İstanbul University-Cerrahpasa, Faculty of Veterinary Medicine, Department of Food Hygiene and Technology[1], İstanbul, Turkey; Eville and Jones[2], Lincoln, UK; Inovatif Biotechnology Chemistry and Health Ltd.[3], İstanbul University Technopark Bld., İstanbul, Turkey

Efficiency of neutral electrolyzed water on quaternary ammonium disinfectant-resistant *Staphylococcus aureus*

Gulay Merve Bayrakal[1a*], Ali Aydin[1b], Cagla Sarimaden[2] and Gurhan Ciftcioglu[3c]

ORCID: a) 0000-0002-2015-7182
b) 0000-0002-4931-9843
c) 0000-0002-5584-517X

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Summary

Disinfectants in the food industry are being replaced by a new generation of agents due to their disadvantages (corrosion, irritation, toxicity, residue problems etc.). Neutral electrolyzed water (NEW) has no negative effects on human, environmental or animal health. It does not alter food quality and is considered a more reliable disinfectant. *Staphylococcus aureus* causes serious health problems, especially food poisoning and intoxications. Quaternary ammonium (QA) disinfectant is commonly used for disinfection in the global food industry. We have investigated the efficacy of NEW at killing quaternary ammonium-resistant and non-resistant *S. aureus* strains *in vitro*, testing different concentrations (20 %, 50 %, 100 %) and treatment times (5, 30 and 120 min). The greatest reductions for both *S. aureus* strains were seen at 100 % concentrations, while no reduction was observed in the tap water used as control. Reductions of more than 6 log CFU/ml were achieved for both strains of *S. aureus* at 50 % and 100 % concentration. After 5 min of treatment, 6 log reductions were achieved for non-resistant *S. aureus* strains at 100 % concentration. QA-resistant strains declined to undetectable levels after 30 min. The results indicate that NEW may be effective in inactivating *S. aureus* strains in the food industry.

Zusammenfassung

Wirksamkeit von neutralem elektrolysiertem Wasser gegenüber *Staphylococcus aureus*, die gegen quaternäre Ammoniumverbindungen resistent sind

Einleitung

Heute werden in der Lebensmittelindustrie eingesetzte Desinfektionsmittel aufgrund ihrer Nachteile (Korrosion, Reizung, Toxizität und Rückstandsprobleme usw.) durch eine neue Generation von Mitteln ersetzt. Neutrales elektrolysiertes Wasser (NEW) hat keine negativen Auswirkungen auf die Gesundheit von Mensch, Umwelt und Tier. Darüber hinaus verändert NEW die Lebensmittelqualität nicht und gilt als zuverlässiges Desinfektionsmittel. *Staphylococcus aureus* verursacht schwerwiegende Gesundheitsprobleme, insbesondere Lebensmittelinfektionen und Vergiftungen. Außerdem wurden in der Lebensmittelindustrie weltweit häufig Desinfektionsmittel verwendet, die quaternäres Ammonium (QA) enthalten. Wir untersuchten QA-resistente und nicht-resistente *S. aureus*-Stämme *in vitro* auf ihre Empfindlichkeit gegen NEW.

Material und Methode

Wir untersuchten die Wirksamkeit von NEW *in vitro* gegen *S. aureus*-Stämme in verschiedenen Konzentrationen (20 %, 50 %, 100 %) und innerhalb von 5, 30 und 120 Minuten Behandlungszeit.

