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RESEARCH ARTICLE

Susceptibility of *Staphylococcus aureus* Isolated from Different Raw Meat Products to Disinfectants

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ABSTRACT

Objective: *Staphylococcus aureus*, a severe public health hazard, causes foodborne diseases from the consumption of contaminated food. Various antimicrobials and disinfectants are used throughout the food chain to reduce microbial contamination or eliminate microorganisms on food contact surfaces. However, little is known about the susceptibility of disinfectants to food pathogens, including *S. aureus*, which can develop resistance to antimicrobials and cause severe diseases.

Materials and Methods: The antimicrobial activity of triclosan, cetyltrimethylammonium bromide (CTAB), acetic acid, citric acid, and lactic acid against 50 *S. aureus* isolates, including multidrug-resistant (MDR) isolates originating from ground beef, chicken, and fish, was investigated using the broth microdilution method.

Results: The minimal inhibitory concentrations (MICs) of triclosan, CTAB, acetic acid, citric acid, and lactic acid against the isolates were 0.125-16 µg/mL, 0.25-32 µg/mL, 102.5-26250 µg/mL, 187.5-12000 µg/mL, and 703-22500 µg/mL, respectively. Almost all MDR isolates showed resistance to triclosan. There was a statistically significant difference in MICs between triclosan and organic acids, as well as between CTAB and organic acids ($p < 0.05$). However, a statistically significant difference was not observed in triclosan and CTAB, as well as in acetic acid and lactic acid ($p > 0.05$). Pearson correlation coefficient revealed a strong relationship between triclosan and multidrug resistance. Based on the multiple linear regression analysis, triclosan had a positive effect on multidrug resistance ($p < 0.05$).

Conclusion: This research gives helpful information on the susceptibility of disinfectants to *S. aureus*, particularly to resistant *S. aureus* isolates from meats, which may help to recommend proper disinfectant use in food production.

Keywords: *Staphylococcus aureus*, Triclosan, Cetyltrimethylammonium bromide, Organic acids, Minimum inhibitory concentration.

INTRODUCTION

Staphylococcus aureus is a significant pathogen that causes a wide range of illnesses including skin and soft tissue infections, foodborne poisoning, pneumonia, bacteremia, endocarditis, osteomyelitis, meningitis, enterocolitis, urinary tract infections, toxic shock syndrome.[1,2] Consumption of contaminated food, such as meat and meat products, with this pathogen is the major source of *S. aureus* foodborne illnesses and poses a serious threat to human health. The presence of foodborne pathogens including *S. aureus* in processed foods or on food processing equipment because of contact by humans or cross-contamination could indicate poor handling or sanitation.[2,3] It is important to avoid cross-contamination of food and possible foodborne disease. It is recommended to clean surfaces; either the organisms must be removed, or they must be inactivated in situ using a disinfection method. Disinfectants and sanitizers are considered more efficient since they make more effective contact with surface-attached microorganisms.[4]

Disinfectants, sanitizing agents, and cleaning chemical agents are widely utilized in the food industry to inhibit the growth or kill microorganisms involved in food spoilage and foodborne diseases on food contact surfaces and reduce them to safe levels in food manufacturing facilities.[3,5] Sanitizers and disinfectants such as triclosan, quaternary ammonium compounds, chlorohexidine, benzalkonium chloride, chlorine and chlorine-based derivatives, hydrogen peroxide, acid anionic agents, peracetic acid, and weak organic acids play essential roles in the food and health care industries.[3,5-7] These sanitizing agents are extensively used in the food industry for disinfecting and cleaning floors, walls, drains, and fields associated

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with livestock and crop production, including farm buildings, equipment, and vehicles, as well as on animals, such as in footbaths or udder cleansing.[5,6] The antimicrobial effectiveness of these chemicals and the amount of residue left on surfaces after application can vary. Several factors that influence antimicrobial efficiency include exposure time, temperature, chemical formulation, concentration, pH, microbiological load and type, microbial adhesion to the surface, and application.[3,5]

Triclosan, 2,4,4'-trichloro-2'-hydroxydiphenyl ether, is a synthetic bisphenol antibacterial agent present in various hygiene products, including soaps and mouthwashes. It has activity against both Gram-positive and Gram-negative bacteria.[8] Triclosan is commonly used in community environments for personal hygiene purposes and to avoid cross-contamination with bacteria in domestic environments and during food processing, as well as in industrial environments.[9,10] It is used as a material preservative in kitchen equipment, including cutting boards, plastic utensils, storage containers, and counters which contact with foodstuffs.[11] The triclosan residue detection in various foodstuffs has previously been reported in China and Spain.[12,13] Furthermore, triclosan has been successfully used in hospital environments to control methicillin-resistant *Staphylococcus aureus* (MRSA) infections and reduce nosocomial outbreaks of antimicrobial-resistant bacteria.[8-10,14] As in clinical settings, disinfectants and preservatives could give selective pressure for the development and isolation of antimicrobial-resistant bacteria.[5,15] Several studies have been published showing the emergence of triclosan resistance in *S.aureus*.[8,15,16] In addition, triclosan resistance has been studied in *Escherichia coli*[17], *Pseudomonas aeruginosa*[18], vancomycin-resistant *Enterococcus faecium*[19], and *Salmonella* strains.[20]

