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# Synergistic potential of aqueous ozone: Sublethal bacterial damage and enhanced antibiotic susceptibility

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**Abstract.** Combat-related injuries are frequently complicated by infections caused by multidrugresistant microorganisms, posing significant challenges to treatment. To improve clinical outcomes in such cases, alternative adjunctive therapeutic strategies are required. This study aimed to assess the antimicrobial potential of electrolytically generated aqueous ozone, focusing on its ability to induce sublethal damage in bacteria and enhance their susceptibility to antibiotics. A total of 357 multidrug-resistant clinical isolates were obtained from wound exudates and blood samples of 284 wounded soldiers. The most frequently isolated pathogens included *Acinetobacter baumannii*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Bacillus cereus*, and coagulase-negative *Staphylococcus* spp. Ozonated water was generated at concentrations ranging from 3.6 to 11.2 mg/L and subsequently standardised to approximately 4 mg/L for bacterial treatment. Selected isolates of *S. aureus* and *Escherichia coli* were exposed to aqueous ozone for 10 and 15 minutes. Sublethal damage was assessed by comparing bacterial growth on selective and nonselective media, revealing up to 89.2% sublethally injured *S. aureus* cells and up to 98.6% injured *E. coli* cells after 15 minutes of exposure. Initial estimations of the minimum inhibitory concentration using the Vitek-2 system were distorted by the combined effect of ozone and free chlorine, which forms during the electrolysis of saline solution. This prompted a shift to the Kirby-Bauer disc diffusion method. The results consistently demonstrated increased antibiotic susceptibility in treated isolates, as evidenced by larger inhibition zone diameters and a reduced number of antibiotics to which the isolates

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remained resistant. Electrolytically generated aqueous ozone effectively compromises bacterial integrity, enhancing their susceptibility to antibiotics. It represents a promising adjunctive strategy for managing multidrug-resistant infections, particularly in resource-limited settings or during wartime

**Keywords:** antibacterial activity; antibiotic resistance; ozonated water; pathogenic bacteria; sublethal damage

## Introduction

Combat-related injuries frequently present with multidrug-resistant infections that pose significant challenges to conventional antibiotic and antiseptic therapies. There is a growing need for alternative, low-cost antimicrobial strategies that can support infection control in resource-limited or high-risk environments. Exploring the potential of electrolytically generated aqueous ozone offers a promising direction for improving bacterial susceptibility and enhancing the effectiveness of standard treatments.

Ozone has long been recognised for its potent antibacterial properties and has been widely adopted across various industries for microbial control. In the food industry, A.C. Khanashyam *et al.* [1] highlighted its efficacy in improving food safety by inactivating a broad range of spoilage and pathogenic microorganisms. Similarly, X. Ren *et al.* [2] emphasised ozone's utility in water treatment, particularly in maintaining microbiological safety. In the wellness sector, A. Nagy [3] described its widespread application in spa and balneotherapy settings, where it contributes to maintaining hygienic conditions and reducing cross-contamination.

Despite these successful applications in non-medical settings, the adoption of ozone in clinical infection control remains limited. Traditional healthcare strategies have long relied on antibiotics and topical antiseptics as the primary tools for managing bacterial infections. However, as S. Lemmen & K. Lewalter [4] reported, the increasing global burden of antibiotic-resistant pathogens has severely undermined the effectiveness of these conventional methods, necessitating the exploration of adjunctive or alternative antimicrobial strategies. Although antiseptics represent a viable alternative to antibiotics, their use as a standalone solution for infection control remains problematic. As noted by A. Roth *et al.* [5], many antiseptics are constrained by limited efficacy in deep-seated infections due to poor tissue penetration, which reduces their utility in more complex clinical scenarios. J. Roy *et al.* [6] further emphasised that the repeated use of antiseptic agents may contribute to the development of microbial tolerance, raising concerns about long-term effectiveness. Additionally, J.J. Pérez-Santonja *et al.* [7] highlighted the cytotoxic effects of several commonly used antiseptics on human tissues, noting that their frequent application can impair wound healing processes. These concerns limit the suitability of antiseptics in sensitive clinical environments, particularly where tissue regeneration and healing are critical. Consequently, the search for alternative antimicrobial approaches that combine broad-spectrum efficacy with biocompatibility remains a key priority in contemporary medicine.