***E-Mail:** merve.bayrakal@iuc.edu.tr

Abbreviations: AEW = Acidic electrolyzed water; BEW = Basic electrolyzed water; CFU = Colony Forming Unit(s); EW = Electrolyzed water; HOCL = Hypochlorous acid; NEW = Neutral electrolyzed water; ND = Not Detectable; ClO^- = Hypochlorite ion; McF = McFarland; OH = Hydroxide; ORP = Oxidation-Reduction Potential; QA = Quaternary Ammonium; QACs = Quaternary Ammonium Compounds; *S. aureus* = *Staphylococcus aureus*; SAEW = Slightly acidic electrolyzed water; StAEW = Strong acidic electrolyzed water; TSA = Tryptic Soy Agar; TSB = Tryptic Soy Broth; TRC = Total Residual Chlorine

Ergebnisse

Die stärksten Reduktionen wurden bei 100 %igen Konzentrationen für beide *S. aureus*-Stämme erzielt, während bei dem als Kontrollprobe verwendeten Leitungswasser keine Reduktion festgestellt wurde. Für beide *S. aureus*-Stämme wurde eine Reduzierung um mehr als 6 $\log_{10}$ KBE/ml bei 50 % und 100 % Konzentration erreicht. Nach 5-minütiger Behandlung wurden eine Reduktion um 6 $\log_{10}$ für nicht resistente *S. aureus*-Stämme bei 100 % Konzentration erreicht, bei QA-resistenten Stämmen dauerte dies 30 Minuten.

Schlussfolgerung

Diese Ergebnisse deuten darauf hin, dass NEW bei der Inaktivierung von *S. aureus*-Stämmen in der Lebensmittelindustrie wirksam sein kann und dass die Wirksamkeit des Desinfektionsmittels mit zunehmender Konzentration und Einwirkzeit zunimmt.

Introduction

Food-borne diseases are a global issue. In the United States alone, 48 million people contract food-borne diseases each year and 3,000 people die due to food-borne diseases (CDC 2020). *Staphylococcus aureus* (*S. aureus*) is one of the major causes of food-borne intoxications worldwide (Fetsch & Johler 2018). It is found in the natural environment, such as in air and dust, as well as on mucosal surfaces such as the larynx and nasal cavity, and the skin and is the main cause of community-associated *S. aureus* infections (Miao et al. 2017; Pal et al. 2020). Not only are people who do not observe hygienic conditions (improper hand washing, no gloves, no hood etc.) carriers of *S. aureus*, they can also transfer bacteria to food during processing, preparation, production, packaging and transport, causing illness and even epidemics (Bencardino et al. 2021). Typical signs of poisoning, characterized by abdominal pain, vomiting and diarrhoea, appear 2–7 hours after ingesting food contaminated with *S. aureus* (Aydin et al. 2011; Denayer et al. 2017). Toxin production and high antibiotic resistance are among other public health risks (Fetsch & Johler 2018; Yildirim et al. 2022).

Staphylococcus aureus is a challenging pathogen for the food industry because it can form biofilms on surfaces, it may produce enterotoxins and resistance to some disinfectants has been reported (Sudagidan & Aydin 2008; Miao et al. 2017; Galie et al. 2018). Consuming contaminated food causes significant economic and health damage. Inadequate and improper disinfection practices lead to food contamination. They also increase the risk of food residues. As a result, biological, physical and chemical risks in food are increasing. Chemicals containing chlorine compounds, peroxide mixtures, quaternary ammonium compounds (QACs), acid anions, hydrogen peroxide and iodine components are commonly used to disinfect food-processing equipment (Al-Qadiri et al. 2016). However, many of them cause damage to the environment, people and animals due to their chemical structure and application process and may lead to the formation of resistant microorganisms. There is now an emphasis on effective and user-friendly chemicals. Neutral electrolyzed water (NEW) is one of them due to its beneficial properties and its broad-spectrum antimicrobial activity.

Electrolyzed water (EW) is obtained by separating diluted salt water from a semi-permeable membrane with an electric current to the negative and positive electrodes (Wang et al. 2019). The electrolyzed water is generally acidic electrolyzed water (AEW, pH 2–3, oxidation-reduction potential (ORP) >1100 mV), basic electrolyzed water (BEW, pH 10–13, ORP of -800 to -900 mV) and NEW (pH 7–8, ORP 750–900 mV) (Rahman et al. 2016). NEW is a popular choice in the food industry due to its stability and environmental friendliness, its low risk to the environment and human health, its strong effect in a short application time, its ease of application and its low cost (Zhao et al. 2021; Mohajer et al. 2022). Unlike acidic and basic EW, NEW is non-corrosive to surfaces and non-irritating to skin membranes, it does not cause changes in the organoleptic properties of food and exhibits less chlorine loss during storage (Monnin et al. 2012; Rahman et al. 2016).