Quaternary ammonium compounds (QACs) are a broad class of chemicals that include central nitrogen, which is bound to four (quaternary) organic groups. The interaction of positively charged quaternary nitrogen with the polar head groups of phospholipids mediates QAC antibacterial activity. This activity restricts the uptake of nutrients into the microbial cell and waste discharge.[9,21] QACs are widely used as disinfectants for the control of bacterial growth in clinical and food production environments because they have antimicrobial effects on a wide variety of microorganisms but have no effect on the spore phase.[3,21] Gram-positive bacteria are more sensitive to QACs at lower concentrations than Gram-negative bacteria.[3,21,22] Cetyltrimethylammonium bromide (CTAB) is a quaternary ammonium compound and cationic detergent that acts as an antibacterial agent by inducing superoxide stress in bacteria.[23,24]

Organic acids are natural compounds present in a variety of fruits and fermented products that have antimicrobial activity against microorganisms.[3,6,25] They can inhibit microorganism growth by decreasing the pH, altering the proton gradient across the membrane, acidifying the cytoplasm, and impeding chemical transport across the cell membrane.[3,7] Organic acids are considered "generally recognized as safe" additives in many foods for people by the U.S. Food and Drug Administration.[3] The major organic acids are acetic, citric, and lactic acids, which can be used in the sanitization process due to their efficacy and cost.[6,7,25-27] Previous studies have demonstrated differences in the antibacterial activity of organic acids such as acetic, lactic, and citric acids against foodborne pathogens.[25-29]

Several chemical sanitizers and disinfectants, including triclosan[8,15,30], CTAB[18,20], and organic acids like acetic, citric, and lactic acid[7,25-27], have been investigated for their efficacy against pathogens in previous studies. These compounds have commonly been used as disinfectants, sanitizers, antiseptics, and surface decontaminants in food and meat processing industries, animal farms, poultry, household cleaning products, hospitals, and other health care settings, and as preservatives in food industries, cosmetics, pharmaceuticals, textiles, and laundry detergents.[10,21,25] Many studies have demonstrated the effects of sanitizers and disinfectants on the growth inhibition of pathogens in food.[7,28,31,32] However, few studies have been conducted to evaluate the effectiveness of disinfectants on multidrug-resistant pathogens from food, which constitute a potential threat to human health.[25,27] Therefore, this study aimed to investigate the susceptibility of *S. aureus* isolates, including multidrug-resistant isolates from meat products to some of the most widely used disinfectants, such as triclosan, CTAB, acetic acid, lactic acid, and citric acid.

MATERIALS AND METHODS

Bacterial Isolates

Fifty *S. aureus* isolates from meat were used in this study. They comprised 20 fish samples: 11 from seawater fish *(Sparus aurata)*, 8 from freshwater fish *(Oncorhynchus mykiss)*, 1 from seawater fish *(Dicentrarchus labrax)*, 17 from ground beef (cow's meat), 13 from chicken meat (breast and leg parts) isolates. All meat samples, including fish samples from local fish markets and supermarkets, ground beef samples from butcher shops, and chicken meat samples from supermarkets and butchers, were collected. The isolates had already been identified using biochemical tests and a PCR for the species-specific fragment (Sa442) and thermonuclease gene *(nucA)*.[1,33,34]

Disinfectants

The disinfectants tested were triclosan, CTAB, acetic acid, citric acid, and lactic acid. Triclosan, CTAB, and citric acid were purchased from Sigma-Aldrich (MO, USA). Acetic acid and lactic acid were obtained from Merck (Darmstadt, Germany). Stock solutions of triclosan and CTAB were prepared in dimethyl sulfoxide (DMSO) (Applichem, Darmstadt, Germany). As in the previous studies [24,30], the level of DMSO in the final working solutions was below 5%. Organic acids were prepared in Mueller-Hinton broth (Merck, Germany). Disinfectants were tested at the following concentrations: triclosan (0.125-64 µg/mL), CTAB (0.25-128 µg/mL), acetic acid (51.45-26250 µg/mL), citric acid (93.75-48000 µg/mL), and lactic acid (43.9-22500 µg/mL). All stock solutions were sterilized before use by syringe filtration through 0.22 µm membrane filters (Sartorius AG, Goettingen, Germany).

Determination of Minimum Inhibitory Concentration (MIC) of Disinfectants

The susceptibility of disinfectants was tested using the broth microdilution method, as defined by the Clinical and Laboratory Standards Institute (CLSI).[35,36] Before the experiment, all *S. aureus* isolates used in this study were activated in Tryptic Soy Broth (TSB) (Merck, Darmstadt, Germany), incubated at 37 ºC for 24 h, streaked on Tryptic Soy Agar (TSA) (Merck, Germany) plates, and incubated overnight at 37 ºC. Several colonies from the TSA plates were taken and suspended in Mueller Hinton broth (Merck) to adjust the bacterial turbidity to 0.5 McFarland standard (approximately 10^8 Colony-Forming Units (CFU)/mL). The isolates were diluted to the final concentration of 1 x 10^6 CFU/mL and added to each well of a 96-microtiter plate (Lp Italiana, U-bottom). The MIC value of *Staphylococcus aureus* ATCC 29213 as a quality control strain according to the CLSI against gentamicin was used in all experiments. The microtiter plates were incubated at 37 ºC for 16-20 h. The optical density was measured at 600 nm using a microplate reader (Thermo Electron Corporation, Vantaa, Finland). MIC experiments were carried out in triplicates. The MIC for each isolate was determined as the lowest concentration that inhibited growth.[35] The MIC was recorded as the lowest concentration of disinfectant at which no visible bacterial growth was considered.[37] The MIC_{50} and MIC_{90} results showed that the MIC values inhibited 50% and 90% of the isolates, respectively. The previously published susceptible/resistant criterion was used to determine triclosan resistance.[38] *S. aureus* was classified as susceptible at MICs of < 0.5 µg/mL, intermediate at MICs of 0.5 to 2 µg/mL, and resistant at MICs of > 2 µg/mL.

