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Antibacterial Activity of Hypertonic Sodium Chloride Solutions Against *Escherichia coli*: An In Vitro Study

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Abstract

Chronic wounds are frequently colonized by bacteria, including *Escherichia coli*, and hypertonic sodium chloride has been proposed as a topical antibacterial agent. This study aimed to evaluate the in vitro antibacterial activity of 7%, 10%, and 13% sodium chloride solutions against *E. coli*. Activity was assessed by agar well diffusion on Mueller–Hinton agar, with sterile distilled water as the negative control and an amoxicillin disc as the positive control (three replicates per group). The net inhibition zone was calculated as the mean of two diameter readings minus the well or disc diameter. Groups were compared by one-way analysis of variance (ANOVA) with Tukey's post hoc test, and the Kruskal–Wallis test with Dunn's test served as a sensitivity analysis. The 7%, 10%, and 13% sodium chloride solutions produced mean net zones of 3.86 ± 2.08, 5.68 ± 1.54, and 4.19 ± 1.35 mm, respectively, compared with 0.00 mm for distilled water and 26.20 ± 1.78 mm for amoxicillin (ANOVA, $p < 0.001$). The 10% and 13% solutions differed from the negative control ($p = 0.007$ and $p = 0.046$), whereas the 7% solution did not ($p = 0.068$), and the three concentrations did not differ from one another. In the sensitivity analysis, no sodium chloride solution differed from the negative control. Under these conditions, hypertonic sodium chloride showed at most weak inhibition of *E. coli*, and its value as an antibacterial agent for chronic wounds remains unproven.

Keywords: agar well diffusion; antibacterial activity; *Escherichia coli*; hypertonic solution; sodium chloride.

Abbreviations: ANOVA, analysis of variance; CI, confidence interval; HSD, honestly significant difference; MHA, Mueller–Hinton agar; Sodium Chloride, sodium chloride; SD, standard deviation.

INTRODUCTION

Chronic wounds fail to heal within an expected time frame and have become an increasingly important clinical problem, owing to their rising incidence and the associated morbidity and socio-economic burden (Falanga et al. 2022). A meta-analysis of observational studies estimated a pooled prevalence of 2.21 per 1000 population for chronic wounds of mixed etiologies and 1.51 per 1000 population for chronic leg ulcers (Martinengo et al. 2018).

A breach in the skin barrier exposes the underlying tissue to microbial contamination, which can lead to infection, delayed healing, and further tissue loss, and the wound microbiota can influence each stage of repair (Uberoi et al. 2024). Interactions between biofilms and the innate immune system have also been implicated in the formation of chronic wounds (Versey et al. 2021).

Gram-negative bacteria are prominent in infected chronic ulcers. In 428 patients with diabetic foot ulcers in Southwest China, specimens from chronic ulcer wounds were primarily Gram-negative bacilli (54.2%) (Wu et al. 2018). A later cross-sectional analysis of 484 patients with diabetic foot infections identified *Escherichia coli* as the most prevalent Gram-negative species (9.88% of 445 pathogens) and reported ceftriaxone resistance of 46.51% among Gram-negative isolates (Zhang et al. 2026). In a genomic analysis, approximately 78% of diabetic foot-associated *E. coli* isolates were multidrug-resistant or extensively drug-resistant (Ajumobi *et al.*, 2026).

At the global level, *E. coli* was the leading pathogen for deaths associated with antimicrobial resistance in 2019 (Antimicrobial Resistance Collaborators 2022). Resistance to aminopenicillins is also common in cultures from chronic wounds: among diabetic ulcer cultures from patients undergoing major limb amputation in the Solomon Islands, 55% showed antibiotic resistance, most frequently to ampicillin and amoxicillin (Anuszewski et al. 2025).