Neutral electrolyzed water is an effective disinfectant due to its pH, high ORP, presence of free available chlorine, hypochlorous acid (HOCl) and hypochlorite ion (OCl^-), and the presence of free hydroxide (OH) groups (Moorman et al. 2017; Chen & Wang 2022). ORP causes the inactivation of bacteria by acting on the inner and outer membranes of the bacteria, active chlorine species help in the inactivation of microbial cells, and radicals produced during electrolysis oxidize tissues, enzymes and DNA/RNA of organisms and

cause their death (Liao et al. 2007; Duan et al. 2016; Rahman et al. 2016; Chen & Wang 2022). In addition, NEW causes the inactivation of viruses by breaking down the viral capsid and reducing receptor binding. It is considered more potent for microbial cell wall penetration and oxidative attack because it predominantly contains HOCl, which, unlike others, is more potent than OCl^- EW (Moorman et al. 2017; Ogunniyi et al. 2019). Furthermore, it should be noted that NEW is not harmful to human health or the environment, as no hazardous substances or chemicals are added during its production.

The effect of different concentrations of NEW on *S. aureus* and QA-resistant *S. aureus* strains was investigated *in vitro* at different times. To our knowledge, this is the first study of the efficacy of NEW against an *S. aureus* strain that is resistant to the quaternary ammonium disinfectants used in the food industry. We evaluated whether NEW can be used in the food industry to inactivate *S. aureus*.

Materials and Methods

Bacterial cultures and preparation of inocula

We used a *S. aureus* strain (ATCC 25923) (Group A) and a *S. aureus* strain resistant to quaternary-based disinfectants (Group B). The QAC-resistant (*qac*C) *S. aureus* strain (Y22 coded) was isolated from food workers' hands in our previous project (Istanbul University-Cerrahpasa, Project Number 17359 YÖP) (Sudagidan et al. 2013). The resistance to a quaternary-based detergent/disinfectant-resistant strain was confirmed by PCR (Veriti, ABI, USA) and gene detection. Genomic DNA of bacterial strains was extracted as described (Sudagidan et al. 2008). The screening of QAC resistance determinant genes *qac*C was performed by PCR using the primers (*qacC-1* **5'→3** GGCTTTTCAAAATTTATACCATCCT *qacC-2* **5'→3** ATGCGATGTTCCGAAAATGT (Sidhu et al. 2002). The thermal protocol was: denaturation for 1 min at 95 °C, annealing for 1 min at between 50 and 55 °C, depending on the primer set, and primer extension for 2 min at 72 °C. The final step was to incubate the reaction mixtures for 10 min at 72 °C. Amplification was run for 30 cycles (Sidhu et al. 2002).

The strains were stored in Tryptic Soy Broth (TSB) supplemented with 20 % glycerol (BD Difco, Sparks, MD, USA) at -80 °C before use. Prior to the study, stock solutions were transferred to Tryptic Soy Agar (TSA) (Oxoid, CM0131, Basingstoke, UK) and incubated at 37 °C for 24 h.

In vitro microbial challenge

Neutral electrolyzed water was obtained from a commercial EW system (Danish Clean Water, T- 20 Series, Denmark). NEW's pH and free active chlorine values were 8.5 and 499.42 mg/l. NEW was diluted to 20 % (1:4 diluted – 99.88 mg/l) or 50 % (1:1 diluted – 249.71 mg/l) with sterile distilled water immediately before analysis or left undiluted 100 % (undiluted). Sterile tap water was used in the control group (0 %).