Electrolytic ozonation offers a compelling alternative by enabling the on-demand generation of ozonated water using only water and electricity, thereby eliminating the need for costly infrastructure or a continuous oxygen supply. H.Y. Li *et al.* [8] demonstrated that this method is both efficient and economical, providing a practical means of ozone production in settings where traditional ozonation systems – dependent on pure oxygen and specialised equipment – are impractical. Moreover, this technique addresses a major safety concern associated with gaseous ozone. As E. Grignani *et al.* [9] noted, exposure to ozone gas at high concentrations poses significant health risks, particularly respiratory toxicity. Electrolytic ozonation mitigates this hazard by avoiding the gas phase altogether, offering a safer alternative for clinical environments. With its advantages in cost, safety, and ease of implementation, electrolytic ozonation is emerging as a valuable tool in the development of novel antimicrobial strategies, particularly in the ongoing battle against antibiotic-resistant pathogens.

This study aimed to evaluate both the bactericidal and sublethal effects of ozonated water on clinical strains, as well as its impact on antibiotic susceptibility.

## Materials and Methods

*Sample collection and microbial identification.* Wound discharge and blood samples were collected from wounded soldiers hospitalised at Ternopil Regional Clinical Hospital, Ternopil, Ukraine, between January and October 2024. Wound discharge samples were obtained using sterile cotton swabs and inoculated onto selective culture media. Blood samples were collected using vacuum blood collection tubes and analysed with the automated blood culture system BACT/ALERT 3D (bioMérieux, Marcy-l'Étoile, France). The resulting cultures were identified using the semiautomated Vitek-2 Compact 15 system (bioMérieux, Marcy-l'Étoile, France). The antibiotic susceptibility of treated and untreated isolates was assessed using the Kirby-Bauer disc diffusion method, alongside minimum inhibitory concentration (MIC) determination via the Vitek-2 Compact 15 system.

*Aqueous ozone production and ozone concentration measurement.* Ozonated water was generated on demand using sterilised tap water and a custom-built pre-production prototype employing water electrolysis on a diamond-coated anode. Ozone concentration in the water was assessed photometrically using a PoolLab 1.0 photometer (Water-i.d., Eggenstein, Germany), based on the intensity of colour change following reaction with N,N-diethyl-p-phenylenediamine sulphate. The ozone concentration was determined by comparing the absorbance of

the coloured light (at wavelengths of 530 and 620 nm) in the sample against that of untreated control, using calibration data programmed into the instrument. Tablet-based reagents were employed for measuring ozone levels. For bacterial treatment, the concentration was standardised to approximately 4 mg/L. When higher concentrations were detected, dilution was carried out to ensure that the treatment did not exceed this threshold.

*Bacterial culture treatment and sublethal damage evaluation.* The isolates were subcultured into meat-peptone broth for overnight incubation at 37°C. The resulting broth cultures were centrifuged at 3000 RPM using an 80-2 Benchtop Universal Laboratory Centrifuge (Jiangsu Jinyi Instrument Technology Co., Changzhou, China). The supernatant was discarded, and the cell pellets were resuspended in a sterile saline solution (0.9% NaCl). To minimise the presence of residual culture medium and reduce the organic load, the centrifugation and resuspension steps were repeated once. For ozone treatment, the supernatant was again discarded, and the pellets were resuspended in freshly prepared ozonated water (ozone concentration ~4 mg/L), followed by incubation at room temperature for 15 minutes. This was followed by serial dilution in sterile saline solution and plating of aliquots onto selective and non-selective culture media. The plates were incubated at 37°C for 24 hours. Sublethal injury of the tested strains was assessed by comparing colony counts between the selective and non-selective media [10]. This evaluation was conducted for *Staphylococcus aureus* and *Escherichia coli* isolates only. For *S. aureus*, non-selective blood agar and selective yolk salt agar (selective due to high salt concentration) were used. For *E. coli*, blood agar served as the non-selective medium, while MacConkey agar was used as the selective medium, with bile salts acting as selective agents by inhibiting the recovery of sublethally damaged cells. The percentage of sublethal injury was calculated using the following equation:

$$\%\ Injured\ cells = \frac{(CFU_{non-selective} - CFU_{selective})}{CFU_{non-selective}} \cdot 100. \quad (1)$$

For antibiotic susceptibility testing, pretreated suspensions of the selected strains were immediately spread onto Mueller-Hinton agar plates, and antibiotic-impregnated discs were applied in accordance with the Kirby-Bauer disc diffusion method. Control samples (untreated) followed the same protocol, using sterile saline in place of ozonated water. Antibiotic resistance status was determined based on the immediate post-treatment response; long-term resistance was not evaluated.

