ISSN: 2717-8161 RESEARCH ARTICLE

NTMS New Trends in Medicine Sciences

New Trend Med Sci 2023; 4(2): 52-57. https://dergipark.org.tr/tr/pub/ntms

The Efficacy of Various Novel Copper-Based Antibacterial Solutions on E. Coli

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Article History
Received 19 July 2022
Accepted 24 Aug 2022
Published Online 26 May 2023

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Doi: 10.56766/ntms.1144829

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Abstract: The continuous rise of infection in households, businesses, and schools has accelerated the need for long-lasting sanitation solutions. Current disinfectants, like Lysol, kill bacteria and other microbes only at initial application and are ineffective under aqueous conditions. Copper (II) ions and Lactic Acid are highly regarded for their synergetic, long-lasting antibacterial properties. Although L-pyroglutamic acid holds similar properties, little research has examined its efficacy with copper metal. The purpose of this experiment is to evaluate various novel antibacterial solutions for instantaneous microbial inhibition and continued inhibition over extended periods of time in aqueous solutions. Two antibacterial solutions utilizing Copper (II) Sulfate (10 ppm) were developed with 1% Lactic Acid (Solution A) and 1% L-Pyroglutamic Acid (Solution B). The extinction rate of Escherichia coli K12 bacteria for each solution and Lysol was recorded. The concentration of E. coli was observed via spectrophotometry at 3-time intervals: Initial Introduction (28 Minutes), Short Term (2 Hours) and Long Term (72 hours). At initial introduction, there was no significant difference between solutions ($p>0.05$) ranging from 22 to 28% E. coli loss from the original sample. Significant growth inhibition ($p<0.05$) occurred in Solution A and Solution B compared to Lysol after 2 hours. Solution B sustained higher efficacy compared to Lysol after 72 hours. Overall, our Copper (II)/Lactic Acid Solution (Solution A) and Copper (II)/L-Pyroglutamic Acid Solution (Solution B) showed significant improvement when compared to the efficacy of Lysol in aqueous solutions over longer periods of time. Both solutions are cheap and long-lasting, making them pragmatic options for the future aqueous household sanitation.

Keywords: Copper; E. Coli; Lactic Acid; Pyrrolidonecarboxylic Acid.

1. Introduction

Copper metal has been highly regarded for its antimicrobial properties in the healthcare industry for the last decade [1]. Ancient civilizations utilized copper water containments and copper medicinal products long before antimicrobial mechanisms were understood [2]. In 2008, The U.S. Environmental Protection

Cite this article as: Chandra A and Thayagabalu S. The Efficacy of Various Novel Copper-based Antibacterial Solutions on E. Coli. *New Trend Med Sci*. 2023; 4(2):52-57. Doi:10.56766/ntms.1144829

program recognized copper and its alloys as the first metallic antimicrobial agent. Within 2 hours of contact, in vitro assays, copper surfaces were found to have eliminated 99.9% of microbes. In recent years, several studies have supported the utility of copper surfaces in the inhibition of antibiotic-resistant bacteria and Healthcare-associated infection in the hospital setting [3-6]. However, the application of copper sanitation to the household environment has been largely unexplored.

Furthermore, copper particles are also considered potent antibacterial agents [7]. The miniscule nature of this copper is typically considered safe for humans, although higher concentrations may lead to toxicity [8]. Usman et al. (2013) found high antibacterial activity on many bacterial strains such as, MRSA, Bacillus subtilis, Salmonella choleraesuis, and P. aeruginosa using chitosan-copper nanoparticles [9]. Antiviral properties of particle copper have also been shown against Hepatitis C Virus, Influenza A virus, and HIV-1 [10-12]. Copper is able to neutralize cellular function via numerous mechanisms including: essential ion substitution, hydrogen peroxide free radical production by membrane-bound copper, enzyme inactivation, and functional group interference [6]. While the exact mechanism for this effect is unknown, smaller particles seem to have higher antibacterial activity due to easier cell penetration [13-14].

Copper has also shown increased antimicrobial action when coupled with organic acid solutions, specifically, Lactic Acid. Used regularly in a biopreservative and food decontaminant, Lactic acid is generally regarded as safe for humans [8, 15]. Beal et al. saw a 10-fold increase in the death rate of Salm. typhimurium after lactic acid (150 mM) and copper sulfate (50 ppm) were added to a liquid pig feed.16 Several other studies exhibited similar results [10, 17]. The reasoning behind increased antibacterial efficacy with a copper/lactic acid combination is unclear. Morphological changes and increased outer membrane permeability in gram negative bacteria are a few of the proposed mechanisms examined in recent studies [8, 15]. L-Pyroglutamic acid holds similar antibacterial properties although little research has been examined on the combined effectiveness with copper.