These trends have renewed interest in simple, low-cost topical agents that do not rely on antibiotics. Hypertonic saline dressings for wounds were described in a 1992 report (Mangete et al. 1992), and sodium

chloride has been proposed to act on bacteria through osmotic stress (al-Khikani 2025). However, the evidence on its direct antibacterial effect against *E. coli* is inconsistent. Sodium chloride alone at concentrations of up to 36% (wt/wt) for 5 minutes did not reduce populations of *E. coli* O157:H7 (Kim and Rhee 2015), and adding 5% Sodium Chloride to 7.5% povidone iodine produced only a modest, non-significant increase in inhibition zones against wound isolates of *E. coli* and *Staphylococcus aureus* in an agar well diffusion assay (al-Khikani 2025). Therefore, this study aimed to evaluate the in vitro antibacterial activity of 7%, 10%, and 13% sodium chloride solutions against *E. coli* using the agar well diffusion method, with sterile distilled water and an amoxicillin disc as negative and positive controls, respectively.

MATERIALS AND METHODS

Study design and materials

This in vitro experimental study assessed the antibacterial activity of hypertonic sodium chloride solutions against *Escherichia coli* using the agar well diffusion method. The test organism was *E. coli* FNCC 0091, obtained from the Food and Nutrition Culture Collection (FNCC), Center for Food and Nutrition Studies, Universitas Gadjah Mada, Yogyakarta, Indonesia, and tested on Mueller–Hinton agar (MHA). The experiments were conducted at the Biomedical Laboratory, Universitas Respati Yogyakarta. Sodium chloride solutions were prepared at concentrations of 7%, 10%, and 13%. Sterile distilled water served as the negative control, and an amoxicillin disc (Oxoid, catalog code CT0061B) served as the positive control. The equipment comprised an autoclave, an incubator, a laminar air flow cabinet, micropipettes, Petri dishes, and a digital caliper. Each treatment was tested in three wells (n = 3 per group).

Procedures

Preparation

All equipment was sterilized by autoclaving. MHA was prepared and poured into Petri dishes. The *E. coli* culture was rejuvenated on agar to ensure that the bacteria were actively growing before testing.

Inoculation and agar well diffusion

The bacterial suspension was adjusted to the turbidity of a 0.5 McFarland standard and spread evenly over the MHA surface using a sterile swab. Wells (6 mm in diameter) were punched into the inoculated agar with a sterile tool and filled with of 7%, 10%, or 13% Sodium Chloride solution or sterile distilled water. For the positive control, an amoxicillin disc was placed on the inoculated agar surface. The plates were incubated at 37 °C for 18–24 h.

Measurement of inhibition zones

After incubation, the diameter of the clear zone formed around each well, including the well itself, was measured twice on the same well using a calibrated digital caliper (D_1 and D_2, in mm). The diameter of the well (6.0 mm) or of the amoxicillin disc (6.5 mm) was recorded as D_3. The net inhibition zone of each well was calculated as:

$$Net\ inhibition\ zone\ (mm) = (D1 + D2) / 2 - D3$$

A value of 0 mm therefore indicates no inhibition beyond the well or disc itself.

Data analysis

The net inhibition zone of each well was the unit of analysis (n = 3 per group). Data are presented as mean ± standard deviation (SD). Statistical analyses were performed using SPSS. Normality of the model residuals was assessed using the Shapiro–Wilk test and homogeneity of variances using Levene's test. As both assumptions were met, differences among the five groups (7%, 10%, and 13% sodium chloride, negative control, and positive control) were analyzed by one-way analysis of variance (ANOVA), followed by Tukey's honestly significant difference (HSD) test for pairwise comparisons. As a sensitivity analysis given the small sample size, the Kruskal–Wallis test followed by Dunn's test with Bonferroni correction was also applied. Statistical significance was set at $p < 0.05$.

RESULTS AND DISCUSSION

Antibacterial activity of Sodium Chloride solutions

The net inhibition zones against *E. coli* are presented in Table 1 and Figure 1. The 7%, 10%, and 13% sodium chloride solutions produced mean net zones of 3.86 ± 2.08, 5.68 ± 1.54, and 4.19 ± 1.35 mm, respectively. No inhibition zone formed around the negative control wells (0.00 mm), whereas amoxicillin produced a mean net zone of 26.20 ± 1.78 mm. Among the sodium chloride solutions, the largest mean zone was observed at 10%, and zone size did not increase with concentration.

Table 1. Net inhibition zone (mm) of sodium chloride solutions and controls against *Escherichia coli*.