Statistical Analysis

The MIC values of the disinfectants against the *S. aureus* isolates were evaluated using one-way analysis of variance (ANOVA) with Tukey's multiple comparison test. Pearson's correlation test was used to evaluate the correlations between disinfectant susceptibility and multidrug resistance. Multiple linear regression was performed using multidrug resistance as the dependent variable and triclosan, CTAB, acetic acid, citric acid, and lactic acid as the independent variables. All analyses were conducted using SigmaPlot 12.3 (Systat Software Inc., USA). The results with a *p*-value of less than 0.05 were regarded as statistically significant.

RESULTS

MICs, MIC_{50}, and MIC_{90} values of disinfectants tested against the *S. aureus* isolates from raw meat products are shown in Table 1. Triclosan MICs ranged between 0.125 and 16 µg/mL. Twenty-eight percent (28%) of the isolates tested positive for triclosan resistance (MIC > 2 µg/mL), while 18% were positive for triclosan intermediate resistance (MIC in the range of 0.5-2 µg/mL). The *S. aureus* isolates (54%) had susceptible triclosan MICs that ranged from 0.125-0.25 µg/mL. The MIC_{50} and MIC_{90} values for triclosan were 0.25 and 4 µg/mL, respectively. Among the tested *S. aureus* isolates, all 13 multidrug-resistant (MDR) isolates exhibited resistance (11 isolates) or intermediate resistance (2 isolates) to triclosan (Figure 1). The Pearson correlation test revealed a significant relationship between triclosan resistance and multidrug resistance in the *S. aureus* isolates ($r = 0.752$, $p < 0.05$). There was no significant relationship between the other disinfectants tested and multidrug resistance ($p > 0.05$). According to the multiple linear regression analysis, only triclosan had a positive effect on multidrug resistance in a 95% confidence level ($R = 0.766$).

The MIC levels for the disinfectant CTAB were between 0.25 and 32 µg/mL. MIC values of CTAB for the 18 isolates (36%) were 1 µg/mL, while 12 isolates (24%) were at 2 µg/mL. The MIC_{50} and MIC_{90} of CTAB were 1 and 8 µg/mL, respectively. The MIC values of CTAB against MDR isolates ranged from 0.5 to 32 µg/mL (Figure 1). Moreover, CTAB MIC values for 75% of the MDR isolates from chicken meat ranged from 4 to 8 µg/mL (Figure 1).

Table 2 shows the triclosan and CTAB susceptibilities among the *S. aureus* isolates from different raw meat products. Resistance to triclosan was detected most frequently in chicken meat at a rate of 46.1% and in ground beef at a rate of 35.3%. On the other hand, the highest sensitivity to triclosan was detected in the isolates from freshwater fish, seawater fish, ground beef, and chicken meat, with 87.5%, 66.7%, 47.1%, and 30.8%, respectively. Considering the effect of CTAB on the susceptibilities of the isolates from meat products, one isolate from ground beef and one from seawater fish showed the highest MIC values at 32 µg/mL and 16 µg/mL, respectively. Among all meat isolates, 41.2% of the ground beef isolates demonstrated activity against CTAB with a MIC of 1 µg/mL.

The MICs of organic acids, including acetic acid, citric acid, and lactic acid against *S. aureus* isolates were determined, and the results are presented in Table 1 and Figure 2. Organic acids tested were efficient against the *S. aureus* meat isolates, with

Table 1. Distributions of disinfectant MIC values for *Staphylococcus aureus* isolates from raw meat products.

Disinfectants	Number of the isolates at each MIC (µg/mL) of disinfectant																								
	0.125	0.25	0.5	1	2	4	8	16	32	102.5	187.5	703	750	820	1406	1500	1641	2812.5	3000	3281	5625	6000	> 6000	MIC_{50}	MIC_{90}
Triclosan	15	12		4	5	**8**	**5**	**1**																0.25	4
CTAB		2	6	18	12	5	5	1	1															1	8
Acetic acid										1				13			23			8			5	1641	3281
Citric acid											1		9			21			13			4	2	1500	6000
Lactic acid												2			29			13			5		1	1406	5625

The concentrations of disinfectants were tested: triclosan (0.125–64 µg/mL), CTAB, cetyltrimethylammonium bromide (0.25–128 µg/mL), acetic acid 51.45–26250 µg/mL), citric acid (93.75–48000 µg/mL), and lactic acid (43.9–22500 µg/mL). Bold numbers indicate triclosan-resistant isolates. *S. aureus* isolates were classified as susceptible to triclosan at MICs of < 0.5 µg/mL, intermediate at MICs of 0.5 to 2 µg/mL, and resistant at MICs of > 2 µg/mL.[38]