Commercially manufactured surface disinfectants currently dominate the household sanitation market. Lysol Disinfecting Spray, a leading product, is widely recognized for its quick killing potential of nearly all microbes on various surfaces. However, disinfectants as such are known to have significantly less antibacterial efficacy in aqueous environments and over longer periods of time [18]. We hypothesized that the effective antibacterial properties, from the combination of copper (II) ions with lactic acid, would address the inadequate nature of Lysol. Thus, the purpose of this study was to (1) determine the efficacy of a copper/lactic acid solution and a copper L-Pyroglutamic Acid solution at inhibiting E. coli K12 growth at initial introduction and (2) the solutions' efficiency over longer periods of time compared to Lysol in an aqueous laboratory condition.

2. Material and Methods

Escherichia coli K12 bacterial strain obtained from Carolina Biologicals was incubated and grown with a nutrient broth as per instruction indicated in manual for 48 hours before experimentation was performed.

Solutions were developed in accordance with safety for regular household use. For this reason, specified amounts of both copper and the acids were used when creating two different solutions (A, B) with various concentrations. The Lysol Solution, the industry standard, was essentially a 2% Lysol dilution with two parts active Lysol per 100 parts distilled water. Solution A was prepared by adding equal parts 10 ppm Copper (II) and 1% L-Pyroglutamic Acid. Solution B consisted of equal parts 10 ppm Copper (II) and 1 % Lactic Acid. Both solutions were synthesized, considering the LD50 values and optimal concentrations of all the agents involved based on accepted literature.

UV spectrophotometry was used to determine changes in E. coli concentration over time. The UV spectrophotometer was used to obtain the percent transmittance of light through each cuvette at 600 nm. For precise concentration calculations, the UV spectrophotometer was utilized [19]. It was calibrated before every cuvette was tested, and the percentage transmittance was output with one decimal precision. Percentage transmittance was converted to absorbance then concentration of E. coli via the Beer Lambert Law [19]. The death rate, the percent loss of E. coli, was calculated using an initial concentration of E. coli obtained from a control sample.

2.1. Procedure

The experimentation was conducted in a constant room temperature environment (21 °C). All surfaces were thoroughly cleaned prior to and after experimentation. The cuvettes were disposed safely, using a 10% bleach solution to eliminate all bacterial growth.

For each experimental group, 7 labelled cuvettes were filled with the initial E. coli broth culture (1.35 mL) and testing solution (0.3 mL) at minute zero in a distinct order. The broth was stirred well before every cuvette was filled as for cultures to be evenly distributed. Each cuvette was filled one minute after the last cuvette. Then, transmittance was recorded via spectrophotometry one minute after the last to maintain consistent reading throughout the experiment.

Spectrophotometry readings of transmittance were taken for all seven cuvettes over the course of 72 hours in the same order, broken into three subcategories of investigation: “initial”, “short term” and “long term.” Initial data represents percent of E. coli loss after 28 minutes from start. Readings were taken at minute 0, 7, 14, 21, and 28. Short term data represents percent of E. coli loss after 127 minutes from start. Readings were taken at minute 120 and 127. Long term data represents the percent of E. coli loss after 72 hours from start. Seven trials were conducted for solution A, solution B and Lysol solution. At each reading point, the average of the seven trials per solution was recorded.

2.2. Statistical Analysis

The data was analyzed by one-sided t-tests on the slopes to measure the positive statistical difference for Solutions A and B compared to Lysol in the percent loss of E. coli. A nonparametric test was not conducted due to insufficient evidence of consistent dispersion. Statistical significance was evaluated where $P<0.05$ was considered significant, and $P<0.001$ highly significant.

3. Results

The efficacy of the Copper (II)/Lactic Acid Solution (Solution A) and Copper (II)/L-Pyroglutamic Acid Solution (Solution B) were similar to that of Lysol in the initial time interval (28 minutes). In the short-term interval (2 hours) both solutions successfully exhibited higher efficacy than that of Lysol. After 72 hours, the long-term interval, Solution B continued to sustain high efficacy compared to Lysol.

