet]. Tuguegarao City; 2019 [cited 2021 Jun 15]. Available from: <https://www.greenhospitals.net/wp-content/uploads/2019/02/Case-Study-Acetic-Acid-St.-Paul-Hospital-Tuguegarao-City.pdf>
12. Cervantes C. Determination of Acetic Acid Content in Vinegar [Research]. Philippines: University of the Philippines Visayas; 2014 Dec 5. Page 4-6
13. Centers for Disease Control and Prevention (US). Chemical Disinfectants [Internet]. USA: CDC. Updated 2016 Sep 18 [cited 2021 Aug 07]. Available from <https://www.cdc.gov/infectioncontrol/guidelines/disinfection/disinfection-methods/chemical.html>
14. Centers for Disease Control and Prevention (US). Rationale and Considerations for Chlorine Use in Infection Control for Non- U.S. General Healthcare Settings [Internet]. USA: CDC. Updated 2019 May 03 [cited 2021 Aug 7]. Available from <https://www.cdc.gov/vhf/ebola/clinicians/non-us-healthcare-settings/chlorine-use.html>
15. Abreu A, Tavares R, Borges A, Mergulhão F, Simões M. Current and emergent strategies for disinfection of hospital environments. *Journal of Antimicrobial Chemotherapy* [Internet]. 2013 Dec [cited 2021 Jun 15]; 68(12):2718–2732. Available from: <https://academic.oup.com/jac/article/68/12/2718/698356?login=true> DOI:10.1093/jac/dkt281
16. Zubair M, Imtiaz S, Zafar A, Aved H, Atif M, Ej Az H, et al. Role of Hospital Surfaces in Transmission of Infectious Disease. *Pakistan Journal of Medical and Health Sciences* [Internet]. 2018 September [cited 2021 Jun 15];12(2):857. Available from: https://www.researchgate.net/publication/327416754_Role_of_Hospital_Surfaces_in_Transmission_of_Infectious_Diseases
17. Rweyendela IH, Patel M, Owen CP. Disinfection of irreversible hydrocolloid impression material with chlorinated compounds. *SADJ* [Internet]. 2009 Jun [cited 2021 Jun 15];64(5):208,210-2. Available from: https://www.researchgate.net/publication/234055339_Disinfection_of_Irreversible_Hydrocolloid_Impressions_with_Chlorinated_compounds