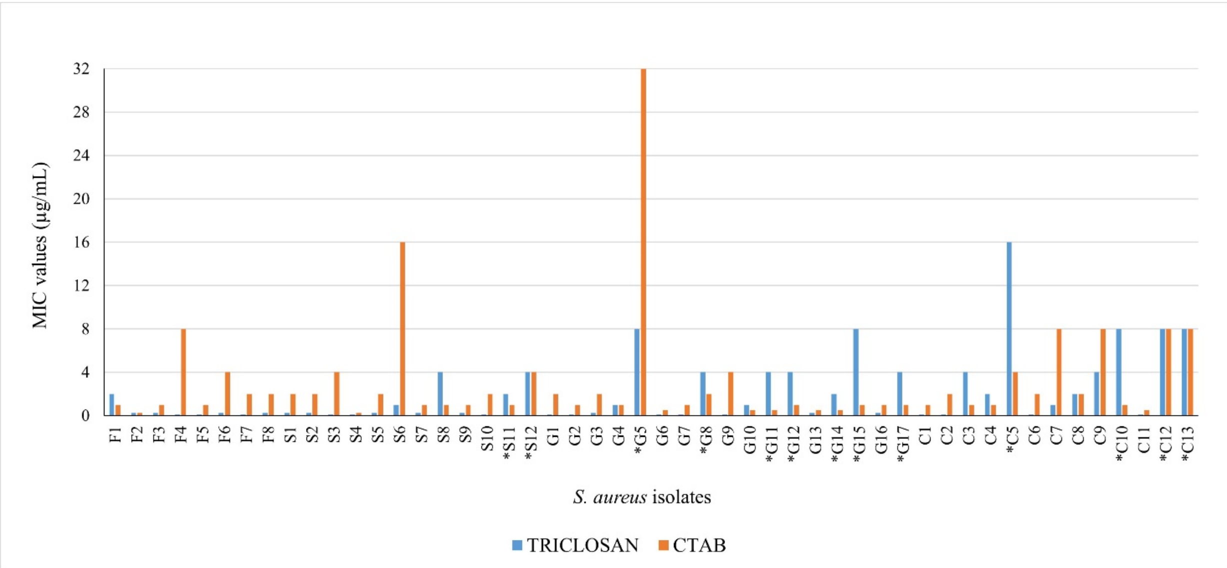

Figure 1. MIC values of triclosan and CTAB (cetyltrimethylammonium bromide) against *Staphylococcus aureus* isolates from meat. Isolate F, freshwater fish; S, seawater fish; G, ground beef; C, chicken meat. The stars (*) indicate the multidrug-resistant isolates.

MICs ranging between 102.5-26250 µg/mL for acetic acid, with MICs ranging between 187.5-12000 µg/mL for citric acid, and with MICs ranging between 703-22500 µg/mL for lactic acid. Acetic acid, citric acid, and lactic acid MICs were 1641 µg/mL for 23 isolates (46%), 1500 µg/mL for 21 isolates (42%), and 1406 µg/mL for 29 isolates (58%), respectively (Table 1). *S. aureus* meat isolates had MIC_{50} values of 1641 µg/mL for acetic acid, 1500 µg/mL for citric acid, and 1406 µg/mL for lactic acid (Table 1). The *S. aureus* meat isolates had MIC_{90} values of 3281 µg/mL for acetic acid, 5625 µg/mL for lactic acid, and 6000 µg/mL for citric acid (Table 1). The MIC > 6000 µg/mL comprised one isolate from ground beef with 22500 µg/mL for lactic acid, one isolate from freshwater fish and one isolate from chicken meat with 12000 µg/mL for citric acid, and one isolate from chicken meat with 6562.5 µg/mL, two isolates from ground beef with 13125 µg/mL, and one isolate from ground beef and one isolate from chicken meat with 26250 µg/mL for acetic acid.

Furthermore, organic acid MICs of *S. aureus* isolated from different meat products are represented in Table 3. In particular, the MIC value of acetic acid for all (100%) freshwater fish isolates was 1641 µg/mL. The acetic acid MIC value of 820 µg/mL was found in more than half of the ground beef isolates (52.9%). In addition, 58.3% of seawater fish isolates had MICs of 1641 µg/mL for acetic acid and 1500 µg/mL for citric acid. More than half of the isolates obtained from ground beef (64.7%), freshwater fish (62.5%), chicken meat (53.8%), and seawater fish (50%) had an MIC of 1406 µg/mL for lactic acid.

The *S. aureus* isolates had lower MICs for the triclosan and CTAB than for the organic acids among the disinfectants tested. Based on the MIC data, there was a statistically significant difference between triclosan, and the organic acids tested (p < 0.05). Similarly, the difference between CTAB and the organic acids tested was statistically significant (p < 0.05). However, the

Table 2. Triclosan and CTAB susceptibilities of the isolates based on raw meat products.

