

## **Study of the Inhibitory Activity of Acetic Acid Compared with Dettol Solution in Sterilizing Study Seats**

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### **Abstract**

The study included the collection of 40 different samples of study seats in the Department of Biology, University of Anbar. The results showed that the seats contaminated with six different types of bacteria, *Pseudomonas* SSP occupied the highest percentage was 44% (56 isolates), followed by *Escherichia coli*, 25% (32 isolates), *Bacillus* SPP recorded 17% (13) isolates, while *Klebsiella* SPP and *Staphylococcus aureus* showed 9% and 5% (12 and 6 isolates), respectively, and lastly *Proteus* SPP recorded 4% (5) isolates. The number of colonies for each of Acetic acid and Dettol was calculated before and after sterilization. The highest value of colonies before sterilization ( $2.93 \times 10^2$  bacteria / 100 ml) and the lowest value was 0.0 after sterilization (acetic acid samples) and the highest value for Dettol samples ( $2.17 \times 10^2$  bacteria / 100 ml). The study showed the superiority of acetic acid with high efficiency in the sterilization process compared with Dettol solution, the acetic acid at a concentration (4-6%) gave a high efficiency in the process of sterilization and disinfection of classrooms by (100%) compared with Dettol solution.

**Key words:** Acetic acid, Dettol, Vinegar, Bacteria and sterilization

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### **Introduction**

The sterilization process aims to kill microorganisms in addition to that it represents the highest level of the process of killing microbes, which makes it a possible process. While the disinfection process, it refers to the use of a liquid chemical substance on surfaces at room temperatures in order to kill microorganisms, so the disinfection process is less effective than the sterilization process because it does not kill bacterial spores (Levinson and Jawetz, 2000, Padovani, 2008). We can prevent the growth of microorganisms by using acids and alkalis such as acetic and citric acid, as these factors cause the killing of microorganisms and others inhibit their growth (Danchaivijitr et al., 2005).

Research and reports indicated during outbreaks of diseases in Hong Kong and Taiwan, people began to use traditional customs, where they used herbs and vinegar to

combat disease, vinegar acts as a germ killer (Hendrickson, 2012). The studies confirmed in a scientifically accurate way, that vinegar has a very great benefit in the process of sterilization and the elimination of germs (Ryssel et al., 2009). Acetic acid, the local name for it (vinegar), is the limit of carboxylic acids. It is an important chemical reagent and an industrial chemical used mainly in the production of acetic acid and is used as an acidity regulator (Pangprasit et al., 2020). Due to the importance of controlling microorganisms in Iraq and other countries of the world, and the dangers posed by pathological microorganisms and other microorganisms to humans and even animals and plants, this study aimed.

### **Materials and Methods**

Sterile cotton swabs containing a carrier medium were used for sampling, taken directly to the laboratory, and cultured on MacConkey agar, mannitol salt agar, Pseudomonas agar, Eosin Methylene Blue, Blood agar and Chocolate agar and the dishes were incubated in the incubator for 24 hours at a temperature of 37 C°. Then single colonies were selected from the first culture media, and the samples were first diagnosed by observing the agricultural characteristics of the developing colonies in terms of colony size and height, and shape and color of its edges.

Thin swabs were prepared from these colonies and dyed with gram stain to observe their ability to stained with this dye and cell shapes and arrangement, then biochemical tests were conducted as in (Collee et al., 1996) and ( MacFaddin, 2000). We obtained acetic acid (Bedouin vinegar) at a concentration of 4-6% from one of the shops in the city of Ramadi. The samples were taken by taking a swab from one of the classrooms, and it was before sterilization. Then the process of wiping the school seat was carried out using a piece of cotton containing acetic acid. With a concentration of 4-6%, then a swab was taken from the school bench by another swab, and this swab was a sample after sterilization.

### **Result and Discussion**

Initially 128 bacterial isolates were diagnosed based on phenotypic traits, after they were grown on each of the culture media used in the study and then diagnosed microscopically. Biochemical tests were also conducted (Levinson, 2016) and they were diagnosed using the Vitek 2 compact system. The results showed that the seats were contaminated with six different bacteria types, as Pseudomonas SSP bacteria occupied the highest percentage was 44% (56 isolates), followed by Escherichia coli 25% (32 isolates).

The study showed that Gram-negative bacteria are more resistant to disinfection processes than Gram-positive bacteria, due to their possession of the outer membrane, which prevents the permeability of antiseptic compounds into the cytoplasm, compared to Gram-positive bacteria that lack this layer (Collee et al., 1996). Bacillus SPP recorded 17% (13) isolates, while Klebsiella SPP and Staphylococcus aureus showed 9% and 5% (12 and 6 isolates), respectively, and lastly Proteus SPP recorded 4% (5) isolates Table (1).

