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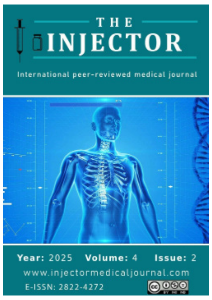

Original Article

The disinfectant effect of sodium hypochlorite on bacteria causing nosocomial infections

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Abstract

Objective: Antiseptics and disinfectants are widely used to prevent and control nosocomial infections (NIs). As with antibiotics, the increasing use of these agents has raised concerns regarding the potential development of resistance to them. In this study, the efficacy of sodium hypochlorite (NaClO) against common NI agents was investigated.

Methods: This study examined bacteria isolated from various clinical specimens obtained from patients hospitalised in the intensive care unit at a microbiology laboratory. The isolate group consisted of two methicillin-sensitive *Staphylococcus aureus* (MSSA), five methicillin-resistant *Staphylococcus aureus* (MRSA), seven *Klebsiella pneumoniae*, two *Escherichia coli*, ten *Pseudomonas aeruginosa,* and eight *Acinetobacter baumannii*. The effectiveness of 1/10, 1/100, and 1/1000 dilutions of NaClO was tested at contact times of 2.5, 5, and 10 minutes against these bacterial isolates. *Staphylococcus aureus* ATCC 25923, *Pseudomonas aeruginosa* ATCC 27853, and *Escherichia coli* ATCC 25922 were used as quality control strains.

Results: The study revealed that 10% NaClO demonstrated a bactericidal effect against all isolates and standard strains when diluted 1/10 at all contact times. As the dilution rate increased, longer contact times were required for the solution to achieve bactericidal activity against an *Acinetobacter baumannii* isolate. However, one *Acinetobacter baumannii* isolate remained resistant to a 1:1000 dilution of NaClO after 10 minutes of contact. Although a slight statistical difference was observed between contact times at higher NaClO dilution rates for *A. baumannii* ($p=0.06$), this difference was not statistically significant ($p>0.05$).

Conclusion: When diluted 1:100, 10% NaClO was found to be effective against common nosocomial pathogens. Microorganism growth was detected at lower NaClO concentrations.

Keywords: Disinfection, disinfectant resistance, nosocomial infection, sodium hypochlorite.

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INTRODUCTION

Nosocomial infections (NIs), also known as hospital-acquired infections (HAIs), represent one of the most significant challenges in modern healthcare. These infections are defined as those that were not present at the time of admission to a healthcare facility but developed during the patient's hospitalization. HAIs increase morbidity and mortality through prolonged hospital stays and impose substantial costs on healthcare providers. Typically, these infections develop within 48–72 hours of admission or within 10 days of discharge (1-3). Although prevalence rates vary according to institution and region, the incidence of HAIs in Turkey ranges from 1% to 16.5% (2). HAIs may originate from either endogenous or exogenous sources. In exogenous HAIs, the causative microorganism is frequently transmitted via cross-contamination from an external source; therefore, decontamination, disinfection, antisepsis, and sterilisation procedures form the foundation of infection control programmes (1-3). Antiseptics and disinfectants are widely used to prevent and control HAIs, particularly given the challenges associated with treatment. The most commonly employed compounds for environmental disinfection include sodium hypochlorite (NaClO), phenol, quaternary ammonium compounds (QACs), and alcohol, or combinations thereof. NaClO exhibits a wide range of antimicrobial activities, including bactericidal, sporicidal, virucidal, and fungicidal properties. It is one of the most commonly used disinfectants owing to its broad-spectrum activity, low cost, ease of application, and non-flammable nature (1-3). Antibiotic resistance has become a significant concern in HAIs. Similar to antibiotics, the increasing use of disinfectants and antiseptics raises concerns regarding the potential emergence of resistance. Extensive application of these agents has been implicated in promoting antimicrobial resistance, thereby contributing to significant ecological and biosafety challenges worldwide (4-6). A growing body of literature emphasizes the role of chlorine-based disinfectants in selecting disinfectant-tolerant strains (5-7). Of particular concern are Gram-negative bacteria such as *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Klebsiella* spp., which are multidrug-resistant (MDR) and commonly found in healthcare environments (4-11). Environmental disinfection has become increasingly important, particularly given that bacteria such as *Acinetobacter* spp., methicillin-resistant *Staphylococcus aureus* (MRSA), *Clostridium difficile*, and vancomycin-resistant *Enterococci* can survive on inanimate surfaces for extended periods, with contaminated environments serving as reservoirs for transmission (2,3,8,9). Although resistance to disinfectants is not currently considered a significant problem, concerns have emerged due to the increasing prevalence of infections caused by MDR bacteria in intensive care units, coupled with high antimicrobial resistance rates (3,9). Difficulties in controlling these resistant organisms within the hospital environment may directly contribute to the incidence of HAIs (1,7,9,11). Given these considerations, periodic evaluation of the efficacy of antiseptics and disinfectants employed in infection-control programs is essential to ensure sustained protection against HAIs (1-3,9-11). Therefore, just as monitoring antimicrobial resistance is mandatory, particularly for infections in intensive care settings, evaluating the effectiveness of commonly used antiseptics and disinfectants against pathogens found in these environments is equally critical.