Origin	MIC (µg/mL)	No. (%) of the isolates	
		Triclosan	CTAB
Freshwater fish (n=8)	0.125	3 (37.5)	-[a]
	0.25	4 (50)	1 (12.5)
	1	-	3 (37.5)
	2	1 (12.5)	2 (25)
	4	-	1 (12.5)
	8	-	1 (12.5)
Seawater fish (n=12)	0.125	3 (25)	-
	0.25	5 (41.7)	1 (8.3)
	1	1 (8.3)	4 (33.3)
	2	1 (8.3)	4 (33.3)
	4	2 (16.7)	2 (16.7)
	8	-	-
	16	-	1 (8.3)
Ground beef (n=17)	0.125	5 (29.4)	-
	0.25	3 (17.6)	-
	0.5	-	5 (29.4)
	1	2 (11.8)	7 (41.2)
	2	1 (5.9)	3 (17.6)
	4	4 (23.5)	1 (5.9)
	8	2 (11.8)	-
	32	-	1 (5.9)
Chicken meat (n=13)	0.125	4 (30.8)	-
	0.5	-	1 (7.7)
	1	1 (7.7)	4 (30.8)
	2	2 (15.4)	3 (23.1)
	4	2 (15.4)	1 (7.7)
	8	3 (23.1)	4 (30.8)
	16	1 (7.7)	-

[a] Not detected.

MIC, minimum inhibitory concentration; CTAB, cetyltrimethylammonium bromide.

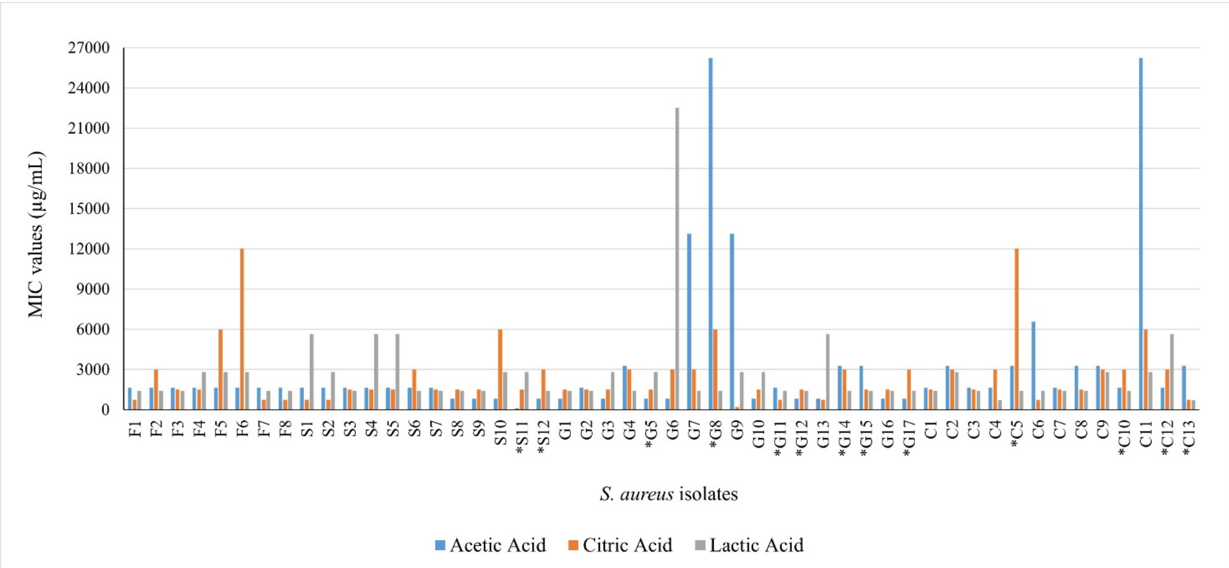

Figure 2. MIC values of three different organic acids against *Staphylococcus aureus* isolates from meat. Isolate F, freshwater fish; S, seawater fish; G, ground beef; C, chicken meat. The stars (*) indicate the multidrug-resistant isolates.

Table 3. Organic acid MICs of *Staphylococcus aureus* isolated from different meat products.

Origin	MIC (µg/mL)	No. (%) of the isolates		
		Acetic acid	Citric acid	Lactic acid
Freshwater fish (n=8)	750	-[a]	3 (37.5)	-
	1406	-	-	5 (62.5)
	1500	-	2 (25)	-
	1641	8 (100)	1 (12.5)	-
	2812.5	-	-	3 (37.5)
	3000	-	-	-
	6000	-	1 (12.5)	-
	> 6000		1 (12.5)	-
Seawater fish (n=12)	102.5	1 (8.3)	-	-
	750		2 (16.7)	-
	820	4 (33.3)	-	-
	1406	-	-	6 (50)
	1500	-	7 (58.3)	-
	1641	7 (58.3)	-	-
	2812.5	-	-	3 (25)
	3000	-	2 (16.7)	-
	5625	-	-	3 (25)
	6000	-	1 (8.3)	-
Ground Beef (n=17)	187.5	-	1 (5.9)	-
	703	-	-	1 (5.9)
	750	-	2 (11.7)	-
	820	9 (52.9)	-	-
	1406	-	-	11 (64.7)
	1500	-	8 (47.1)	-
	1641	2 (11.8)	-	-
	2812.5	-	-	4 (23.5)
	3000	-	5 (29.4)	-
	3281	3 (17.6)	-	-
	5625	-	-	1 (5.9)
	6000	-	1 (5.9)	-
	> 6000	3 (17.6)	-	-
Chicken meat (n=13)	703	-	-	2 (15.4)
	750	-	2 (15.4)	-
	1406	-	-	7 (53.8)
	1500	-	4 (30.7)	-
	1641	6 (46.1)	-	-
	2812.5	-	-	3 (23.1)
	3000	-	5 (38.5)	-
	3281	5 (38.5)	-	-
	5625	-	-	1 (7.7)
	6000	-	1 (7.7)	-
	> 6000	2 (15.4)	1 (7.7)	-

a Not detected.
MIC, minimum inhibitory concentration.

difference in MIC values between triclosan and CTAB, as well as acetic acid and lactic acid, was not considered significant ($p > 0.05$).