*Statistical analysis.* Data were collected and tabulated using MS Excel 2013. Categorical data were presented as percentages and proportions, while CFU counts were expressed in logarithmic values. Mean values and standard deviations (M ± SD) were calculated and used for statistical analysis. The equality of mean values between the two groups was assessed using Student's t-test. For comparisons among multiple groups, one-way analysis of variance (ANOVA) was performed using Statistica 8.0 software (StatSoft Inc., Tulsa, Oklahoma, USA).

*Ethical approval.* The research was conducted following the principles set out in the Declaration of Helsinki [11]. Ethical approval for the publication of this case report was obtained from the Ethics Committee of Ternopil National Medical University (Protocol No. 81, 3 April 2025).

## Results and Discussion

Ozonated water, generated through electrolytic ozonation, had ozone concentrations ranging from 3.6 to 11.2 mg/L. A total of 357 clinical isolates were obtained from wound discharge and blood samples were collected from 284 wounded soldiers. The most frequently isolated pathogens included *Acinetobacter baumannii, Klebsiella pneumoniae,* and *Pseudomonas aeruginosa* among Gramnegative bacteria, while *S. aureus, Bacillus cereus,* and coagulase-negative *Staphylococcus* spp. were the predominant Gram-positive isolates. The distribution of these pathogens, along with the number and percentage of cases in which they were identified, is presented in Table 1.

**Table 1.** Distribution of isolated pathogens from wound discharge and blood samples

| Pathogen | Wound discharge (n, %) | Blood isolates (n, %) |
|---|---|---|
| *Acinetobacter baumannii* | 78 (21.85%) | 5 (1.40%) |
| *Bacillus cereus* | 28 (7.84%) | |
| *Enterobacter cloacae* | 6 (1.68%) | |
| *Enterococcus* spp. | 13 (3.64%) | 1 (0.28%) |
| *Escherichia coli* | 11 (3.08%) | |
| *Klebsiella pneumoniae* | 51 (14.29%) | 2 (0.56%) |
| *Proteus mirabilis* | 6 (1.68%) | |
| *Pseudomonas aeruginosa* | 38 (10.64%) | |
| *Rothia kristinae* | 6 (1.68%) | |
| *Staphylococcus aureus* | 64 (17.93%) | 3 (0.84%) |
| *Staphylococcus epidermidis* | 11 (3.08%) | |
| *Staphylococcus* spp. | 13 (3.64%) | |
| Other | 21 (5.88%) | |
| **Total, n = 357** | **346 (96.92%)** | **11 (3.08%)** |

**Source:** compiled by the authors

During preliminary testing of antibiotic susceptibility using the Vitek-2 Compact system, bacterial suspensions were prepared in 0.45% NaCl, in accordance with the manufacturer's protocols. When assessing antibiotic susceptibility, the MICs of the untreated isolates were successfully determined using the Vitek-2 system. All 357 isolates demonstrated multidrug resistance, exhibiting resistance to three or more classes of antibiotics, thereby confirming the challenging nature of antimicrobial therapy in these cases. To evaluate the impact of aqueous ozone pretreatment, the required 0.45% sodium chloride solution was subjected to electrolysis using a portable ozone generator. However, chemical analysis revealed that the process generated not only ozone but also high concentrations of free chlorine (≥30 mg/L), another potent oxidising agent. This dual oxidative stress likely disrupted bacterial membranes and enzymatic systems. As a result, the Vitek-2 system misidentified *Staphylococcus aureus* as *Granulicatella adiacens* and *Erysipelothrix rhusiopathiae* – an unexpected but noteworthy observation. However, since the presence of chlorine introduced a confounding variable, the MIC results could no longer be attributed to the effect of ozone alone. For this reason, the Vitek-2 system was excluded from further ozone-specific susceptibility testing, and the Kirby-Bauer disc diffusion method was used instead. Representative results, illustrating the increased susceptibility of *S. aureus* to nitrofurantoin following ozonated water pretreatment, are shown in Figure 1.