**Table (1) Types, numbers and percentage of bacterial isolates**

Isolates	Numbers	Percentage %
<b>pseudomonas SSP.</b>	<b>56</b>	<b>44%</b>
<b>Escherichia coli</b>	<b>32</b>	<b>25%</b>
<b>Bacillus SPP.</b>	<b>17</b>	<b>13%</b>
<b>Staphylococcus aureus</b>	<b>12</b>	<b>9%</b>
<b>Klebsiella SPP.</b>	<b>6</b>	<b>5%</b>
<b>Proteus SPP.</b>	<b>5</b>	<b>4%</b>
<b>Total</b>	<b>128</b>	<b>100%</b>

The statistical analysis of the data showed that there was a statistically significant effect of the type of bacterial isolates on the rates of infection for all bacterial species during the specified period in terms of ( $\chi^2$ ) values  $P < 0.05$ . Study seats in the Department of Life Sciences were randomly selected, as swabs were taken from the surface of the classroom before disinfection, as they were purified by acetic acid disinfectant (vinegar) at a concentration of 4-6%.

From Table (2), the highest value was recorded before disinfection for school seats No. 5 and 10, which amounted to ( $2.15 \times 10^2$  -  $2.93 \times 10^2$ ) bacteria / 100 ml, and after disinfection it reached ( $0.11 \times 10^2$  -  $0.10 \times 10^2$ ) bacteria / 100 ml, and the lowest value ( $0.25 \times 10^2$  -  $0.40 \times 10^2$ ) bacteria/100 ml for the study seat No. 8 and 19, respectively, before disinfection.

After disinfection, it reached (0.0 -0.0), meaning there was no growth, while the school seat No. (3,4,7,8,16) the sterilization rate in them was (0.0), meaning there was no bacterial growth, as in Table (2). The acetic acid disinfectant used in the study was compared

with Dettol disinfectant and that the highest value produced after disinfection for class seats (8,1) was  $(0.27 \times 10^2 - 0.30 \times 10^2)$  bacteria / 100 ml, respectively, while the lowest value was  $(0.45 \times 10^2 - 0.50 \times 10^2)$  bacteria / 100 ml for the class seats (10,12).

**Table (2) Total number of bacteria obtained from the class seats before and after disinfection by acetic acid**

Total number of bacteria x 10 <sup>2</sup> after disinfection	Total number of bacteria x 10 <sup>2</sup> before disinfection	Sample No.
0.11	19.6	1
0.17	0.45	2
0.0	1.06	3
0.0	0.96	4
0.11	2.15	5
0.20	1.09	6
0.0	1.72	7
0.0	0.25	8
0.07	1.46	9
0.10	2.93	10
0.02	1.73	11
0.06	0.80	12
0.04	0.75	13
0.03	0.71	14
0.01	1.31`	15
0.0	0.67	16
0.01	1.79	17
0.01	0.50	18
0.03	0.40	19
0.04	0.55	20
<b>0.913</b>		<b>L.S.D 0.05</b>

1.248	L.S.D 0.01
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L.S.D 0.05 = the smallest significant difference at the 5% probability level.

L.S.D 0.01 = the smallest significant difference at the 1% probability level.

**Mean squares analysis of variance table**

						ANOVA TABLE
P-value	RESULT	F	MS	SS	DF	SOURCE
0	**	48.93566	9.305935	167.5068	18	TREATMENTS
			0.190167	3.613168	19	ERROR
				171.12	37	TOTAL

\* Significant

\*\* Highly significant

**Before Sterilization**

**After Sterilization**

[Figure 1: shows the total number of bacteria obtained from the study seats before and after disinfection by acetic acid.](#)

The results of Table (2) showed the superiority of acetic acid in its efficiency in the sterilization process compared to Dettol solution, acetic acid (4-6%) gave a high efficiency in sterilizing study seats, the sterilization rate in killing microorganisms was 100% compared to Dettol solution and this is due to the deadly action of organic acids, where these acids work

on the coagulation of cellular protein and thus the elimination of microorganisms, and this is consistent with what was stated by (Tawre et al., 2021).