DISCUSSION

Sanitizers and disinfectants are necessary for ensuring food safety. They are critical for controlling pathogen spread and are beneficial to public health.[5] Several parameters influence disinfectant effectiveness, including concentration, bacterial state, and the presence of interfering components such as organic waste.[3,6,9] The concentration of disinfectant utilized is the most essential component in pathogen control, together with other physical and chemical parameters such as temperature, pH, humidity, and organic load.[3,25]

Triclosan, as a disinfectant and antiseptic, is widely used in personal hygiene and disinfection and has good antimicrobial activity against a broad range of microorganisms, including antimicrobial-resistant bacteria, since it has a specific bacterial cellular target.[8,15] Many investigations have reported resistance to triclosan in *S. aureus* and other bacteria.[16-18,24] In the present study,[23] (46%) *S. aureus* isolates demonstrated intermediate resistance or resistance to triclosan (MIC ≥ 1 µg/mL) (Table 1). All (13) MDR isolates had MICs to triclosan ranging from 2 to 16 µg/mL among the 50 *S. aureus* isolates from raw meat products (Figure 1). All MDR isolates obtained from chicken meat were resistant to triclosan, with MICs of 8 to 16 µg/mL.

Pearson's test showed a strong correlation (r = 0.752) between triclosan and multidrug resistance. In contrast to our study, all *S. aureus* strains from swine mandibular lymph node tissue and commercial pork sausage meat were susceptible to triclosan.[24] All *Pseudomonas aeruginosa* veterinary isolates were resistant to triclosan.[18] Similarly, 99% of *Campylobacter jejuni* strains recovered from broiler chicken house environments were triclosan resistant.[30]

CTAB, a quaternary ammonium compound, is a widely used antimicrobial cationic surfactant.[3,18,24,30] The MICs for CTAB ranged between 0.25 and 32 µg/mL among the *S. aureus* meat isolates. The MIC_{50} and MIC_{90} values for the CTAB were 1 µg/mL and 8 µg/mL, respectively (Table 1). The disinfectant CTAB MICs in this study were higher than the MICs (range from 0.25 to 2 µg/mL) for the *S. aureus* strains isolated from swine feces reported by Beier et al.[24] In contrast to the current study, the researchers observed higher MIC_{50} values for CTAB (4 µg/mL) in *C. jejuni* strains from the litter of broiler chicken houses.[30] However, in this study, the isolates from chicken meat with a rate of 38.5% demonstrated high CTAB MIC values (≥ 4 µg/mL) (Table 2).

Organic acids have strong bactericidal effects and can be applied in the sanitization process of food and food environments.[7,25,27] The susceptibility of three organic acids, including acetic acid, citric acid, and lactic acid, against *S. aureus*, isolates from various types of meat are shown in Table 1 and Figure 2. The MIC_{50} results of acetic, lactic, and citric acids against *S. aureus* isolates were 1641, 1406, and 1500 µg/mL, respectively (Table 1). The MICs for acetic, lactic, and citric acids against *S. aureus* isolates that inhibited 90% of isolates (MIC_{90}) were 3281, 5625, and 6000 µg/mL, respectively (Table 1).

In overall, the antimicrobial effectiveness of acids follows the order acetic > lactic > citric under similar conditions[3], consistent with the results of this study. In a study, acetic, lactic, and citric acids all had high MICs (1024 to 4096 µg/mL) against *E. coli* O157:H7 strains from cattle carcasses, feces, hides, and ground beef.[17] Acetic acids, also known as vinegar (5 to 10%), have antibacterial activity at low concentrations and have been used commonly in food and medicine.[3,29] In the present study, freshwater fish (100%), seawater fish (58.3%), ground beef (52.9%), and chicken meat (46.1%) had the highest percentages of acetic acid with MICs of 1641 µg/mL and 820 µg/mL (Table 3). In et al.[28] reported that acetic acid exhibited relatively high antimicrobial activity (the lowest MIC among organic acids) against all *Shigella* species, which was in parallel to our findings. Furthermore, the results showed that acetic acid had greater germicidal activity against most pathogens than lactic acid.[29] Humayoun et al.[25] found that the MIC_{50} of the tested multidrug-resistant *Salmonella* was 1640 µg/mL, which was like our acetic acid results. However, they found higher MIC_{50} values for lactic acid (5664 µg/mL) and citric acid (3156 µg/mL). In a study documented by Hussain et al.[31], citric acid was a more efficient agent than lactic acid at the tested concentrations in reducing *E. coli* and *Salmonella* growth. Acetic and citric acids were effective in inhibiting *Salmonella typhimurium*, *E. coli* O157:H7, and *S. aureus* in tabbouleh salad.[32] The organic acid disinfectants, including lactic acid and citric acid, exhibited good bactericidal activity against drug-resistant foodborne pathogens, including *Salmonella* and *Campylobacter*.[27] This study did not indicate a relationship between multi-drug resistance and susceptibility to organic acids in the *S. aureus* isolates from raw meat products. In medicine, Burns et al.[26] reported that all organic acids tested had antibacterial properties against the uropathogens, *Proteus mirabilis, S. aureus, E. coli*, and *P. aeruginosa*.