Group	Well 1	Well 2	Well 3	Mean ± SD
7% sodium chloride	1.61	4.26	5.72	3.86 ± 2.08
10% sodium chloride	4.79	7.46	4.80	5.68 ± 1.54
13% sodium chloride	3.71	5.72	3.14	4.19 ± 1.35
Negative control (distilled water)	0.00	0.00	0.00	0.00 ± 0.00
Positive control (amoxicillin disc)	28.24	24.92	25.45	26.20 ± 1.78

Note: Net inhibition zone = $(D_1 + D_2) / 2 - D_3$, where D_3 is the well diameter (disc diameter for the positive control); n = 3 per group.

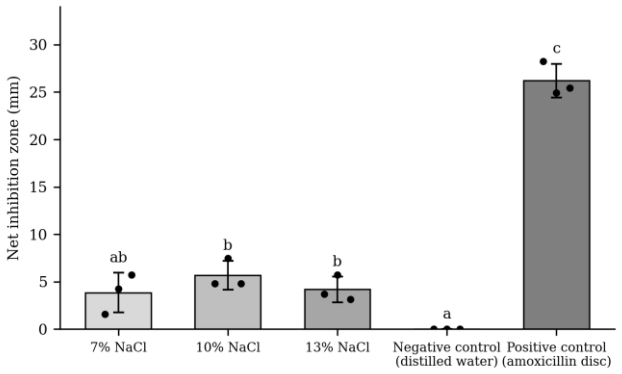


Figure 1. Mean net inhibition zone (bars, mean ± SD) and individual wells (dots) of Sodium Chloride solutions and controls against *E. coli* (n = 3 per group). Different letters indicate significant differences (Tukey's HSD, $p < 0.05$).

Comparison among groups

One-way ANOVA indicated a significant difference among the five groups ($F(4, 10) = 138.39$, $p < 0.001$). The model residuals were normally distributed (Shapiro–Wilk $W = 0.929$, $p = 0.265$) and the variances were homogeneous (Levene's test, $p = 0.086$). Tukey's HSD showed that amoxicillin differed from all other groups ($p < 0.001$; Table 2). Compared with the negative control, the 10% and 13% Sodium Chloride solutions produced larger zones (mean difference 5.68 mm, 95% CI 1.57 to 9.79, $p = 0.007$; and 4.19 mm, 95% CI 0.08 to 8.30, $p = 0.046$, respectively), whereas the 7% solution did not (3.86 mm, 95% CI −0.25 to 7.97, $p = 0.068$). The three sodium chloride concentrations did not differ from one another ($p \geq 0.609$).

In the sensitivity analysis, the Kruskal–Wallis test also indicated a difference among groups ($H(4) = 11.53$, $p = 0.021$). In Dunn's pairwise comparisons with Bonferroni correction, only amoxicillin differed from the negative control (adjusted $p = 0.010$); none of the sodium chloride solutions differed from the negative control (adjusted $p \geq 0.349$).

Table 2. Tukey's HSD pairwise comparisons of net inhibition zone (mm).

Comparison	Mean difference	95% CI	Adjusted p
7% sodium chloride vs. 10% sodium chloride	−1.82	−5.93 to 2.29	0.609
7% sodium chloride vs. 13% sodium chloride	−0.33	−4.44 to 3.78	0.999
7% sodium chloride vs. negative control	3.86	−0.25 to 7.97	0.068
7% sodium chloride vs. positive control	−22.34	−26.45 to −18.23	<0.001
10% sodium chloride vs. 13% sodium chloride	1.49	−2.62 to 5.61	0.754
10% sodium chloride vs. negative control	5.68	1.57 to 9.79	0.007
10% sodium chloride vs. positive control	−20.52	−24.63 to −16.41	<0.001
13% sodium chloride vs. negative control	4.19	0.08 to 8.30	0.046
13% sodium chloride vs. positive control	−22.01	−26.13 to −17.90	<0.001
Negative control vs. positive control	−26.20	−30.31 to −22.09	<0.001

Note: Mean difference = first group − second group; CI, confidence interval; n = 3 wells per group.