Staphylococcus aureus was cultured on TSA plates at 37 °C for 24 h. After incubation, the *S. aureus* test strains were swabbed onto 9 ml of sterile saline. The inocula were adjusted to 0.5 McF (1.5 x 10^8 CFU (Colony Forming Unit)/ml) using a McFarland (McF) densitometer (Biosan, Latvia). To verify the McF value, 1 ml (0.3+0.3+0.4 ml) suspensions were spread on three TSA plates and plates were incubated at 37 °C for 24 h (detection limit ≥ 1 log CFU/ml).

One ml of each bacterial suspension was added to the test solution containing 9 ml mixture of NEW and deionized water. In the control group, 1 ml of bacterial solution was transferred to tubes containing 9 ml of sterile tap water. All tubes were vortexed and incubated at room temperature (21–22 °C) for 5, 30 and 120 min for inactivation. At the end of this time, 1 ml of each sample was transferred to a tube containing 9 ml of neutralizing solution (phosphate-buffered saline, Oxoid, BR0014G), vortexed and incubated for 5 min at room temperature to stop the activity of NEW. Decimal serial dilutions were prepared in 0.1 % peptone water (Oxoid, LP0049). 1 ml of each dilution were spread on TSA and incubated at 37 °C for 24 h, then colonies on the Petri dishes were counted. All analysis was performed in duplicate (TSE EN 1276, 2019).

Statistical analyses

We used the Shapiro-Wilk test to ascertain whether the data exhibited a normal distribution. The results indicated that this was not the case, so we applied non-parametric tests. In the initial step, the data sets of the resistant and non-resistant strains were analysed separately. A Kruskal-Wallis test and a Mann-Whitney U test were conducted to assess the statistical significance of differences between the groups (0 %, 20 %, 50 %, 100 %) at each measurement time. Kruskal-Wallis and Mann-Whitney U tests were used to compare the measurement time in each group. In the second stage, we used the Mann-Whitney U test to assess the differences between the resistant and non-resistant strains for each concentration x time subgroup. In the calculations, the statistical significance level was set to $p < 0.05$. We used the SPSS statistical package program (IBM SPSS for Windows, version 26) for the analysis.

Results

We studied the effect of NEW on quaternary ammonium-based disinfectant-resistant *S. aureus* and ATCC

S. aureus strains *in vitro*. We counted viable bacteria after disinfectants at concentrations of 20 %, 50 %, and 100 % had been in contact with *S. aureus* strains for 5, 30 and 120 min at room temperature. Figures 1, 2 and 3 show the surviving populations of the groups (Groups A and B) after treatment with the NEW solution and the control. Table 1 shows the statistical difference between resistant and non-resistant *S. aureus* when exposed to NEW and control solutions at different exposure times and concentrations.

We observed a slight decrease in *S. aureus* strains in contact with NEW for 5 min. Although we noted reductions after 30 min of exposure, the bacteria decreased to undetectable levels at 50 % and 100 % concentrations at 120 min. The two groups showed similar results when treated with 20 % NEW. In the control group, similar results were observed in time-based measurements and no significant decrease in bacterial counts was found. A reduction was observed at 20 % but a greater reduction (of 6 logs) was seen at 100 %.

In group A, we obtained efficacy data at 50 % and 100 % concentrations with no surviving bacteria at 30 and 120 min (Fig. 1). While no significant microbial reduction was observed at 20 %, 5 log CFU/ml microbial reductions were achieved at a concentration of 50 % in 5 min. There was a statistically significant difference between the groups at 5, 30 and 120 min ($p < 0.05$).