Sloss et al., (1993) also pointed out that the deadly action is due to the destruction of its enzyme system, and that the acids increase the concentration of hydrogen ions and thus disrupt the permeability of cell membranes. There are samples in which the number of colonies appeared after sterilization with a ratio of (0.10, 0.11, 0.17, 0.20) to acetic acid. And samples in which the ratio of the number of colonies to Dettol appeared (0.14, 0.15, 0.16, 0.19, 0.20, 0.27, 0.30) and this is due to the presence of microorganisms such as molds and fungi that are resistant to increasing or decreasing the pH, as they can resist the increase or decrease of acidity or alkalinity and thus grow at very high pH and this is what was indicated by (Marc-Kevin & Bockmühl, 2021) and (Ganavadiya et al., 2014). Figure (1) shows the total number of bacteria obtained from study seats before disinfection with acetic acid, recorded about 2.083, and after disinfection, reached 0.051.

Each specific enzyme system in the cell has a specific range of pH value that operates within its limits, and its activity has an optimal degree that leads to a slowdown in the activity of the enzyme and this is reflected on its growth rate. The pH range of one enzyme may also vary according to the type of cell containing it, and this may be due to its chemical interaction with amino acids and proteins, and this is what was indicated by (Pangprasit et al., 2020). Scott, (2013) indicated that the deadly action is due to its oxidizing effect in addition to the concentration of hydrogen ion (Gajadhar et al., 2003). Figure (2) shows the total number of bacteria obtained from the study seats before disinfection by Dettol, which amounted about 0.998, while after disinfection 0.135.

Vinegar has been shown to have some antiseptic properties. conducted in 2010 found that 10% of barley vinegar was effective against the influenza virus. Another study conducted in 2014 published in the Journal of the American Society for Microbiology found that a 10% solution can kill the bacteria that cause tuberculosis (Pangprasit et al., 2020). Vinegar has been used as a food preservative for hundreds of years due to its antibacterial properties. It can act effectively as food preservative by controlling the microorganisms that attack the food and causing it to decompose and to eliminate *S. aureus* (Scott, 2009).

**Table (3) The total number of bacteria obtained from the study seats before and after disinfection by Dettol**

<b>Total number of bacteria x 10<sup>2</sup> after disinfection</b>	<b>Total number of bacteria x 10<sup>2</sup> before disinfection</b>	<b>Sample No.</b>
0.27	2.17	1
0.15	1.12	2
0.20	1.80	3
0.11	0.75	4
0.09	0.95	5
0.10	1.19	6
0.19	1.70	7
0.30	1.10	8
0.15	0.68	9
0.10	0.45	10
0.16	0.97	11
0.15	0.50	12
0.05	0.51	13
0.06	0.75	14
0.07	1.06	15
0.08	1.13	16
0.14	0.98	17
0.10	0.60	18
0.13	0.75	19

0.10	0.80	20
1.686279		L.S.D 0.05
2.300154		L.S.D 0.01

L.S.D 0.05 = the smallest significant difference at the 5% probability level.

L.S.D 0.01 = the smallest significant difference at the 1% probability level.

**Mean squares analysis of variance table**

						ANOVA table
P-value	RESULT	F	MS	SS	DF	SOURCE
0.00105	**	4.301534	2.811053	53.41	19	TREATMENTS
			0.6535	13.07	20	ERROR
				66.48	39	TOTAL

\* Significant

\*\* Highly significant

Before Sterilization

After Sterilization

Figure 2: shows the total number of bacteria obtained from the study seats before and after disinfection by Dettol



It can be concluded that acetic acid at a concentration of 3% has an excellent bactericidal effect, and therefore it appears to be suitable as a local antiseptic agent (Sloss, 1993).

### Conclusions

1 - The current study showed that the study seats in the College of Education were contaminated with different numbers and types of microorganisms, as *Pseudomonas* SSP. occupied the highest percentage 44% (56 isolates).

2- The study benches (5,10) had the highest value before disinfection, as the percentage of colonies was ( $2.93 \times 10^2$  bacteria / 100 ml), while the percentage of the number of colonies after sterilization was ( $0.11 \times 10^2$ ,  $0.10 \times 10^2$  bacteria / 100 ml).

3- Study seats (8,19) had the lowest value before sterilization, which amounted to ( $0.25 \times 10^2$ ,  $0.40 \times 10^2$  bacteria/100 ml) and after sterilization, the sterilization percentage was (0.0), meaning no growth.

4- Some bacterial species showed high and multiple resistance towards acetic acid.

5-Acetic acid is highly efficient in the process of sterilization and disinfection against bacterial species compared to Dettol.

### References

**Collee, J.G.; Franser, A.G; Mmarmion, B.P. and Sinmones, A.(1996).** Mackie and Maccartney practical Medical microbiology 14th. ed. Churchill Living ston, London.

**Danchaivijitr S, Dhiraputra C, Rongrungruang Y, Srihapol N, umsuwan V (2005).** Microbial contamination of antiseptics and disinfectants. J Med Assoc Thai 88(Suppl 10):S133– S139.