This study aimed to investigate the effectiveness and duration of action of NaClO, a disinfectant commonly used in general hospital areas, against 34 resistant bacterial species isolated from HAIs in our hospital.

MATERIALS AND METHODS

The study was conducted retrospectively using nosocomial strains isolated from clinical specimens of patients hospitalised in the ICU. A total of 34 clinical isolates were included in the study. The distribution of the strains included two methicillin-sensitive *Staphylococcus aureus* (MSSA) strains, five MRSA strains, seven *Klebsiella pneumoniae* (*K. pneumoniae*) strains, two *Escherichia coli* (*E. coli*) strains, ten *Pseudomonas aeruginosa* (*P. aeruginosa*) strains, and eight *Acinetobacter baumannii* (*A. baumannii*) strains. The bacteria were identified using conventional methods and the API (Biomerieux, France) systems for Gram-negative and Gram-positive bacteria. Three standard American Type Culture Collection (ATCC) strains were used as quality control: *S. aureus* ATCC 25923, *P. aeruginosa* ATCC 27853, and *E. coli* ATCC 25922. The qualitative suspension method was used to investigate the time-dependent effects of 10% NaClO (HYPNOS, Turkey) on test bacteria and standard strains (12-13). Fresh cultures of all bacteria (18–24 hours old) on Tryptic Soy Agar (TSA) (BBL, Becton Dickinson, USA) were inoculated into Tryptic Soy Broth (TSB) (BBL, Becton Dickinson, USA) and incubated at 37 °C for 24 hours. The 24-hour-old cultures, which had been grown in liquid media, were resuspended in sterile broth. Microbial suspensions with a turbidity equivalent to 0.5 McFarland were obtained. Dilutions of NaClO were prepared using distilled water at ratios of 1:10, 1:100, and 1:1000. Initially, 5 mL of each disinfectant solution was transferred to the tubes. Then 0.2 mL of the microorganism suspension was added. The bacterial-disinfectant mixtures were maintained at room temperature for contact times of 2.5, 5, and 10 minutes. At the end of these periods, 0.5% sodium thiosulfate (Merck, Germany) was added to the mixture to neutralise the disinfectant effect. The inoculated tubes were incubated at 37°C for 48 hours. Following incubation, a sample from each tube was transferred to Petri dishes containing tryptic soy agar. All inoculations were performed using a standard 4 mm diameter inoculation loop. The dilution ratios and contact times associated with bacterial growth in the 48-hour cultures at 37 °C were recorded. Cultures from the grown microorganisms were examined by Gram staining and identified. Once it was confirmed that they were the same microorganism, the growth of bacteria at these dilution rates and contact times with the disinfectant was considered positive.

Statistical analysis

All statistical analyses were performed using IBM SPSS Statistics version 22.0 (IBM Corp., Armonk, NY, USA), with $p<0.05$ considered statistically significant. Descriptive statistics included frequency and percentage distributions. The associations between categorical variables were assessed using Fisher's exact test. Differences between species were compared using the chi-squared test, while pairwise differences were explored using Fisher's exact test with Bonferroni correction. Differences in dilution and contact times for each species were assessed using Kruskal–Wallis tests. Gram-negative species were compared using the Kruskal–Wallis test, and Gram-positive versus Gram-negative groups were compared using the Mann–Whitney U test. One-way analysis of variance (ANOVA) was used to evaluate the effectiveness of NaClO among the three main bacterial groups included in the study. Statistical significance was set at $p < 0.05$ for all analyses.

RESULTS

The study utilized a total of 34 patient isolates and three ATCC strains. Table 1 shows the distribution of the clinical isolates and standard strains used. NaClO demonstrated bactericidal activity against all isolates and standard strains at 1/10 dilution at all contact times. One *A. baumannii* isolate was not eliminated by 1/100 dilution after 5 minutes of contact, while it was effectively killed at 10 minutes of contact. At a dilution ratio of 1/1000, the solution exhibited bactericidal activity against one MRSA isolate after 5 minutes, and against one *K. pneumoniae* and one *A. baumannii* isolate after 10 minutes. However, one *A. baumannii* isolate remained viable at a dilution ratio of 1/1000 despite a 10-minute contact time. Table 2 presents the efficacy of NaClO against clinical isolates and standard strains, as determined by dilution ratios and contact times.