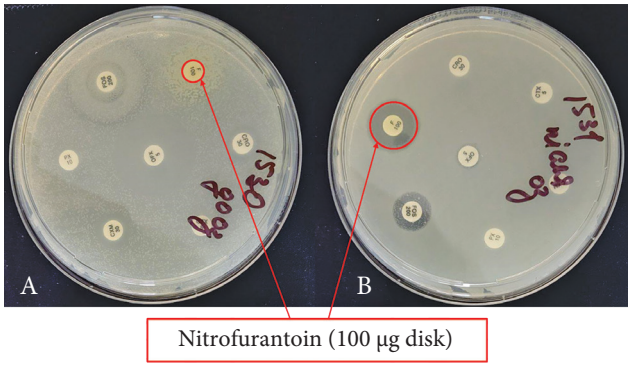


**Figure 1.** Results of the Kirby-Bauer disc diffusion method showing the effect of ozonated water pretreatment on antibiotic susceptibility of *S. aureus*
**Notes:** conversion to susceptibility is highlighted in red. A – untreated control; B – after 15-minute pretreatment with ozonated water
**Source:** compiled by the authors

To quantify this effect across all tested strains, the number of antibiotics to which each isolate was resistant was compared before and after ozonation. As summarised in Table 2, ozonated water pretreatment consistently reduced the number of antibiotics to which isolates exhibited resistance, highlighting its potential role in the susceptibility of multidrug-resistant bacteria to conventional antibiotics.

**Table 2.** Susceptibility of the isolates to antibiotics before and after treatment with ozonated water

| Pathogen | Number of antibiotics to which the strain is resistant | |
|---|---|---|
| | before treatment with ozonated water | after treatment with ozonated water |
| *Acinetobacter baumannii* | 12.64 ± 3.69 | 8.86 ± 3.81** |
| *Enterobacter cloacae* | 9.50 ± 3.79 | 6.50 ± 3.37** |
| *Enterococcus* spp. | 6.17 ± 1.58 | 5.11 ± 2.01* |
| *Escherichia coli* | 5.76 ± 3.35 | 3.77 ± 1.89** |
| *Klebsiella pneumoniae* | 12.42 ± 2.41 | 10.14 ± 3.39* |
| *Proteus mirabilis* | 7.12 ± 2.13 | 5.57 ± 1.98* |
| *Pseudomonas aeruginosa* | 11.57 ± 3.48 | 9.36 ± 3.54** |
| *Staphylococcus aureus* | 10.71 ± 2.58 | 8.77 ± 2.64** |
| *Staphylococcus epidermidis* | 8.21 ± 1.42 | 5.93 ± 1.10** |

**Notes:** * – $p < 0.05$; ** – $p < 0.01$
**Source:** compiled by the authors

The extent of sublethal damage caused by ozonated water was quantified by comparing bacterial counts on selective and non-selective media for *S. aureus* and *E. coli*. For *S. aureus*, the initial concentration was approximately 8.9 log CFU/mL. After 10 minutes of treatment, there was no detectable reduction in blood agar, but a 1.04 log CFU/mL decrease was observed on yolk-salt agar, indicating that 90.97% of the surviving cells were sublethally injured. Following 15 minutes of treatment, a 1.65 log CFU/mL reduction was recorded on blood agar, and an additional 0.96 log CFU/mL reduction on yolk-salt agar was noted, corresponding to 89.19% sublethal injury among the treated population (Fig. 2).

For *E. coli*, the initial bacterial load was 8.97 log CFU/mL. After 10 minutes of ozonation, a reduction of 0.82 log CFU/mL on blood agar was observed, while a 1.59 log CFU/mL decrease was recorded on MacConkey agar, indicating that 97.44% of the population was sublethally damaged. With 15 minutes of treatment, the total reduction on blood agar reached 2.04 log CFU/mL, and the additional reduction on MacConkey agar was 1.77 log CFU/mL, representing 98.57% sublethally injured cells (Fig. 3).

