Development of an organic acid compound disinfectant to control food-borne pathogens and its application in chicken slaughterhouses

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ABSTRACT During poultry slaughter, cross-contamination of chicken carcasses with microorganisms (including drug-resistant bacteria) can occur because of incomplete disinfection during the pre-cooling process, and surface contact with contaminated tools and equipment. The use of disinfectants is the most common way to reduce the risk of cross-contamination and bacterial spread, as they can effectively reduce the number of bacteria. We developed a disinfectant consisting of organic acids and sodium dodecyl sulfate (**SDS**) and tested its bactericidal effects at different concentrations against *Salmonella* and *Campylobacter*. The main effective components in the disinfectant were citric acid, lactic acid, and SDS, and together they exerted a synergistic bactericidal effect. The bactericidal efficacy of the disinfectant increased with increasing concentrations of the 3 active ingredients. To reach a 100% reduction rate during a 15-s treatment in vitro, for *Salmonella*, the lowest concentrations of citric acid, lactic acid, and SDS were 0.06, 0.08, and 0.02%, respectively; and for *Campylobacter*, the lowest concentrations were 0.02, 0.025, and 0.0125%, respectively. The disinfectant remained effective in presence of interfering substances (e.g., 15% fetal bovine serum). Further experiments showed that the disinfectant inactivated sensitive bacteria as well as 23 drug-resistant strains of *Salmonella* and *Campylobacter*. Treatment with the disinfectant for 15 s decreased the concentrations of all tested strains by more than 4.7 log colony forming units per mL, and the reduction rate was as high as 100%. In on-site disinfection tests in chicken slaughterhouses, the disinfectant significantly reduced the number of pathogenic bacteria on carcasses during the pre-cooling process, and on tools (such as knives and gloves) during the segmentation process. Thus, this disinfectant has potential uses in preventing cross-contamination of food-borne pathogens (including resistant bacteria) in slaughterhouses.

Key words: organic acids, disinfectant, food-borne pathogen, cross-contamination, resistance

INTRODUCTION

Salmonella and *Campylobacter* are common zoonotic pathogens that cause gastroenteritis and food poisoning in humans, with symptoms such as abdominal pain, vomiting, and diarrhea. These illnesses can be life-threatening in severe cases. In 2015, the World Health Organization released a report, "Estimation of the Global Burden of Foodborne Diseases", stating that more than half of the types of food-borne diseases are diarrheal diseases that are usually caused by the consumption of foods contaminated with *Campylobacter*, *Salmonella*, and pathogenic *Escherichia coli*. These pathogens cause about 550 million people to become sick and 23,000 deaths annually. Food-borne pathogens have a high prevalence rate in chicken products in the retail market (Jerngklinchan et al., 1994; Uyttendaele et al., 1998; Zhao et al., 2001; Capita et al., 2003; Yang et al., 2011), and poisoning incidents occur from time to time. There are several risk factors for the spread of pathogenic bacteria on farms. Control measures implemented to reduce contamination of poultry flocks on farms include vaccination of the breeding chickens, competitive exclusion, the use of prebiotics, acidification of feed and drinking water, and strict hygiene measures (Doyle and Erickson, 2006). Even so, food-borne pathogens can contaminate carcasses during processing in the slaughterhouse (Corry et al., 2010). Because of the wide range of sources of chickens in slaughterhouses and their different health conditions, cross-contamination can easily occur during slaughtering and processing. Previous

studies have explored the risk factors for contamination, and have identified how carcasses become contaminated during slaughter. First, incomplete disinfection during the pre-cooling process can lead to residual pathogens on the carcasses. Second, bacteria that survive on the processing equipment can be an important source for carcass-contamination (Olsen et al., 2003; Rasschaert et al., 2006, 2008; Christian et al., 2020). For example, during the segmentation process after pre-cooling disinfection, the cutting tools and gloves that come into contact with chicken carcasses can serve as vectors to cause secondary cross-infection by pathogenic bacteria. Consequently, pre-cooling and segmentation are the key processes to control the cross-contamination of food-borne pathogens, otherwise, the pathogens will spread into the retail market via contaminated chicken products (Olsen et al., 2003; Rasschaert et al., 2006). Many studies have found that the strains of *Salmonella* and *Campylobacter* isolated from broiler slaughter plants in different countries and areas are generally resistant or even multidrug resistant (Wang et al., 2013; Mainali et al., 2014; Youn et al., 2017). The spread of these drug-resistant bacteria would be devastating for human health (Pèrez- Boto et al., 2014).

Disinfectant treatments (especially process disinfection) are the main method to reduce the risk of cross-contamination by surface contact. The disinfectants commonly used on slaughter lines include sodium hypochlorite, peracetic acid, quaternary ammonium salts, and organic acids (Northcutt et al., 2007; Chaine et al., 2013; European Commission, 2013; European Food Safety Authority, EFSA Panel on Biological Hazards 2014; Nhung et al., 2015; Scott et al., 2015; Christian et al., 2020). However, these disinfectants have certain limitations. For example, the by-products of sodium hypochlorite have potential tertiary risks (carcinogenicity, teratogenicity, and reproductive toxicity), and quaternary ammonium disinfectants can cause a range of bacteria to become resistant to this class of compounds (Russell Scott and Axtell Stephen, 2005; Corry et al., 2010; Han et al., 2019). High concentrations of organic acids negatively affect the flavor of chicken (Smulders and Greer, 1998), and organic substances can weaken the disinfection effect of single-component organic acid disinfectants (Christian et al., 2020).

