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Efficacy of stable liquid chlorine dioxide in the cleaning of drinking systems for laboratory rats in non-standardised vivarium conditions

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Abstract
The study evaluated the disinfection efficacy of stabilised liquid chlorine dioxide (Dioxy Active Supra, 0.3%.) on water dispensers used for laboratory rats in a vivarium operating outside the standards prescribed by the European Council Directive 2010/63/EU. Swabs were taken from all drinkers before and after disinfection and inoculated on three microbiological media: nutrient agar, MacConkey agar, and XLD agar. Before treatment, abundant and diverse bacterial growth was recorded, including colonies of Enterobacteriaceae and other opportunistic microorganisms. After immersion of the drinkers and metal taps in the liquid chlorine dioxide solution for 15 minutes, a significant reduction or complete inhibition of bacterial colonies was observed on all media. Quantitatively, the disinfectant achieved more than 95% efficiency on nutrient agar and 100% on MacConkey and XLD agar. These findings confirm the strong bactericidal properties of stable liquid chlorine dioxide, attributable to its oxidative mechanism and biofilm-disrupting capacity. The disinfectant proved effective against both Gram-positive and Gram-negative bacteria, without producing odours or residues that could adversely affect animal welfare. In conclusion, stabilised liquid chlorine dioxide represents a safe, highly efficient and practical solution for maintaining the hygienic integrity of drinking systems in laboratory animal facilities, thereby ensuring reliable experimental outcomes and compliance with welfare standards.

Keywords: *Laboratory rats, vivarium hygiene, stable liquid chlorine dioxide, disinfection, Enterobacteriaceae, animal welfare.*

Introduction

Laboratory animals are increasingly used in biomedical research as models for studying and understanding human and animal diseases. Interest among researchers from various medical disciplines in experimenting on laboratory animals is growing. Above all, the most commonly used laboratory animal is the mouse, followed by the laboratory rat. For experimental studies to proceed smoothly and for the results obtained from scientific research to be valid and applicable, it is essential to use completely healthy animals with ensured welfare.[1]

The vivarium where laboratory animals are bred must ensure proper management of the animals, their housing, and health. Furthermore, the vivarium must provide necessary veterinary care to maintain animal health and welfare. It is essential to ensure regular and proper maintenance of the facility and equipment, as well as the health and safety of employees, professional associates, researchers, and technical staff involved in conducting animal experiments. The best guidelines in this regard are those outlined in the European Council Directive[2], with emphasis on Annexe A, the principles of Good Laboratory Practice (GLP), and harmonised with the legal regulations on animal welfare in Bosnia and Herzegovina.

However, countries outside the European Union, particularly transitional countries, often lack sufficient material resources and are unable to fully implement all recommendations from DIRECTIVE 2010/63/EU, which relate to specified parameters for temperature, ventilation, relative humidity, and light/dark cycles (zoohygiene, circadian rhythm). Additionally, the use of certified bedding for cage changes twice a week is often neglected in conventional cages. According to the Directive mentioned above, 2010/63/EU, procedures for cleaning, washing, decontaminating, and desinfecting cages, tools, bottles, and other equipment are precisely defined. However, in practice, water bottles and their accessories are often inadequately cleaned, and deposits of fine particles can be observed at the bottom of the bottles with the naked eye.

Therefore, it is necessary to select an appropriate disinfectant that will remove any potential microbial buildup on such items after mechanical cleaning and manual

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washing with detergents. One such disinfectant of choice is stabilised liquid chlorine dioxide, which is assumed to have broad-spectrum bactericidal effects and, after application on work surfaces, does not leave any odour or create an unfavourable environment for laboratory animals, and does not require rinsing after use.[3-5] This same preparation has also proven effective in the safe sanitation of drinking water and the water distribution system on farms for laying hens producing table eggs.[6]

The aim of this study was to determine the effectiveness of stabilised liquid chlorine dioxide on the potential bacterial status of specific equipment segments in a laboratory animal vivarium, which did not operate according to the recommendations outlined in DIRECTIVE 2010/63/EU.