Table 1. The distribution of the clinical isolates and standard strains used.

Bacteria	Strains n (%)	ATCC strains	Number of ATCC
Gram-positive strains			
MSSA[a]	2 (5.88)	*S. aureus* ATCC 25923	1
MRSA[b]	5 (14.7)	*E. coli* ATCC 25922	1
Fermentative Gram-negative strains			
Klebsiella pneumoniae	7 (20.6)	*P. aeruginosa* ATCC 27853	1
Escherichia coli	2 (5.88)		
Non-fermentative Gram-negative strains			
Pseudomonas aeruginosa	10 (29.4)		
Acinetobacter baumannii	8 (23.5)		
Total strains	**34 (100)**	**Total ATCC**	**3**

Abbreviations: ATCC: American Type Culture Collection, MSSA[a]: Methicillin-sensitive *Staphylococcus aureus,* MRSA[b]: Methicillin-resistant *Staphylococcus aureus.*

Table 2. The efficacy of NaClO against clinical isolates and standard strains, depending on dilution rate and contact time

	The dilution rate for 10% NaClO								
	1/10			**1/100**			**1/1000**		
	Contact times (min) n (%)			**Contact times (min) n (%)**			**Contact times (min) n (%)**		
Strains n (%)	**2.5**	**5**	**10**	**2.5**	**5**	**10**	**2.5**	**5**	**10**
Gram-positive strains									
MSSA[1] n: 2 (5.88)	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
MRSA[2] n: 5 (14.7)	**0**	**0**	**0**	**0**	**0**	**0**	**1 (2.9)**	**0**	**0**
Fermentative Gram-negative strains									
***Klebsiella pneumoniae* n:7 (20.6)**	**0**	**0**	**0**	**1 (2.9)**	**0**	**0**	**2 (5.9)**	**1 (2.9)**	**0**
***Escherichia coli* n: 2 (5.88)**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
Non-fermentative Gram-negative strains									
***Pseudomonas aeruginosa* n: 10 (29.4)**	**0**	**0**	**0**	**1 (2.9)**	**0**	**0**	**1 (2.9)**	**0**	**0**
***Acinetobacter baumannii* n:8 (23.5)**	**0**	**0**	**0**	**1 (2.9)**	**1 (2.9)**	**0**	**2 (5.9)**	**1 (2.3)**	**1 (2.9)**
Total strains n: 34 (100)	**0**	**0**	**0**	**3 (8.8)**	**1 (2.9)**	**0**	**6 (17.6)**	**2 (5.9)**	**1 (2.9)**
Quality Control Strains									
***Staphylococcus aureus* ATCC 25923**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
***Escherichia coli* ATCC 25922**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
***Pseudomonas aeruginosa* ATCC 27853**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**

Abbreviations: The number of isolates remaining viable after exposure to the disinfectant is indicated in the relevant boxes. ATCC: American Type Culture Collection, MSSA[1]: Methicillin-sensitive *Staphylococcus aureus,* MRSA[2]**:** Methicillin-resistant *Staphylococcus aureus.*

Table 3. Statistical comparison of the effectiveness of sodium hypochlorite dilutions and contact times against healthcare-associated bacteria

Comparison	Species/Group	Statistical test	p-Value	Interpretation
MSSA vs MRSA. All dilutions (1/10, 1/100, 1/1000) and contact times (2.5, 5, 10 min)	Between MSSA and MRSA	Fisher's exact test after Bonferroni correction	1.00	No statistically significant difference
GP bacteria versus GN bacteria for all dilution rates and contact times	MSSA+MRSA vs Gram-negative (all four GN species)	Mann–Whitney U	0.074	Gram-negative isolates showed slightly higher resistance than the Gram-positive isolates, but this difference was not statistically significant.
Fermenters GN vs non-fermenters GN bacteria for all dilution rates and contact times	*E. coli, K. pneumoniae* versus *P. aeruginosa, A. baumannii.*	Mann–Whitney U Kruskal–Wallis, Fisher's exact tests after Bonferroni correction	0.1757 Ns	No statistically significant difference
Overall comparison among the three bacterial groups	Gram-positive, fermentative GN, non-fermentative GN	One-way ANOVA	0.071	No statistically significant difference
Among GN species for all dilution rates and contact times	*K. pneumoniae*, *E. coli*, *P. aeruginosa*, *A. baumannii*	Kruskal–Wallis	0.060	No statistically significant difference. Possible species-specific variation, mainly driven by *A. baumannii.*
Effect of dilution and contact times on bactericidal activity	MSSA	Kruskal–Wallis	> 0.10	No statistically significant difference
	MRSA	Kruskal–Wallis	> 0.10	No statistically significant difference
	K. pneumoniae	Kruskal–Wallis	0.24	No statistically significant difference
	E. coli	Kruskal–Wallis	-	No statistically significant difference
	P. aeruginosa	Kruskal–Wallis	0.10	No statistically significant difference
	A. baumannii	Kruskal–Wallis	0.06	Borderline significant effect of dilution on survival.
A. baumannii (1/10 vs 1/100 for 2.5 min.)		Fisher exact Mann–Whitney U	0.07 0.06	Higher NaClO concentration reduced survival (borderline)
P. aeruginosa (1/100 vs 1/1000 for 5 min.)		Fisher exact	0.082	Possible concentration-related effect, not significant