Discussion

The 7%, 10%, and 13% sodium chloride solutions produced only small net inhibition zones against *E. coli*, and the evidence for an effect depended on the analysis. Relative to sterile distilled water, which formed no zone, the 10% and 13% solutions differed in Tukey's test and the 7% solution did not, whereas none of the three differed after Dunn's test with Bonferroni correction. Zone size did not rise with concentration, so no dose–response relationship was observed. With three wells per group, these results are best read as a weak and uncertain signal rather than confirmed antibacterial activity.

This pattern agrees with earlier reports that sodium chloride alone has a limited direct effect on *E. coli*. Sodium chloride at up to 36% (wt/wt) for 5 min did not reduce populations of *E. coli* O157:H7 (Kim and Rhee 2015), and adding 5% Sodium Chloride to povidone iodine only modestly and non-significantly enlarged inhibition zones against wound isolates (al-Khikani 2025). These studies differ from ours in strain, exposure time, and test format, so the comparison is qualitative.

A plausible explanation lies in the osmoregulatory capacity of *E. coli*. Cells control their hydration by accumulating solutes at high osmolality, and *E. coli* is a

paradigm for studies of this process (Romantsov et al. 2009); Shiga toxin-producing *E. coli* has also been reported to survive hyperosmotic conditions (Dos Santos Rosario et al. 2020). Osmotic adaptation could blunt the effect of hypertonic sodium chloride and confine it to bacteria closest to the well, where the salt concentration is highest. In addition, salt may diffuse readily through agar and flatten the concentration gradient around the well, which could explain why 13% sodium chloride did not outperform 10%. Both explanations are hypotheses that this experiment cannot test.

The amoxicillin disc produced a large net zone, indicating that the strain was susceptible and the diffusion assay was functional. However, the disc and the wells deliver the agent differently, so the zone of the positive control should not be compared quantitatively with those of the sodium chloride solutions. Agar diffusion is a widely used screening bioassay with recognized advantages and limitations (Balouiri et al. 2016), and zone size reflects the diffusion of the agent as well as its antibacterial potency.

These findings do not support hypertonic sodium chloride as a stand-alone antibacterial agent against *E. coli* in chronic wounds, where biofilm formation and the wound microbiota complicate treatment (Versey et al. 2021; Uberoi et al. 2024). Any benefit of hypertonic saline dressings seen in practice cannot be inferred from this agar-based assay, which does not model biofilm or wound exudate.

This study has several limitations. Each group comprised three wells, so statistical power was low and estimates are imprecise. Only one reference strain was tested, and clinical or multidrug-resistant isolates may respond differently. Minimum inhibitory or bactericidal concentrations, time–kill kinetics, biofilm models, and cytotoxicity were not assessed. Future work should include broth microdilution, time–kill assays, biofilm models, several clinical isolates, and cytotoxicity testing on fibroblasts. Under the conditions tested, 7–13% sodium chloride solutions showed at most weak inhibition of *E. coli*, and their value as wound antibacterial agents remains unproven.

CONCLUSIONS

Under the conditions tested, 7%, 10%, and 13% sodium chloride solutions produced small net inhibition zones against *E. coli* (3.86–5.68 mm) with no dose–response relationship, and the difference from sterile distilled water was inconsistent across the analyses. The amoxicillin disc confirmed that the strain was susceptible and the assay was functional. These findings do not support hypertonic sodium chloride as a stand-alone antibacterial agent against *E. coli*. Studies with more replicates, determination of minimum inhibitory and bactericidal concentrations, biofilm models, and clinical isolates are needed before any wound-care application is considered.

Authors' Contributions: Januar Rizqi led the study, coordinated the team, obtained the research permits, supervised the laboratory work, performed the in vitro assays, analyzed the data, and wrote the manuscript. Adi Sucipto performed the in vitro assays, assisted with data analysis, and contributed to the manuscript. Puspita Mardika Sari performed the in vitro assays, analyzed the experimental results, and contributed to the manuscript. Fransiska Lanni strengthened the theoretical framework and contributed to the analysis and the manuscript. Rido Muhajir prepared the materials and equipment and assisted with the in vitro assays. Samuel Juanli assisted with material preparation and the laboratory experiments and documented the research. All authors read and approved the final manuscript.

Competing Interests: The authors declare that there are no competing interests.

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