Material and Methods

All water dispensers in the vivarium were included in the procedure for microbiological examination, which did not operate according to the principles of Directive 2010/63/EU. Sampling was conducted so that aggregate samples, such as swabs, were taken from all drinkers in the vivarium. Swabs were collected using sterile cotton swabs moistened with sterile saline (0.9% NaCl). Immediately after sampling, inoculation onto suitable nutrient media commenced. Following the initial sampling, the drinkers and their associated metal taps were mechanically cleaned and washed with detergent in lukewarm water and then immersed in a solution of stable liquid chlorine dioxide (Dioxy Activ Supra, 0.3%, produced by the Bosnian company ITR d.o.o.) for 15 minutes before drying. The sampling process was then repeated for all drinkers. To isolate and differentiate microorganisms, three types of standard microbiological media were employed.

- Nutrient agar – to determine the total number of aerobic mesophilic bacteria.
- MacConkey agar – a selective-differential medium for the isolation of bacteria from the family

Enterobacteriaceae, especially Escherichia coli and other Gram-negative bacteria.

- XLD (Xylose Lysine Deoxycholate) agar – selective medium for the isolation of enteropathogenic bacteria of the genus Salmonella and Shigella.

The microbiological media are prepared following the manufacturer's instructions, sterilised by autoclaving at 121 °c for 15 minutes, then poured into sterile Petri dishes and stored in a refrigerator (+4 °C) until use. The swabs are rotated directly on the media surface using sterile techniques. Each medium is labelled according to the sample type and an ordinal number. After seeding, Petri dishes are incubated for 24–48 hours at 37 °C under aerobic conditions. Following incubation, the plates are visually examined. Colonies are classified based on morphological characteristics (colour, shape, size, edge, and surface), and the colony count provides a qualitative indicator of bacterial presence before and after treatment.

The results were analysed descriptively, and the differences in the number of colonies before and after treatment were shown comparatively (both visually and in description). A quantitative assessment of chlorine dioxide's effectiveness is expressed as a percentage reduction in bacterial growth.

Results

The results shown in Table 1 and Pictures 1–4 clearly demonstrate a significant reduction in the microbiological load before and after treatment with stable liquid chlorine dioxide. All samples exhibited almost complete or complete inhibition of bacterial growth following the application of stable liquid chlorine dioxide, confirming its strong bactericidal properties. In the photographs labelled "before disinfection" on all types of media, a notable amount of microbiological growth was observed. Conversely, after treatment with chlorine dioxide, colonies marked "after disinfection" were scarce or absent, indicating the high disinfecting potential of this compound.

Results on nutrient agar and MacConkey agar

Before treatment, a dense growth of diverse bacterial colonies was observed on nutrient agar. The colonies varied in appearance – ranging from tiny, white, smooth forms to larger, creamy, convex shapes. This suggests the presence of a wide range of saprophytic and potentially opportunistic bacteria that colonise surfaces, including the genera *Bacillus*, *Micrococcus, Pseudomonas*, and others.

After treatment with stable liquid chlorine dioxide, the number of colonies was significantly reduced; only individual colonies were visible. (Picture 1.) This result confirms the bactericidal effect of chlorine dioxide at the concentration and exposure time of 15 minutes, in accordance with previous findings from the literature.[7,8]

MacConkey agar, a selective and differential medium used for isolating Gram-negative bacteria, displayed noticeable differences between treated and untreated drinkers. Before disinfection, numerous pink colonies were observed, indicating the presence of lactose-positive bacteria from the genera *Enterobacter*, *Klebsiella,* or *Escherichia* (Picture 2).

After chlorine dioxide treatment, no bacterial growth was observed – the surface of the media is sterile, with no visible colonies or discolouration of the substrate. This finding confirms that stable liquid chlorine dioxide is effective against Gram-negative bacteria, including the aforementioned bacterial species.

Results on XLD agar

On XLD agar before treatment, moderate growth of yellow colonies was observed, with no black centres, indicating the presence of bacteria that ferment xylose but do not produce H_2S – most likely members of the genus *Enterobacter spp.* or *Escherichia spp.* At the same time, Salmonella spp. and Shigella spp. were not detected. No colony development was observed after chlorine dioxide treatment, indicating complete inactivation of Gram-negative enteropathogens. These findings align with studies confirming the high effectiveness of chlorine dioxide against bacteria of the genus *Salmonella spp*. and *Escherichia coli* on surfaces.[3,4,9]

Before the treatment, a dense growth of various cream-coloured bacterial colonies is visible (Figure 1a), while after disinfection, the number of colonies is significantly reduced, with some isolated colonies (Figure1a).