Abbreviations: MSSA: Methicillin-sensitive *Staphylococcus aureus,* MRSA**:** Methicillin-resistant *Staphylococcus aureus,* GP: Gram positive, GN: Gram negative, Ns: No significant.

The 10% NaClO solution was found to be effective against most of the bacteria tested at dilutions of 1/10, 1/100, and 1/1000 after a 2.5-minute contact time—only one *Acinetobacter* spp. The isolate was found to be resistant to the 1/1000 dilution of NaClO after 10 minutes of contact time. No significant differences were observed among contact times (p>0.05). *A. baumannii* showed a near-significant variation across NaClO dilutions (p=0.06). Among Gram-negative species, overall differences approached significance (p=0.06). The Gram-negative group tended to show slightly higher survival than the Gram-positive isolates (p = 0.07). A one-way ANOVA (analysis of variance) was conducted to assess the efficacy of NaClO among the three bacterial groups included in the study. No statistically significant difference was found (p> 0.05) (Table 3).

DISCUSSION

NaClO is a potent oxidising agent with broad-spectrum antimicrobial action and is classified as a low- or medium-level biocidal agent depending on the concentration. It has been used for the disinfection of equipment, surfaces, and laundry, as well as for treating drinking water. Despite its broad-spectrum and bactericidal activity, bacterial resistance to chlorine-releasing compounds has been reported (1,10-11,14). In our study, the efficacy of NaClO, which is widely used in the community and in our hospital, was investigated. Various studies have tested different stock concentrations of NaClO and contact times. In our study, the efficacy of 10% NaClO was analyzed using a total of 34 isolates of various Gram-positive and Gram-negative bacteria at 1/10, 1/100, and 1/1000 dilutions after contact times of 2.5, 5, and 10 minutes.