Figure 1 (Initial interval) represents the average percent loss of E. coli from its initial concentration to the end of the 28-minute period. There was no significant difference found between the effectiveness of both solution A ($P=0.149$, $t=1.365$) and B ($P=0.063$, $t=2.012$) compared to Lysol. Both solutions as well as Lysol exhibited relatively equal antibacterial efficiency with respect to loss of E. coli.

Figure 2 (Short-term interval) denotes the average percent loss of E. coli from its initial concentration over 128 minutes. We found significant differences between the effectiveness of both solution A ($P<0.001$, $t=23.053$) and B ($P<0.001$, $t=58.415$) compared to Lysol. Solution A reached 46.5 % bacterial death and Solution B reached 42.3 % bacterial death by the two-hour mark highlighted in yellow. Lysol remained rather stagnant over this two-hour period holding at 27 % E. coli loss.

Figure 3 (Long-term interval) presents the average percent loss of E. coli from 128 minutes till 72 hours. Our results indicated significant differences between the effectiveness of both solution A ($P<0.001$, $t=-0.318$) and B ($P<0.001$, $t=2.650$) compared to Lysol. The slope of Solution A, the orange line, peaks after two hours and approaches a constant rate during this interval. Solution B, on the other hand, the gray line, continued eliminating E. coli for the duration of 72 hours peaking at 58% bacterial death at the 4320 minutes. Lysol also has an increase in bacterial death, peaking its percentage loss at 42%. The long-lasting high efficacy of Solution B in comparison to Solution A and Lysol is exhibited in this analysis.

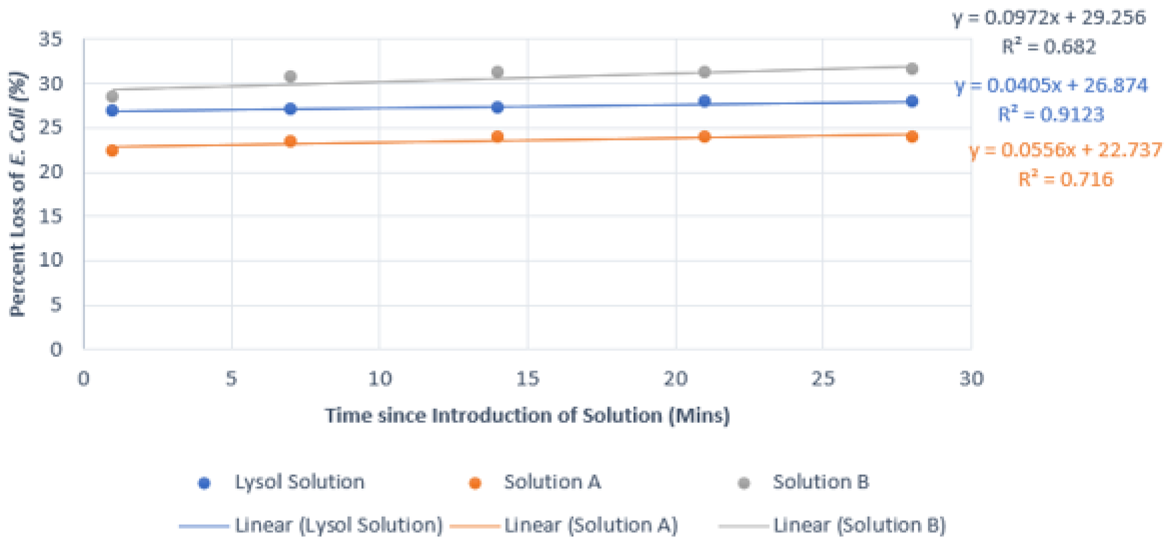

Figure 1: Average Percent Loss of E. Coli Over 28 Minutes. The scatterplot presents the immediate change in E. Coli concentration once each solution is introduced.