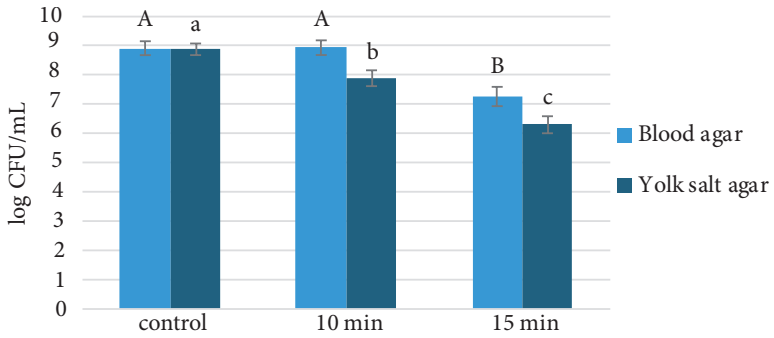


**Figure 2.** Effect of electrolytically generated aqueous ozone (~4 mg/L) on *S. aureus*
**Notes:** error bars indicate the standard deviation of the mean; letters above the bars denote a significant difference ($p < 0.05$)
**Source:** compiled by the authors

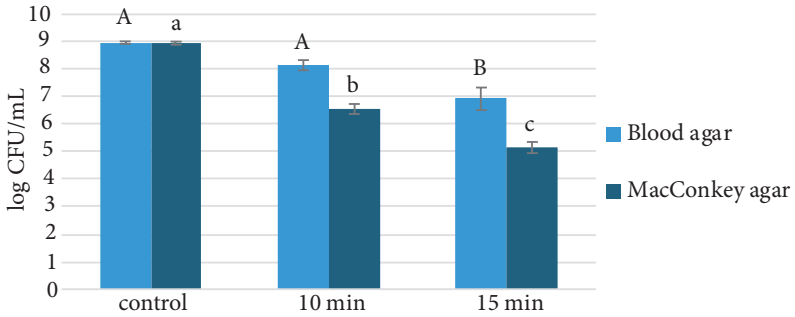


**Figure 3.** Effect of electrolytically generated aqueous ozone (~4 mg/L) on *E. coli*
**Notes:** error bars indicate the standard deviation of the mean; letters above the bars denote a significant difference ($p < 0.05$)
**Source:** compiled by the authors

The diversity of microbial isolates observed in this study is consistent with previous reports describing the microbiological landscape of combat-related injuries. I. Trutyak *et al.* [12] reported frequent isolation of *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and members of the *Enterobacteriaceae* family from mine blast and gunshot wounds, which aligns with the findings of the present study. G. Loban' *et al.* [13] highlighted the high prevalence of multidrug resistance among *A. baumannii* and *K. pneumoniae* isolates collected since the onset of the full-scale war in Ukraine, with resistance rates reaching 75%-80%. Likewise, K. Moussally *et al.* [14] documented substantial multidrug resistance in bacterial isolates from osteomyelitis cases in conflict zones, particularly among *S. aureus* and *P. aeruginosa*. The pathogen profile and resistance patterns observed in this cohort reflect broader trends in military medicine and underscore the ongoing challenge of managing infections in battlefield conditions. The high prevalence of multidrug-resistant pathogens necessitates the exploration of adjunctive antimicrobial strategies capable of enhancing bacterial susceptibility and disrupting resistance mechanisms.

The ozone concentrations obtained in this study (3.6-11.2 mg/L) are consistent with those reported in previous studies on electrolytic ozonation. Similar ranges (up to 5 mg/L) have been documented by E.I. Epelle *et al.* [15], where electrolytic ozonation was employed for microbial decontamination. However, some studies have reported higher ozone concentrations of 20-25 mg/L [16]. Differences in ozone yield may be attributed to variations in electrolysis parameters, electrode materials, water composition, and applied voltage. Compared with traditional ozonation systems, electrolytic ozonation offers the advantage of generating ozone on demand, without requiring a pure oxygen supply, making it a more cost-effective and scalable approach for antimicrobial applications [17].