**Gajadhar T, Lara A, Sealy P, Adesiyun AA (2003).** Microbial contamination of disinfectants and antiseptics in four major hospitals in Trinidad. Rev Panam Salud Publica 14(3):193–200.

**Ganavadiya R, Chandra Shekar, B.R, Saxena V, Tomar P, Gupta R, Khandelwal ,G. (2014).** Disinfecting efficacy of three chemical disinfectants on contaminated diagnostic instruments: a randomized trial. J Basic Clin Pharm.;5:98–104.

**Hendrickson. Cram.(2012).** Hammond organic Chemistry Third Edition.

**Levinson, W. and Jawetz, E. (2000).** Sterilization and disinfection, in medical microbiology and immunology, examination and broad review, 6th ed. : 78-81. McGraw-Hill medical publishing division.

**Levinson, W.(2016).** Review of Medical Microbiology and Immunology. 14thed.McGraw-Hill education,Inc.PP821.

**MacFaddin, J.E. (2000).** Biochemical test for identification of medical bacteria. "3rd ed.". Lippn Cott. Williams and Wilkins. Philadelphia. U.S.A.

**Marc-Kevin Z. & Bockmühl D. (2020).** Evaluating the antibacterial, antifungal and antiviral efficacy of acetic acid for home care procedures. BMC.

**Padovani C.M, Graziano KU, Goveia VR (2008).** Microbiological evaluation of different antiseptic povidone-iodine and chlorhexidine formulations after intentional contamination of containers. Rev Lat Am Enfermagem 16(6):1038–1041.

**Pangprasit, N., Srithanasuwan A, Suriyasathaporn W, Pikulkaew S, Bernard JK, Chaisri W. Pathogens. (2020).** Antibacterial Activities of Acetic Acid against Major and Minor Pathogens Isolated from Mastitis in Dairy Cows. 19;9(11).

**Ryssel ,H. ;, Kloeters, O.; Germann,G.; Schäfer,T.; G Wiedemann,G. & Oehlbauer,M.( 2009).**The antimicrobial effect of acetic acid--an alternative to common local antiseptics, Epub .35(5):695-700.

**Rusin P, Orosz-Coughlin P, Gerba C.( 1998).** Reduction of faecal coliform, coliform and heterotrophic plate count bacteria in the household kitchen and bathroom by disinfection with hypochlorite cleaners. J Appl Microbiol.;85(5):819–828.

**Saha AK, Haque MF, Karmaker S, Mohanta MK (2009).** Antibacterial effects of some antiseptics and disinfectants. J Life Earth Sci;3:19–21.

**Scott, E, Bloomfield SF, Barlow CG. (1982).** An investigation of microbial contamination in the home. J Hyg (Lond);89(2):279–293.

**Scott E, Duty S, McCue K. A ( 2009).** Critical evaluation of methicillin-resistant Staphylococcus aureus and other bacteria of medical interest on commonly touched household surfaces in relation to household demographics. Am J Infect Control.;37(6):447–453.

**Scott E. (2013).** Community-based infections and the potential role of common touch surfaces as vectors for the transmission of infectious agents in home and community settings. *Am J Infect Control.*;41(11):1087–1092.

**Sloss ,J. M.; Cumberland, N. and Milner, S.M.(1993).** Acetic Acid used for the elimination of *Pseudomonas aeruginosa* from Burn and soft tissue wounds . *J .Army med corps*, 139 (3): 139.

**Tawre M.S., Kamble, E.E., Kumkar, S.N, Mulani M.S .,& Pardesi K.R. (2021).** Antibiofilm and antipersister activity of acetic acid against extensively drug resistant *Pseudomonas aeruginosa* PAW1. *2;16(2)*.

**Moghadasi, S., Elveny, M., Rahman, H.S. Et Al(2021).** A Paradigm Shift In Cell-Free Approach: The Emerging Role Of Mscs-Derived Exosomes In Regenerative Medicine. *J Transl Med* 19, 302 <https://doi.org/10.1186/S12967-021-02980-6>

**Jalil, A. T., Dilfy, S. H., Karevskiy, A., & Najah, N. (2020).** Viral Hepatitis In Dhi-Qar Province: Demographics And Hematological Characteristics Of Patients. *International Journal Of Pharmaceutical Research*, 12(1)

**Dilfy, S. H., Hanawi, M. J., Al-Bideri, A. W., & Jalil, A. T. (2020).** Determination Of Chemical Composition Of Cultivated Mushrooms In Iraq With Spectrophotometrically And High Performance Liquid Chromatographic. *Journal Of Green Engineering*, 10, 6200-6216