Tab. 1: Effect of time, concentration and strain on *S. aureus* viability / Einfluss von Einwirkzeit, Konzentration und Bakterienstamm auf das Überleben von *Staph. aureus*

Strain/ Gruppe	Time/ Einwirkdauer	Concentration /Konzentration				
		20 % Mean/Mittelwert ±SD, Median (Min-Max)	**50 %** Mean/Mittelwert ±SD, Median (Min-Max)	**100 %** Mean/Mittelwert ±SD, Median (Min-Max)	**0 % Control/ Kontrolle** Mean/Mittelwert ±SD, Median (Min-Max)	**# p value**
A (non-resistant)	5 min	5.97±0.185 6.00 [a] (5.78-6.15)	1.30±0.043 1.30 [b, y] (1.26-1.34)	0.00±0.0 0.00 [c] (0.00-0.00) ND*	6.01±0.023 6.00 [a] (6.00-6.04)	**0.022**
A	30 min	5.99±0.088 6.00 [b] (5.90-6.08)	0.00±0.0 0.00 [c, z] (0.00-0.00) ND*	0.00±0.0 0.00 [c] (0.00-0.00) ND*	6.12±0.018 6.11 [a] (6.11-6.15)	**0.014**
A	120 min	5.95±0.014 5.95 [b] (5.94-5.97)	0.00±0.0 0.00 [c, z] (0.00-0.00) ND*	0.00±0.0 0.00 [c] (0.00-0.00) ND*	6.10±0.089 6.11 [a] (6.00-6.18)	
◊ p value		0.739	**0.022**	1.000	0.156	**0.014**
B (resistant)	5 min	5.99±0.088 6.00 [a] (5.90-6.08)	4.30±0.021 4.30 [b, x] (4.28-4.32)	1.30±0.021 1.30 [c] (1.28-1.32)	6.11±0.067 6.11 [a] (6.04-6.18)	**0.019**
B	30 min	6.01±0.023 6.00 [b] (6.00-6.04)	3.95±0.014 3.95 [c, y] (3.94-3.97)	1.30±0.065 1.30 [d] (1.23-1.36)	6.11±0.033 6.11 [a] (6.08-6.15)	**0.015**
B	120 min	5.84±0.024 5.85 [b] (5.82-5.87)	0.00±0.0 0.00 [c, z] (0.00-0.00) ND*	0.00±0.0 0.00 [c] (0.00-0.00) ND*	6.01±0.023 6.00 [a] (6.00-6.4)	**0.014**
◊ p value		0.061	**0.024**	0.060	0.113	

ND = not detectable; no detectable survivors by a direct plating procedure; SD = standard deviation; # = Significance level of difference between groups in the same period (a, b, c, d = shows differences according to Mann-Whitney U test); ◊ = Significance level of the difference between periods in the same group (x, y, z = shows differences according to Mann-Whitney U test); / ND = nicht nachweisbar; kein Koloniewachstum auf den Agarplatten; SD = Standardabweichung; # =Signifikanzlevel zwischen den Gruppen bei gleicher Einwirkzeit (a, b, c, d = signifikante Unterschiede nach dem Mann-Whitney U test); ◊ = Signifikanzlevel des Unterschiedes zwischen den Einwirkzeiten innerhalb einer Gruppe (x, y, z = Unterschiede nach dem Mann-Whitney U test).

The bacterial counts in the control group and the 20 % concentration group were similar and significantly higher than in the other groups, indicating that the 20 % concentration was not effective. The lowest bacterial counts were observed at 50 % and 100 %. The *S. aureus* in the control samples showed no reduction.

There was no significant decrease at the 20 % concentration in the *S. aureus* strain that is resistant to quaternary-based disinfectants. Approximately 2 log CFU/ml reductions were achieved at 50 % concentration and 5 log CFU/ ml reductions at 100 % at 5 and 30 min (Fig. 2). After 120 min, the number of surviving bacteria decreased to undetectable levels at 50 % and 100 %, while no decrease was observed in the control group. Statistical analysis revealed a significant difference in group B at 5, 30 and 120 min ($p < 0.05$). At concentrations of 50 % and 100 %, the 5 min and 30 min measurements were consistent, with a significant difference at 120 min.