Ozone is widely recognised for its antimicrobial properties, but its effects on bacterial physiology extend beyond direct inactivation. In this study, the authors observed that exposure to ozonated water not only reduced bacterial viability but also induced sublethal damage, as evidenced by differences in colony counts on selective and non-selective media. The observed differences in sublethal injury levels between Gram-positive and Gram-negative bacteria align with existing literature, suggesting that Gram-negative bacteria may be more susceptible to oxidative damage caused by ozone. This heightened vulnerability of Gram-negative bacteria may be attributed to the structural characteristics of their outer membrane, which contains lipopolysaccharides and unsaturated fatty acids that are particularly prone to oxidative attack. These results are consistent with earlier findings showing that reactive oxygen species, including ozone, cause significant damage to bacterial membranes, proteins, and DNA, ultimately leading to cell death or sublethal injury depending on the intensity and duration of exposure [18]. This suggests that, while some bacterial

cells survived ozone treatment, their cellular integrity and metabolic functions were compromised, making them more susceptible to additional stressors such as antibiotics. These findings support the idea that ozonated water could serve as an adjunct to conventional antimicrobial treatments, enhancing their efficacy against resistant pathogens.

Misidentification of microorganisms by the Vitek-2 system has been documented in several studies. S. Kim *et al.* [19] reported a case in which *Acinetobacter baumannii* was misidentified as *Alcaligenes faecalis*. Similarly, N. De Lappe *et al.* [20] described the misidentification of *Listeria monocytogenes*, while Z. Zong *et al.* [21] found that *Burkholderia pseudomallei* was incorrectly identified as *Burkholderia cepacia*. T.S. Park *et al.* [22] observed a misidentification of *Aeromonas veronii* biovar *sobria* as *Vibrio alginolyticus*, and T.K.F. Wang *et al.* [23] noted that a mucoid strain of *Salmonella enterica* serotype *Choleraesuis* was misidentified as *Hafnia alvei*. These examples illustrate the limitations of automated identification systems, particularly when dealing with atypical strains or those subjected to environmental stressors. In the present study, the misidentification of *Staphylococcus aureus* following treatment was likely influenced by oxidative stress caused by the combined presence of ozone and chlorine generated during the electrolysis of saline solution. While the specific contribution of each oxidant cannot be precisely determined, this outcome supports, at least partially, the hypothesis that oxidative treatments, including ozone exposure, may induce sublethal damage to bacterial cells. Such damage may interfere with biochemical identification systems and compromise diagnostic accuracy, highlighting the need for further investigation.

Although the observed increase in inhibition zone diameters and the reduction in the number of antibiotics to which the isolates appeared resistant suggest enhanced susceptibility, these changes should not be interpreted as a permanent reversal of antibiotic resistance. The effect is more accurately attributed to sublethal cellular damage induced by oxidative stress, which compromises bacterial defence mechanisms and temporarily sensitises the cells to antibiotics. This is consistent with previous findings in food microbiology, where reactive oxygen species such as ozone were shown to cause bacterial injury that is reversible under favourable conditions [24]. It is likely that, if the bacteria were provided with time and nutrients to recover, their original resistance profiles would re-emerge. Therefore, aqueous ozone should be viewed not as a standalone solution but as a temporary adjunct that enhances antibiotic efficacy during the acute phase of infection.

While this study demonstrates that pretreatment with aqueous ozone can enhance bacterial susceptibility to antibiotics through sublethal injury, recent findings also urge caution regarding the broader implications of sublethal ozonation. A study by C. Bai *et al.* [25] reported that sublethal concentrations of ozone (0-1.0 mg/L for 10 minutes) increased the frequency of conjugation and transformation among *E. coli* strains carrying resistance genes. These findings suggest that oxidative stress may promote horizontal gene transfer, potentially accelerating the spread of antibiotic resistance in aquatic environments. Although the ozone concentrations and experimental settings in this study differ, and the treatment was designed to support infection control in a clinical context, this evidence highlights the need for careful optimisation of ozone-based interventions. Ensuring adequate ozone dosing to achieve complete bacterial inactivation may be critical, particularly when applying ozone in environmental or water treatment settings, where sublethal exposure could have unintended ecological consequences.