**Jalil, A. T., Al-Khafaji, A. H. D., Karevskiy, A., Dilfy, S. H., & Hanan, Z. K. (2021).** Polymerase Chain Reaction Technique For Molecular Detection Of HPV16 Infections Among Women With Cervical Cancer In Dhi-Qar Province. *Materials Today: Proceedings*

**Marofi, F., F. Abdul-Rasheed, O., Sulaiman Rahman, H., Setia Budi, H., Jalil, A. T., Valerievich Yumashev, A., ... & Jarahian, M. (2021).** CAR-NK Cell In Cancer Immunotherapy; A Promising Frontier. *Cancer Science*

**Widjaja, G., Jalil, A. T., Rahman, H. S., Abdelbasset, W. K., Bokov, D. O., Suksatan, W., ... & Ahmadi, M. (2021).** Humoral Immune Mechanisms Involved In Protective And Pathological Immunity During COVID-19. *Human Immunology*

**Jalil, A.T., Kadhun, W.R., Faryad Khan , M.U. et al. (2021).** Cancer Stages And Demographical Study Of HPV16 In Gene L2 Isolated From Cervical Cancer In Dhi-Qar Province, Iraq. *Appl Nanosci.* <https://doi.org/10.1007/S13204-021-01947-9>

**Sarjito, I., Elveny, M., Jalil, A. T., Davarpanah, A., Alfakeer, M., Bahajjaj, A. A. A., & Ouladsmame, M. (2021).** CFD-Based Simulation To Reduce Greenhouse Gas Emissions From Industrial Plants. *International Journal Of Chemical Reactor Engineering*

**Turki Jalil, A., Hussain Dilfy, S., Oudah Meza, S., Aravindhan, S., M Kadhim, M., & M Aljeboree, A. (2021).** CuO/ZnO Nanocomposites: Facile Synthesis, Characterization And Photocatalytic Degradation Of Tetracycline Antibiotic. *Journal Of Nanostructures*

**Hanan, Z. K., Saleh, M. B., Mezal, E. H., & Jalil, A. T. (2021).** Detection Of Human Genetic Variation In VAC14 Gene By ARMA-PCR Technique And Relation With Typhoid Fever Infection In Patients With Gallbladder Diseases In Thi-Qar Province/Iraq. *Materials Today: Proceedings*

**Vakili-Samiani, S., Jalil, A. T., Abdelbasset, W. K., Yumashev, A. V., Karpisheh, V., Jalali, P., ... & Jadidi-Niaragh, F. (2021).** Targeting Wee1 Kinase As A Therapeutic Approach In Hematological Malignancies. *DNA Repair*, 103203

**Ngafwan, N., Rasyid, H., Abood, E. S., Abdelbasset, W. K., Al-Shawi, S. G., Bokov, D., & Jalil, A. T. (2021).** Study On Novel Fluorescent Carbon Nanomaterials In Food Analysis. *Food Science And Technology*

**Marofi, F., Rahman, H. S., Al-Obaidi, Z. M. J., Jalil, A. T., Abdelbasset, W. K., Suksatan, W., ... & Jarahian, M. (2021).** Novel CAR T Therapy Is A Ray Of Hope In The Treatment Of Seriously Ill AML Patients. *Stem Cell Research & Therapy*, 12(1), 1-23

**Jalil, A. T., Shanshool, M. T., Dilfy, S. H., Saleh, M. M., & Suleiman, A. A. (2021).** Hematological And Serological Parameters For Detection Of Covid-19. *Journal Of Microbiology, Biotechnology And Food Sciences*, E4229. <https://doi.org/10.15414/jmbfs.4229>

**Abosooda, M., Majid, W. J., Hussein, E. A., Jalil, A. T., Kadhim, M. M., Abdullah, M. M., ... & Almashhadani, H. A. (2021).** Role Of Vitamin C In The Protection Of The Gum And Implants In The Human Body: Theoretical And Experimental Studies. *Int. J. Corros. Scale Inhib*, 10(3), 1213-1229

**Dilfy, S. H., Al-Bideri, A. W., & Hanawi, M. J. (2020).** Effect Of *Pleurotus ostreatus* Extract On Epidermal Growth Factor Receptor Expression During Healing Of Aspirin-Induced Peptic Ulcer In Male Rats. *Jordan Journal Of Biological Sciences*, 13

**Hanawi, M. J., Dilfi, S. H., & Albideri, A. W. (2020).** Histological Study Of Therapeutic Effect Of *P. Ostreatus* On Gastric Ulcer In Male Rats. *Systematic Reviews In Pharmacy*, 11(1), 26-34.