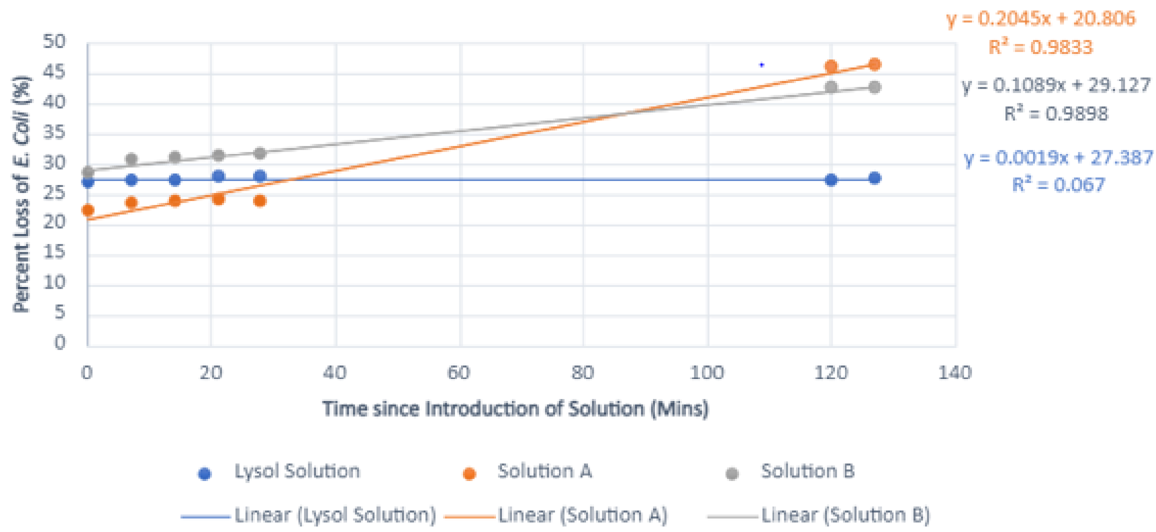

Figure 2: Average Percent Loss of E. Coli Over 128 Minutes. The scatterplot presents the "Short-term" change in E. Coli concentration once each solution is introduced.

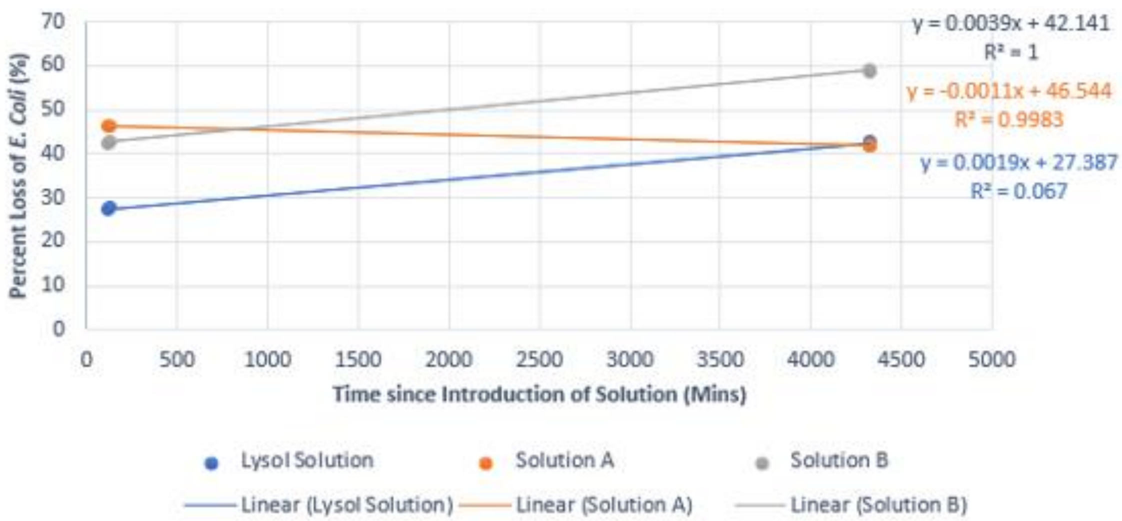

Figure 3: Average Percent Loss of E. Coli Over 72 Hours. The scatterplot presents the "Long- term" change in E. Coli concentration once each solution is introduced.

4. Discussion

Overall, our Copper (II)/Lactic Acid Solution (Solution A) and Copper (II)/L-Pyroglutamic Acid Solution (Solution B) showed significant improvement when compared to the efficacy of Lysol in aqueous solutions over longer periods of time.

Our results are fairly consistent with Gyawali et al. (2011) and Al-Holy (2010). Gyawali et al. evaluated the combination of lactic acid and Copper (II) on E. coli O157:H7. On both lettuce and tomato samples as well as laboratory medium, significant growth inhibition was observed [20]. Al-Holy studied the natural antimicrobial effect of Copper (II), lactic acid, and Monolaurin on Cronobacter. With low concentrations of these components, Al-Holy was able to achieve complete elimination of Cronobacter [21]. Diminished antibacterial properties were observed after a 24-hour time period in both studies. Our study presents similar results as our Copper (II)/Lactic Acid Solution had comparable high efficacy after 2 hours, but severely decreased efficacy after 72 hours. We believe the synergetic inhibitory activity of Solution A to attributed to Lactic Acid permeabilizing the outer membrane of Gram-negative bacteria, as suggested in the prior literature [22]. Free copper (ii) ions are then able to permeate the membrane, disabling enzymes and inactivating functional groups of proteins [22].