CONCLUSION

Sanitizers and disinfectants are widely used to prevent contamination by foodborne pathogens. Different disinfectants have varying degrees of antibacterial activity against numerous bacteria. In this study, susceptibility values of *S. aureus* originating from raw meat products to various disinfectants were determined. Triclosan and CTAB were more effective than organic acids, including acetic, lactic, and citric acids against the *S. aureus* isolates from meats. Resistance to triclosan was detected in 46% of the isolates, but almost all multidrug-resistant *S. aureus* isolates were triclosan-resistant. Among the organic acids tested, acetic acid had the most inhibitory effect on the *S. aureus* isolates from raw meats. The difference in MIC values between triclosan and organic acids, as well as between CTAB and organic acids, was statistically significant. *S. aureus* isolates from meat with high MIC values against disinfectants may pose a risk of growing antimicrobial resistance. The results of this study might be useful for evaluating the efficacy of disinfectants against *S. aureus* as a food pathogen and determining the development of resistance to them.

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Peer Review: Externally peer-reviewed.

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REFERENCES

1. Götz F, Bannerman T, Schleifer KH. The genera *Staphylococcus* and *Macrococcus*. In: Dworkin M, Falkow S,E, Rosenberg E, Schleifer KH, Stackebrandt E, eds. *The Prokaryotes*. 3rd ed. New York, N.Y.: Springer; 2006:5-75.
2. Bhunia AK. *Foodborne microbial pathogens: Mechanisms and pathogenesis*. New York, N.Y.: Springer; 2008.
3. Ray B. *Fundamental Food Microbiology*. 3rd ed. Boca Raton, Florida: CRC press; 2004.
4. Bloomfield SF, Exner M, Fara GM, Nath KJ, Scott EA. Hygiene procedures in the home and their effectiveness: A review of the scientific evidence base. International Scientific Forum on Home Hygiene. 2013. https://ifh-homehygiene.org/review-best-practice/hygiene-procedures-home-and-their-effectiveness-review-scientific-evidence-base/.
5. Donaghy JA, Jagadeesan B, Goodburn K, et al. Relationship of sanitizers, disinfectants, and cleaning agents with antimicrobial resistance. *J Food Prot.* 2019;82(5):889-902.
6. Beuchat LR. Surface decontamination of fruits and vegetables eaten raw: A review. World Health Organization. 1998.
7. Lepaus BM, Rdcha JS, Sadjse JFB. Organic acids and hydrogen peroxide can replace chlorinated compounds as sanitizers on strawberries, cucumbers and rocket leaves. *Food Sci Technol.* 2020;40:242-249.
8. Suller MTE, Russell AD. Triclosan and antibiotic resistance in *Staphylococcus aureus. J Antimicrob Chemother.* 2000;46:11-18.
9. Sheldon AT. Antiseptic “Resistance”: Real or perceived threat? *Clin Infect Dis.* 2005;40:1650-1656.
10. Jones RD, Jampani HB, Newman JL, Lee AS. Triclosan: A review of effectiveness and safety in health care settings. *Am J Infect Control*. 2000;28:184-196.
11. Glaser A. The ubiquitous triclosan: A common antibacterial agent exposed. *Pesticides and You NCODM*. 2004;24(3):12-17.
12. Yao K, Wen K, Shan W, Jiang H, Shao B. An immunoaffinity purification method for the simultaneous analysis triclocarban and triclosan in foodstuffs by liquid chromatography tandem mass spectrometry. *J Agric Food Chem*. 2019;67:9088-9095.
13. Azzouz A, Colon LP, Hejji L, Ballesteros E. Determination of alkylphenols, phenylphenols, bisphenol A, parabens, organophosphorus pesticides and triclosan in different cereal-based foodstuffs by gas chromatography–mass spectrometry. *Anal Bioanal Chem*. 2020;412:2621-2631.
14. Coia JE, Duckworth GJ, Edwards DI, et al. Guidelines for the control and prevention of meticillin-resistant *Staphylococcus aureus* (MRSA) in healthcare facilities. *J Hosp Infect*. 2006;63S:S1-S44.
15. Ciusa ML, Furi L, Knight D, et al. A novel resistance mechanism to triclosan that suggests horizontal gene transfer and demonstrates a potential selective pressure for reduced biocide susceptibility in clinical strains of *Staphylococcus aureus. Int J Antimicrob Agents.* 2012;40:210-220.
16. Fan F, Yan K, Wallis NG, et al. Defining and combating the mechanisms of triclosan resistance in clinical isolates of *Staphylococcus aureus. Antimicrob Agents Chemother.* 2002;46(11):3343-3347.