Our findings indicate that NaClO was effective against all *E. coli* and MSSA isolates at all concentrations and contact times. A 1/10 dilution with a 2.5-minute exposure was sufficient to achieve bactericidal activity for all species. Lower dilutions (1/100 and 1/1000) were also effective against resistant Gram-negative strains (*K. pneumoniae*, *P. aeruginosa*, *A. baumannii*) with longer contact times. Although not statistically significant, Gram-negative bacteria showed a trend toward greater resistance to NaClO, particularly *A. baumannii*. These findings suggest the existence of possible species-specific tolerance mechanisms. Given the limited sample size, the results should be interpreted cautiously; however, they support the general effectiveness of NaClO as a disinfectant. Fabrizio et al. identified a significant number of antimicrobial resistance genes in carbapenem-resistant *K. pneumoniae* and multidrug-resistant *A. baumannii* isolates using whole-genome sequencing methods. However, they found fewer disinfectant resistance genes in the resistant Gram-negative bacteria group compared to MRSA and MSSA. Additionally, they discovered that NaClO exhibited significant antimicrobial activity against both planktonic cells and biofilms. Their findings highlighted the potential of NaClO as a powerful antimicrobial agent against difficult-to-treat biofilm infections at concentrations lower than those found in commercial disinfectants (15). In one study, Rostamani and colleagues reported that 5% NaClO was the most effective disinfectant against multidrug-resistant (MDR) and extensively drug-resistant (XDR) *A. baumannii* isolates, which have a high capacity for biofilm production. They found that 91.3% of all isolates formed biofilms and reported the minimum bactericidal concentration of NaClO as a 1/16 dilution (16). Another study conducted on clinical isolates of MDR and XDR *P. aeruginosa* found that 97.5% of strains produced biofilms. According to the results of the disinfectant susceptibility test, NaClO was reported to have a minimum bactericidal concentration of a 1/32 dilution, making it the most effective disinfectant (17). Yiğit et al. reported that 0.08% NaClO eliminated ATCC strains of *S. aureus*, *S. epidermidis*, and *E. coli* within two minutes, whereas *P. aeruginosa* required five minutes of exposure. In our study, 1/100 and 1/1000 dilutions of 10% NaClO similarly inhibited *P. aeruginosa* after five minutes. Notably, the inclusion of resistant clinical isolates from ICU patients, in addition to ATCC reference strains, allowed a more clinically relevant assessment of NaClO efficacy (18). Köhler et al. (19) investigated the disinfectant efficacy of NaClO separately in the presence and absence of organic matter by using both qualitative and quantitative suspension methods. Similar to our study, they examined MDR *P. aeruginosa*, *Acinetobacter* spp., and *Klebsiella* spp. Unlike their study, which included contact times up to 30 minutes, our investigation was conducted only under clean conditions using a qualitative suspension method, without assessing the 30-minute exposure. They observed reduced susceptibility in *P. aeruginosa* during qualitative tests, but no significant differences were found in quantitative assays. They concluded that NaClO remained effective against all tested species, particularly in the absence of organic matter, with no overall increase in tolerance among MDR-GNB (19). In contrast, one *A. baumannii* strain in our study showed resistance to a 1/1000 NaClO dilution after 10 minutes of contact. However, the absence of quantitative testing limited direct comparison with the findings of Köhler et al. (19). Avcı et al. reported that they detected a bactericidal effect of 5% NaCl at a 1/10 dilution against resistant bacteria after a one-minute contact. However, they only detected a bactericidal effect at a dilution of 1/100 after an exposure time of at least five minutes. Although the efficacy of 10% NaClO was investigated in our study, we observed a bactericidal effect after longer contact times as the dilution ratio increased (20). Hinenoya et al. reported that 100 ppm NaClO significantly decreased the amount of MDR isolates compared to 10 ppm NaClO. Similarly, NaClO was found to be more effective at lower dilution rates in our study. In contrast, it was found ineffective against one *Acinetobacter* isolate at the highest dilution rate after 10 minutes (21). Ogunsola et al. investigated the presence of bacterial growth in 130 disinfectant solutions and found that 82 (63.1%) of the solutions were contaminated with bacteria. Of the 134 isolated strains, 120 were Gram-negative, and *Pseudomonas* sp. was the most frequently isolated bacterium (67.2%). This high contamination rate was attributed to stock solutions being stored under inappropriate conditions, the use of large-volume packages beyond their capacity, and the use of diluted disinfectant solutions beyond their expiry date (22). Wang and colleagues reported that exposing various bacteria to 2% and 6% NaClO for 3 minutes resulted in greater bacterial death in the root canal compared to 1-minute exposure (23). Anari et al. observed that 5% NaClO was the most effective biocide against S. maltophilia isolates among five biocides tested (24). Rakshit et al. reported that the MIC values they determined for 1% NaClO against *K. pneumoniae* and *P. aeruginosa* isolates obtained in the hospital environment were between 0.25% and 1%, which was the lowest MIC value compared to other disinfectants (25). Although numerous studies indicate that NaClO is more effective at higher concentrations, its use is limited due to corrosion and inactivation by organic matter. In healthcare settings, optimising the concentration of NaClO and the contact time could improve the effectiveness of disinfection while reducing material damage.

Limitations:

This study has several limitations. The number of strains tested was small, and some resistant microorganisms, such as VRE and pan-resistant GNB, were not included. Furthermore, the small number of MDR isolates limited the correlation analysis between antimicrobial resistance and the efficacy of NaClO. Disinfectant resistance genes reported in pan-resistant bacteria were not investigated in our study. Differences in analytical methods, dilution ratios, and contact times may also account for inconsistencies with other studies. Additional comprehensive studies, including a broader range of resistant Gram-positive and Gram-negative bacteria, are needed to evaluate the efficacy of NaClO accurately.

CONCLUSION

NaClO was effective against the vegetative forms of common HAI pathogens at contact times of 2.5 and 5 minutes. Only one *Acinetobacter* isolate was resistant to a 1/1000 dilution after 10 minutes. These findings underscore the importance of adhering to the recommended contact times and dilutions. The correct selection and use of disinfectants and antiseptics are critical for limiting the emergence and spread of resistant strains in hospitals and other settings. Regular monitoring of disinfectant efficacy using recent hospital-acquired isolates with updated resistance profiles is essential for effective infection control.

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