When comparing the A and B groups, higher levels of the ATCC *S. aureus* strain decreased to undetectable levels by 5 min, while the QA-resistant strain decreased to undetectable levels after 30 min (Fig. 3). The decrease in the B group at 100 % in 30 min and to an undetectable level in 120 min shows the strong effect of the disinfectant against the QA-resistant strain. The number of viable survivors at 50 % and 100 % concentrations and 5 and 30 min was higher in group B than in group A. The difference in the 5-0, 5-20, 30-0, 30-20, 120-0, 120-50 and 120-100 groups was statistically significant, while the difference between the 5–50, 5-100, 30-50, 30-100 and 120-20 groups was not. The data from both groups showed that the number of viable bacteria decreased as the concentration of disinfectant increased.

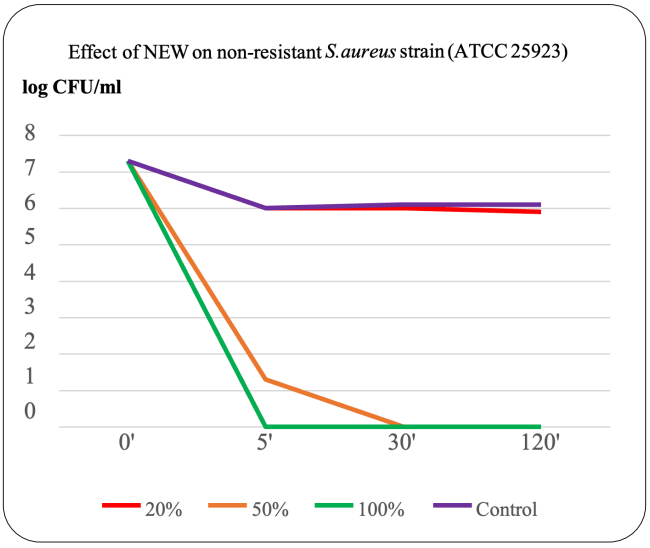

Fig. 1: Non-resistant *S. aureus* strain ATCC 25923 viable counts after treatment with NEW (log CFU/ml) / Keimzahlen des nicht-QAV resistenten *S. aureus* Stamms ATCC 25923 nach Behandlung mit neutralem elektrolysiertem Wasser (log_{10} KbE/ml)

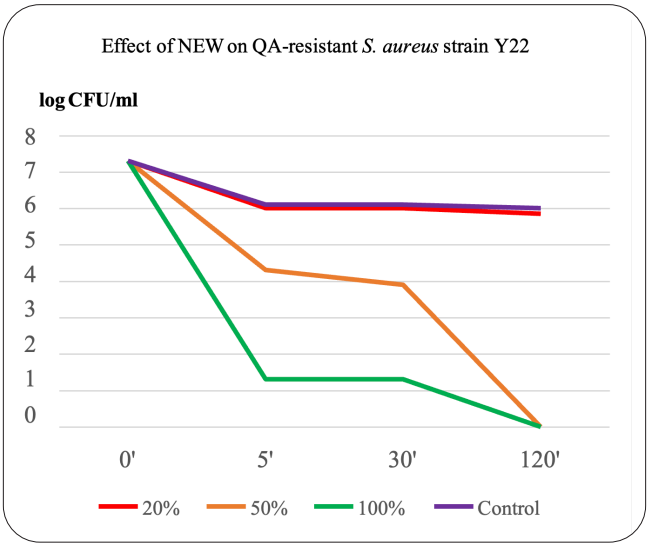

Fig. 2: Quaternary-ammonium disinfectant resistant *S. aureus* strain Y22 viable counts after treatment with NEW (log CFU/ml) / Keimzahlen des QAV resistenten *S. aureus* Stamms Y22 nach Behandlung mit neutralem elektrolysiertem Wasser (log_{10} KbE/ml)