Current research on antibacterial properties of L-Pyroglutamic acid is rather limited. Two studies, Tan et al. [23] and Gang et al. [24], presented preliminary evidence of antibacterial and antifungal properties of L-Pyroglutamic acid. However, we were unable to find any relevant literature utilizing the combination of L-Pyroglutamic acid and Copper (II) ions as a synergetic antibacterial agent. While we did not evaluate the antibacterial action mechanisms of L-Pyroglutamic acid, our study pioneers the involvement of L-Pyroglutamic acid in the disinfecting space. Our results from Solution B highlight the potential of L-Pyroglutamic acid over longer periods of antibacterial inhibition along with copper particles, although further research should be conducted.

The data from our experiment supported both of our hypotheses. Hypothesis 1 was supported from our initial testing phase where we saw similar percent loss across all three solutions. Hypothesis 2 was supported from the short term and long-term testing phases. After 2 hours the Copper/L pyroglutamic acid solution was most effective and after 72 hours Copper/Lactic Acid solution was most effective. Given the high availability, low cost, and low concentration of key ingredients, our cost analysis indicates cheap commercial production of both solutions. As for safety, our solutions utilize concentrations (g/L) of Copper (II) Sulfate, L-Pyroglutamic Acid, and Lactic Acid that are less than 4% of the LD50 value per average adult body mass (g). Both solutions are suitable for household use based on LD50 values comparison and all-natural ingredients. In summary, both solutions A and B are strong contenders as antibacterial alternatives to Lysol. The research presented has practical applications in regard to disinfection. Because of the low cost, our solutions can be applied in two main avenues: Aqueous Disinfecting and Household Disinfecting. Our sanitation formula, having been tested in aqueous environments, will combat microbial growth in standing waters and wastewaters continuously. Our product is most practical as a household disinfectant via wipes and aerosol spray cans. The aqueous nature of this sanitation formula makes our solution easy to implement in a variety of antibacterial products offered to the average consumer. Although we do not speculate any health concerns, the human safety of both solutions should be formally regulated before household implementation. Since this method is cheap, easy to implement, and long-lasting, it is a pragmatic option for future household sanitation.

In the future, we wish to continue testing our solutions with varied concentrations to maximize the longevity of their antibacterial potential. We would like to further research the antiviral potential of our solutions on other molecules including COVID-19. A study conducted by Govind et al. (2021) demonstrated promising results on the antiviral capabilities of copper through its production of certain toxins which combat COVID-19 viral attacks [25]. Another study by Kassaa et al. (2014) analyzed the antiviral potential of lactic acid bacteria through direct probiotic-virus interaction and other mechanisms [26]. The antifungal properties of L-pyroglutamic acid may also be explored.

5. Conclusions

We synthesized copper-based aqueous antibacterial solutions with 1% Lactic Acid (Solution A) and 1% L Pyroglutamic Acid (Solution B). The efficacy of the solutions reflected in the average percent loss of E. coli in comparison to 2% Lysol dilution over 28 minutes, 128 minutes, and 72 hours' time-intervals. Optimal efficacy was exhibited in Solution B given its long-lasting antibacterial activity. Coupled with low production cost, our results foreground the potential of L-pyroglutamic Acid/copper solutions in aqueous household disinfecting. Future research must be conducted with an array of microorganisms and health risks must be considered.

Limitations of the Study

Our research was limited by time constraints for data collection. Thus, the testing-intervals were restricted to 28 minutes, 128 minutes, and 72 hours. To increase the range of data collection, longer time-intervals are recommended.

Acknowledgement

We would like to thank Mr. Jay Rosenberg of Cypress Bay High School for his valuable input and contributions.

Conflict of Interests

Authors did not have any conflicts.

Financial Support

It was partially supported by Cypress Bay High School.

Author Contributions

Both authors had equal contributions.

Ethical Approval

No ethical approval was needed for this study.

Informed Consent

Informed consent form was submitted to the journal.

Availability of Data and Materials

Not applicable for this study.

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