## Conclusions

This study demonstrates the promising potential of aqueous ozone, generated via electrolysis, as an effective adjunct in combating multidrug-resistant pathogens isolated from combat-related wounds. Among the Gram-negative bacteria, the most frequently isolated pathogens were *Acinetobacter baumannii*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa*. For Gram-positive bacteria, *Staphylococcus aureus*, *Bacillus cereus*, and coagulase-negative *Staphylococcus* spp. predominated. All isolates exhibited multidrug resistance, displaying resistance to three or more classes of antibiotics, highlighting the urgency for alternative or adjunctive antimicrobial strategies. The results indicated that pretreatment with electrolytically generated ozonated water (~4 mg/L) induced significant sublethal injury in both Gram-positive and Gram-negative bacteria. Specifically, *S. aureus* exhibited approximately 89% sublethal injury after 15 minutes of exposure, while *E. coli* showed an even higher level of damage, with nearly 99% of cells sublethally injured. Notably, this treatment enhanced the antibiotic susceptibility of multidrug-resistant isolates, as evidenced by the consistent reduction in the number of antibiotics to which the bacteria appeared resistant, and by increased inhibition zones in Kirby-Bauer disc diffusion tests. These findings highlight the potential of aqueous ozone as a complementary approach that can temporarily sensitise resistant pathogens and improve the effectiveness of existing treatments. Importantly, electrolytic ozonation offers practical advantages: it is low-cost, generates ozone on demand without the need to handle gaseous ozone, and can be implemented in resource-limited or field settings. However, the study also underscores the need for careful optimisation, as oxidative treatments may have unintended effects, such as temporarily altering bacterial identification profiles. Future research should explore the long-term clinical outcomes of combined ozone-antibiotic therapies and establish standardised protocols for safe and effective use in both civilian and military healthcare contexts.

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**Conflict of Interest**
None.

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# Синергічний потенціал водного розчину озону: сублетальні пошкодження бактерій та підвищена чутливість до антибіотиків

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**Анотація.** Бойові поранення часто ускладнюються інфекціями, спричиненими мультирезистентними мікроорганізмами, що створює значні проблеми для лікування. Для покращення результатів у таких випадках необхідні альтернативні допоміжні стратегії лікування. Метою цього дослідження було оцінити антимікробний потенціал електролітично згенерованого водного розчину озону, зосереджуючись на його здатності викликати сублетальне пошкодження бактерій та підвищувати чутливість до антибіотиків. Загалом було отримано 357 мультирезистентних клінічних ізолятів з виділень з ран та зразків крові 284 поранених військових. Найчастіше виділялися такі збудники, як *Acinetobacter baumannii*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Bacillus cereus* та коагулазонегативні *Staphylococcus* spp. Озоновану воду було згенеровано в концентраціях від 3,6 до 11,2 мг/л, потім стандартизувано до ~4 мг/л для бактеріальної обробки. Відібрані ізоляти *S. aureus* та *Escherichia coli* були піддані впливу водного розчину озону протягом 10 і 15 хвилин. Сублетальні пошкодження оцінювались шляхом порівняння росту бактерій на селективних та неселективних середовищах, виявивши до 89,2 % сублетально пошкоджених клітин *S. aureus* та до 98,6 % пошкоджених клітин *E. coli* через 15 хвилин. Попередні оцінки мінімальної інгібуючої концентрації з використанням системи Vitek-2 були спотворені комбінованим впливом озону та вільного хлору, що утворюється під час електролізу сольового розчину, що спонукало до переходу на диско-дифузійний тест Кірбі-Бауера. Результати послідовно показували підвищену чутливість до антибіотиків у оброблених ізолятах, про що свідчить збільшення діаметрів зон інгібування та зменшення кількості антибіотиків, до яких ізоляти залишалися стійкими. Електролітично згенерований водний розчин озону ефективно порушує цілісність бактерій, підвищуючи їхню чутливість до антибіотиків. Він пропонує перспективну додаткову стратегію для лікування мультирезистентних інфекцій, особливо в умовах обмежених ресурсів або у воєнний час

**Ключові слова:** антибактеріальна активність; стійкість до антибіотиків; озонована вода; патогенні бактерії; сублетальні пошкодження