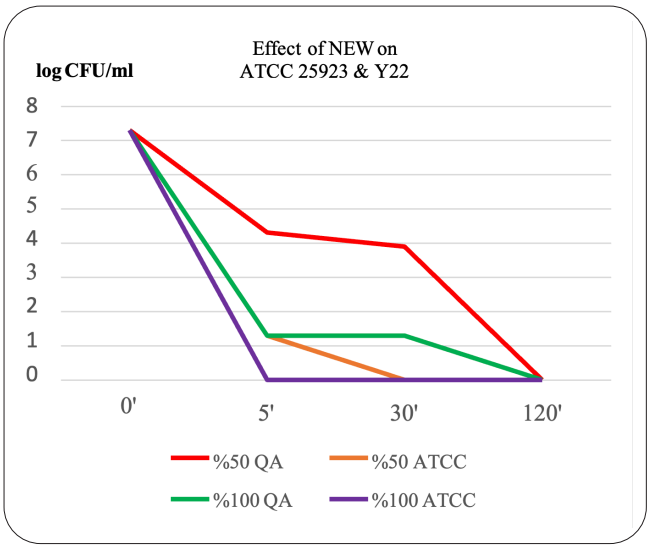

Fig. 3: Comparison of the number of colonies of the non-resistant ATCC 25923 (ATCC) and the resistant *S. aureus* strain Y22 (QA) after treatment with NEW (log CFU/ml) / Vergleich der Keimzahlen des nicht-resistenten ATCC 25923 (ATCC) und des QA-resistenten *S. aureus* Stamms Y22 (QA) nach NEW Behandlung (log_{10} KbE/ml)

Discussion

Rationale for the use of NEW as a disinfectant in food industry

Staphylococcus aureus can cause various diseases such as systemic infections, severe skin infections, sepsis and food poisoning (primarily because of contaminated foods containing staphylococcal enterotoxins, SEs). *S. aureus* contamination in the food industry must be avoided to prevent foodborne

staphylococcus-related diseases (Cheung et al. 2021), so disinfection processes should be carried out correctly and effectively. As long as disinfectants are used in the appropriate dosage, time and form, they inactivate microorganisms. However, many widely used disinfectants cause corrosion to surfaces, tools and equipment and affect food quality (Wang et al. 2019; Zhao et al. 2021). For this reason, NEW - which is environmentally friendly and safe for animals and humans and does not alter food quality - is more widely used than most other disinfectants (Han et al. 2018). The declaration that electrolyzed water can be used as an effective disinfectant for SARS-CoV-2, even in the COVID-19 pandemic, again proved the effectiveness of this disinfectant (Takeda et al. 2020; Sarada et al. 2020).

Neutral electrolyzed water is widely used in the food industry because it has few disadvantages and a strong effectiveness. It has proven effective on surfaces and equipment such as cutting boards (Monnin et al. 2012; Al-Qadiri et al. 2016), stainless steel surfaces (Jiménez-Pichardo et al. 2016) for food preparation and chopping. NEW has been used to control bacterial contamination of foods such as carcasses (Han et al. 2018; Hernández-Pimentel et al. 2020), juices (Huang et al. 2019), eggs (Medina-Gudiño et al. 2020) and apples (Sheng et al. 2020) and many studies show that it can be used effectively in the decontamination of foods.

Effect of NEW on *Staphylococcus aureus* and factors affecting its effectiveness

We observed 6 log CFU/ml reductions for QA-resistant *S. aureus* at 100 % concentration after 5 and 30 min and for non-resistant *S. aureus* strains at 50 % concentration after 5 min. Previous studies on the inactivating effect of NEW on *S. aureus* showed a 100 % effect in 10 min on spinach leaves at 100 TRC (Total Residual Chlorine) concentration (Guentzel et al. 2008). *S. aureus* strains decreased by 4 log CFU/50 cm^2 after soaking wooden boards for 5 min in NEW with 63 mg/l active chlorine and by more than 6 log CFU/50 cm^2 on stainless steel and glass surfaces after soaking for 1 min (Deza et al. 2005, 2007). Another study showed a decrease of both QA and NEW *S. aureus* strains of about 5 log CFU/ml on cutting boards after 5 min (Al-Qadiri et al. 2016).