Pink colonies of lactose-fermenting bacteria (E. coli, Enterobacter spp.) are observed on the samples before treatment (Figure 1b). In contrast, after the treatment, the substrate remains sterile, without visible microbiological growth (Figure1b).

Before treatment, the growth of yellow colonies without black centres was noted, most likely members of the genus *Enterobacter spp*. or *Escherichia spp.* (Figure 2a). After treatment, no growth was recorded, which confirms the complete decontamination of the surface of the drinkers (Figure 2a).

Table 1. Qualitative and semi-quantitative presentation of bacterial growth before and after treatment with stable liquid chlorine dioxide.

Type of substrate	Samples before disinfection	Samples after disinfection	Evaluation of the effectiveness of disinfection
Nutrient agar	Abundant growth of different colonies (heterogeneous microflora) – **+++**	Single colonies or complete absence of growth **– – / +**	Very high efficiency (>95%)
MacConkey agar	Numerous pink colonies of lactose-positive bacteria (*Enterobacteriaceae*) – **++/**+++	No visible growth, substrate sterile **– –**	Complete elimination of Gram-negative bacteria (≈100%)
XLD agar	Moderate growth of yellow colonies without black centers (xylose fermentation, absence of *Salmonella* spp.) – **++**	No colonies **– –**	Complete inhibition of the growth of enteropathogens (≈100%)

Legend:
+++ = abundant growth (over 100 colonies);
++ = moderate growth (30–100 colonies);
+ = minimal growth (1–30 colonies);
– = without growth.

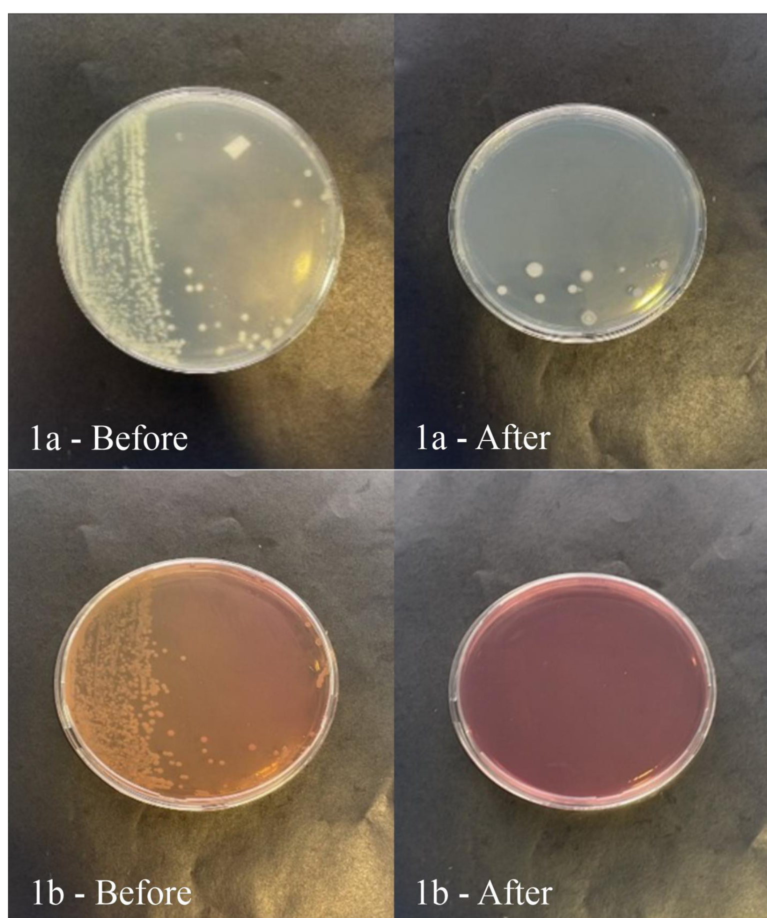

Figure 1. 1a before and 1a after Nutrient agar – before and after disinfection of drinkers with stable liquid chlorine dioxide. 1b before and 1b after MacConkey agar – before and after disinfection.