17. Beier RC, Poole TL, Brichta-Harhay DM, et al. Disinfectant and antibiotic susceptibility profiles of *Escherichia coli* O157:H7 strains from cattle carcasses, feces, and hides and ground beef from the United States. *J Food Prot*. 2013;76:6-17.
18. Beier RC, Foley SL, Davidson MK, et al. Characterization of antibiotic and disinfectant susceptibility profiles among *Pseudomonas aeruginosa* veterinary isolates recovered during 1994-2003. *J Appl Microbiol*. 2014;118:326-342.
19. Beier RC, Duke SE, Ziprin RL, et al. Antibiotic and disinfectant susceptibility profiles of vancomycin-resistant *Enterococcus faecium* (VRE) isolated from community wastewater in Texas. *Bull Environ Contam Toxicol*. 2008;80:188-194.
20. Beier RC, Callaway TR, Andrews K, et al. Disinfectant and antimicrobial susceptibility profiles of *Salmonella* strains from feedlot water-sprinkled cattle: Hides and feces. *J Food Chem Nanotechnol*. 2017;3:50-59.
21. McBain AJ, Ledder RG, Moore LE, Catrenich CE, Gilbert P. Effects of quaternary-ammonium–based formulations on bacterial community dynamics and antimicrobial susceptibility. *Appl Environ Microbiol*. 2004;70(6):3449-3456.
22. To MS, Favrin S, Romanova N, Griffiths MW. Postadaptational resistance to benzalkonium chloride and subsequent physicochemical modifications of *Listeria monocytogenes. Appl Environ Microbiol.* 2002;68(11):5258-5264.
23. Nakata K, Tsuchido T, Matsumura Y. Antimicrobial cationic surfactant, cetyltrimethylammonium bromide, induces superoxide stress in *Escherichia coli* cells*. J Appl Microbiol.* 2011;110:568-579.
24. Beier RC, Andrews K, Hume ME, et al. Disinfectant and antimicrobial susceptibility studies of *Staphylococcus aureus* strains and ST398-MRSA and ST5-MRSA strains from swine mandibular lymph node tissue, commercial pork sausage meat and swine feces. *Microorganisms*. 2021;9:2401. doi.org/10.3390/microorganisms9112401.
25. Humayoun SB, Hiott LM, Gupta SK, et al. An assay for determining the susceptibility of *Salmonella* isolates to commercial and household biocides. *PloS one*. 2018;13(12):e0209072. doi.org/10.1371/journal.pone.0209072.
26. Burns J, McCoy CP, Irwin NC. Synergistic activity of weak organic acids against uropathogens. *J Hosp Infect.* 2021;111:78-88.
27. Bai Y, Ding X, Zhao Q, et al. Development of an organic acid compound disinfectant to control food-borne pathogens and its application in chicken slaughterhouses. *Poult Sci.* 2022;101:101842. doi.org/10.1016/j.psj.2022.101842.
28. In YW, Kim JJ, Kim HJ, Oh SW. Antimicrobial activities of acetic acid, citric acid and lactic acid against *Shigella species. J Food Saf.* 2013; 33:79-85.
29. Pangprasit N, Srithanasuwan A, Suriyasathaporn W, et al. Antibacterial activities of acetic acid against major and minor pathogens isolated from mastitis in dairy cows. *Pathogens*. 2020; 9:961. doi:10.3390/pathogens9110961.
30. Beier RC, Byrd JA, Andrews K, et al. Disinfectant and antimicrobial susceptibility studies of the foodborne pathogen *Campylobacter jejuni* isolated from the litter of broiler chicken houses. *Poult Sci*. 2021;100:1024-1033.
31. Hussain G, Rahman A, Hussain T, Uddin S, Ali T. Citric and lactic acid effects on the growth inhibition of *E. coli* and *S. typhymurium* on beef during storage. *Sarhad J Agric.* 2015;31(3):183-190.
32. Al-Rousan WM, Olaimat AN, Osaili TM, Al-Nabulsi AA, Ajo RY, Holley RA. Use of acetic and citric acids to inhibit *Escherichia coli* O157:H7, *Salmonella Typhimurium* and *Staphylococcus aureus* in tabbouleh salad. *Food Microbiol*. 2018;73:61-66.

33. Brakstad OG, Aasbakk K, Maeland JA. Detection of *Staphylococcus aureus* by polymerase chain reaction amplification of the nuc gene. *J Clin Microbiol*. 1992;30(7):1654-1660.
34. Bannerman TL, Peacock SJ. *Staphylococcus, Micrococcus*, and other catalase-positive cocci. In: Versalovic J, Carroll KC, Funke G, Jorgensen JH, Landry ML, Warnock DW, eds. Manual of clinical microbiology, Washington, D.C: ASM Press; 2011:308-330.
35. Clinical and Laboratory Standards Institute (CLSI), Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically; Ninth edition, M7-A9, in, Wayne, PA, USA, 2012.
36. Clinical and Laboratory Standards Institute (CLSI), Performance standards for antimicrobial susceptibility testing; 32nd edition, in, Malvern, PA, USA, 2022.
37. Andrews JM. Determination of minimum inhibitory concentrations. *J Antimicrob Chemother*. 2001;48:5-16.
38. Heath RJ, Rock CO. A triclosan-resistant bacterial enzyme. *Nature*. 2000;406:145-146.

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