Changes in the pH of EW alter the effectiveness of the disinfectant and its duration of action. The reduction in *S. aureus* achieved with strongly acidic electrolyzed water (StAEW) was significantly higher than that with slightly acidic electrolyzed water (SAEW) at all exposure times (30 s, 60 s, 90 s), with the strongest decrease observed in 90 seconds (Issa-Zacharia et al. 2010a). In other *in vitro* work, a 60-second treatment of *S. aureus* with StAEW and SAEW resulted in a reduction of 5.93 log CFU/ml and 4.83 log CFU/ml, respectively (Issa-Zacharia et al. 2010b). SAEW (pH 6.1) achieved a 5.83 log CFU/ml reduction of *S. aureus* in 30 sec (Liao et al. 2017). SAEW is more effective in inactivating *S. aureus* when applied for 3 or 5 min instead of 1 min (Forghani et al. 2015) and therefore also EW can be used effectively (Ding et al. 2016). Despite the effectiveness of acidic and basic electrolyzed water, we used electrolyzed water with neutral pH to avoid the disadvantages associated with high or low pH (corrosive and irritating etc.).

We achieved 5 log CFU/ml reductions in QA-resistant *S. aureus* at 100 % concentration after 5 and 30 min and in non-resistant *S. aureus* strains at 50 % concentration after 5 min. NEW is 100 % effective at 20, 50, 100 and 120 ppm TRC concentration after 10 min at room temperature *in vitro* (Guentzel et al. 2008) and no bacteria were detected after 5 min of *in vitro* treatment with NEW containing 60 mg/l active chlorine (Deza et al. 2005). NEW (pH 6.5, containing 500–700 ppm chlorine) affects *S. aureus* in 1 min at 100 % concentration, while a 1/20 concentration is ineffective even after 30 min (Yanik et al. 2015). *In vitro* studies of the efficacy of NEW on *S. aureus* strains showed results similar to ours, confirming that NEW can be used as an effective disinfectant. We also investigated the effect of NEW against a QA-resistant *S. aureus* strain and showed that even inactivates strains resistant to QA, a powerful disinfectant. Our study highlights the effectiveness of NEW as a safe and reliable disinfectant.

In addition to pH, many physico-chemical variables such as water hardness, presence of organic matter, salt concentration, storage conditions, the ambient temperature and time of use affect the effectiveness of electrolyzed water on microorganisms (Kim et al. 2019; Ogunniyi et al. 2019; Block et al. 2020). NEW is nearly 100 % effective at high concentrations (Moorman et al. 2017; Han et al. 2018). As the length of exposure to the disinfectant increases, the contact with the microorganism also increases and there is more effect on the microorganisms (Al-Qadiri et al. 2019; Shiroodi et al. 2021). Our findings are consistent with previous work and confirm that the onset of action is faster with increasing concentration of disinfectant. At lower concentrations, the time of contact with the disinfectant should be increased.

Conclusion

Neutral electrolyzed water can be used even against *S. aureus* resistant to quaternary ammonium-based disinfectant, which is widely used in the food industry but has many disadvantages. NEW is more effective than the QA disinfectant and can be reliably used in the food industry due to the absence of harmful effects on the environment, humans, animals and food. It should be noted that the results are based on a limited sample size of only two strains, so the findings should be considered preliminary. Further testing with

a larger panel will be necessary to confirm the results. Nevertheless, our research has the potential to make a significant contribution to food safety.

Fazit für die Praxis:
Neutrales, elektrolysiertes Wasser gilt als sicheres Desinfektionsmittel für Umwelt, Mensch und Tier und hat weniger negative Auswirkungen auf die Lebensmittelqualität. Nach den Ergebnissen dieser Studie ist NEW bei der Inaktivierung sowohl quaternärer Ammonium-resistenter als auch nicht-resistenter *S. aureus*-Stämme wirksam. Einschränkend muss angemerkt werden, dass jeweils nur ein Stamm untersucht wurde.

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Conflict of interest
The authors declare no conflict of interest.

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