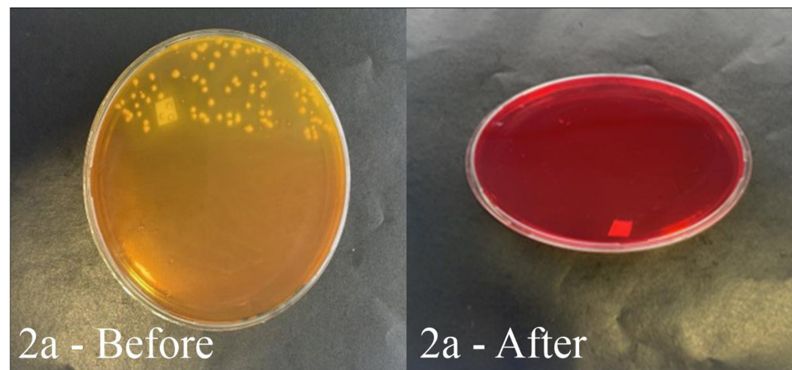

Figure 2. 2a and 2a XLD agar – before and after disinfection

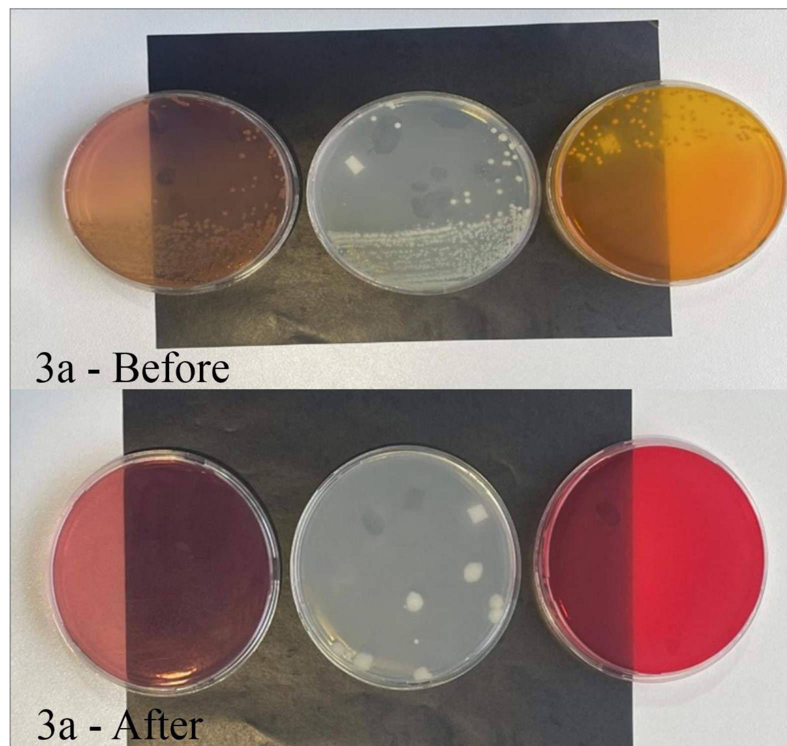

Figure 3. 3a before and 3a after. A comparative review of all three types of substrates before and after treatment.

Discussion

The results of the conducted research confirm an extremely high level of efficiency of stable liquid chlorine dioxide (*Dioxy Activ Supra*, 0.3%) in decontaminating drinking systems for laboratory rats. After treatment with this disinfectant, an almost complete absence of microbial growth was observed on all tested nutrient media, clearly indicating a strong bactericidal effect. These findings are consistent with previous research demonstrating that chlorine dioxide, due to its oxidative mechanism, effectively reduces and even eradicates microorganisms, including bacteria, fungi, and viruses, and breaks down biofilm formations on and within treated surfaces.[4,6]

On nutrient agar before treatment, abundant growth of heterogeneous microflora was observed, indicating the presence of saprophytic and potentially opportunistic bacteria such as *Bacillus spp.*, *Micrococcus* spp., and *Pseudomonas* spp. After applying chlorine dioxide, the number of bacterial colonies was markedly reduced. In most samples, only single colonies were found, indicating a microbiological load reduction of more than 95%. These findings confirm that a concentration of 0.3% and an exposure time of 15 minutes are the optimal parameters to achieve complete disinfection without damaging the power supply system. On MacConkey agar, which selectively supports the growth of Gram-negative bacteria from the *Enterobacteriaceae family*, a large number of pink colonies were observed before treatment, indicating the presence of lactase-positive bacteria such as *Escherichia coli*, *Enterobacter,* and *Klebsiella* spp. After treatment, no growth was detected, confirming the complete eradication of these bacteria and the sterility of the substrate. This outcome is significant because Gram-negative bacteria, along with others, often contribute to biofilm formation in water systems, which is a layer of microorganisms protected by a matrix of polysaccharides, making them resistant to most common disinfectants. On XLD agar**,** before disinfection, a moderate growth of yellow colonies without black centres was noted, which indicates the presence of bacteria that ferment xylose, but do not produce H_2S – probably members of the genera *Enterobacter* or *Escherichia*. Af-

ter treatment, colony growth is absent, which confirms the complete inactivation of enteropathogenic bacteria and the effectiveness of the disinfection procedure in removing microorganisms that are potentially pathogenic to laboratory animals.

Although all types of laboratory animal keeping, as stated by Ališah[9], require high standards of hygiene and cleanliness especially in a vivarium such as regular cage washing, substrate changing, and disinfecting the premises, the presence of ambient microflora remains an unavoidable risk factor. Ambient micromism refers to the presence of microorganisms in the air, water, and on surfaces within the environment, which can spontaneously inhabit the space even under strict hygiene measures. It indicates a constant microbiological presence due to the circulation of dust, aerosols, and moisture, and is mainly observed in enclosed systems where heat, moisture, and organic material coexist. Such conditions are common in vivariums, so despite regular hygiene practices, contamination in the water supply system can still be expected.

Conclusion

Although the presence of ambient microbiota in a vivarium environment that does not operate in accordance with the principles of the European Council Directive (DIRECTIVE 2010/63/EU) is a natural and unavoidable phenomenon, it arises from the continuous circulation of microorganisms through air, humidity, and dust, which spontaneously colonize surfaces and water systems. This issue can be effectively and easily controlled by applying stable liquid chlorine dioxide. The results of this study confirm that this disinfectant is highly effective in eliminating microbial contamination, including bacteria from the Enterobacteriaceae family and other opportunistic microorganisms, without adverse effects on animal welfare, technical equipment, or personnel handling the disinfectant. Due to its stability, high biological safety, and broad-spectrum antimicrobial activity, stable liquid chlorine dioxide is an optimal solution for maintaining the hygienic integrity of drinking systems, thereby ensuring animal health, welfare, and the reliability of experimental outcomes in laboratory animal research.

References

1. Katica M, Delibegovic S. Laboratorijske životinje: pokusni modeli u biomedicini. Veterinaria. 2019; 68(3): 121-124. (In Bosnian).
2. Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes Text with EEA relevance. Official Journal of the European Union. Date 20.10.2025. https://eur-lex.europa.eu/eli/dir/2010/63/oj/eng
3. Ališah A, Bešić A, Nenadović K. et al. Assessment of the hygienic correctness of drinking water on the farm of South American chinchilla before and after "shock" treatment with stable liquid chlorine dioxide: A case report. Veterinaria. 2024a; 72(3):321-328.
4. Ališah A, Kapo-Dolan N, Pivić A. et al. Efficiency of stable liquid chlorine dioxide (CLO2) in sanitation of water distribution system in poultry production- synthesis of theoretical and experimental findings. Veterinaria, 2025;74Suppl(1), 39-47.
5. Gagić A, Selimović S, Jukić S, et al. Dileme savremene dezinfekcije: Hlor ili stabilni tečni hlor dioksid. Veterinaria, 2013; 62 (3-4), 229-240. (In Bosnian).
6. Gagić, A. Hadžiabdić S, Vrabelj D. The use of stabilized liquid chlorine dioxide (ClO2) for internal water supply system sanitation of farm with laying flock affected by colisepticemia.Veterinaria. 2013b; 62(3-4), 145-156.
7. Ališah A. Higijenski, zdravstveno-tehnološki, etološki i ekonomski aspekti novog koncepta opšte profilakse tova brojlerskih pilića. Doctoral dissertation. University of Sarajevo - Veterinary Faculty. 2020. (In Bosnian).
8. Gagić A. Zoohigijenski aspekti borbe protiv BSE, slinavke i šapa, bruceloze i Q -groznice. Veterinaria, 2000; 49(1-2): 197-209. (In Bosnian).
9. Ališah A, Bešić A, Jusić A. Social Behaviour Analysis of Wistar laboratory rats in their environment. Rev Rom Med Vet. 2024b; 34(3): 5-11.