Previous studies have demonstrated that lactic acid (**LA**) and citric acid (**CA**) have strong bactericidal effects (Dibner and Buttin, 2002; Mroz, 2005). As permitted food-additives (GB 1886.173-2016, 2016; GB 1886.235-2016, 2016), lactic acid and citric acid comply with food standards. Recent studies have shown that combining a surfactant with organic acids enhances the bactericidal activity of organic acids. For example, sodium dodecyl sulfate (**SDS**) was found to enhance the bactericidal activity of an organic acid against *Salmonella* derived from chicken and cantaloupes (Zhao et al., 2011; Webb et al., 2013), and against *Salmonella* and *Escherichia coli* on dressed chicken skin (Zhao et al., 2009; Hamdy et al., 2015;). Also, SDS is a permitted ingredient in a food disinfectant (The Ministry of Health of China, 2009), and has generally recognized as safe (**GRAS**) status (FDA, 2007). To date, there have been almost no reports on the bactericidal effect of organic acids against resistant bacteria. Previous studies have mainly focused on chicken cages, preharvest poultry, cantaloupes, and processing water, but few have focused on events during poultry slaughter and processing in slaughterhouses.

The main goal of this study was to develop a highly effective disinfectant that is compliant with food standards, which can control the spread of food-borne pathogens (including drug-resistant bacteria) when used, for example, in slaughterhouses.

MATERIALS AND METHODS

Materials

Citric acid, lactic acid, SDS, and fetal bovine serum (**FBS**) were purchased from Yuanye Bio-Tech Co., Ltd. (Shanghai, China). Nutrient agar plates were purchased from Aoboxing Bio-Tech Co., Ltd. (Beijing, China). Columbia blood plates were purchased from Niupu Bio-Tech Co. (Beijing, China). Brilliance agar plates and the culture test kits for *Campylobacter* were purchased from the Zhongchuanghuike Biotechnology Co., Ltd. (Qingdao, China). Gram-negative aerobe/anaerobe susceptibility plates were purchased from Xingbai Biotechnology Co., Ltd. (Shanghai, China). All other solvents and chemicals were of analytical grade and were used without further purification. The presence of resistance genes in bacterial strains was determined by sequencing, which was conducted by the Alvesen Sequencing Co., Ltd. (Beijing, China).

Bacterial Strains

Laboratory tests were carried out on *Salmonella* and *Campylobacter* strains isolated from chicken slaughterhouses in Shandong, Hebei, and Henan Provinces. All samples were collected using sterile cotton swabs. The swabs were packed in sterile plastic bags, transported to the laboratory under cooled conditions, and processed the same day.

Bacteriological Cultures

For *Salmonella*, samples were added to selenite cysteine broth and incubated at 37°C for 18 h. Then, 0.2 mL culture was inoculated onto Brilliance agar plates and the plates were incubated at 37°C for 18 h. Suspected colonies were subcultured onto nutrient agar plates, and incubated at 37°C for 24 h.

For *Campylobacter*, samples were added to selenite cysteine broth and incubated at 42°C for 24 h in micro-aerobic environment. Then, 0.3 mL culture was inoculated onto filter membranes of the culture test kit (*Campylobacter*) and incubated under the same conditions for 36 h. Single colonies were subcultured onto Columbia blood plates, and the plates were incubated at 42°C for 36 h.

All strains were identified and confirmed by polymerase chain reaction (**PCR**) using *Salmonella*-specific and *Campylobacter*-specific primers. *Salmonella* (forward, 5'-GTGAAATTATCGCCACGTTCGGGCAA-3'; reverse, 5'-TCATCGCACCGTCAAAGGAACC-3'), Campylobacter jejuni (forward, 5'-CATCTTCCCTAGTCAAGCCT-3'; reverse, 5'-AAGATATGGCACTAGCAAGAC-3'), Campylobacter coli (forward, 5'-AGGCAAGG-GAGCCTTTAATC-3'; reverse, 5'-TATCCCTATCTA-CAAATTCGCTATCCCTATCTACAAATTCGC-3').

Antibiotic Sensitivity Test

The resistance characteristics of bacterial isolates were analyzed according to the standard of the United States Committee for Clinical Laboratory Standardization (**CLSI**) (Arendrup et al., 2017). *Salmonella* isolates were treated with 16 kinds of antibacterial drugs (ampicillin, amoxicillin/clavulanic acid, gentamycin, spectinomycin, tetracycline, florfenicol, sulfafurazole, trimethoprim/ sulfamethoxazole, ceftiofur, ceftazidime, enrofloxacin, ofloxacin, meropenem, apramycin, colistin, mequindox) by the micro broth-dilution method. The *Salmonella* serotypes identified were randomly selected for resuscitation culture, wherein 2 to 3 single colonies were selected and placed in 5 mL sterilized normal saline. Turbidity was measured using a 0.5 MacFarland turbidimetric tube, and the concentration of the liquid was about 1×10^7 to 1×10^8 colony forming units CFU/mL. For each of the above bacterial solutions, 60 μL was added to 12 mL drug-sensitive culture medium, and the mixture was then mixed and diluted. An automatic microbial sampling instrument was used to add 100 μL of the mixture to each well of a 96-well Gram-negative aerobic bacteria drug-sensitive plate. The plates were incubated in a constant temperature incubator at 37°C for 18 to 24 h. On the premise that the minimum inhibitory concentration of the quality control strains was within the specified range, the sensitivity of the tested strains was judged according to the criteria specified in the kit's instructions, and the drug resistance results were analyzed.

The same method was used to determine the resistance of *Campylobacter* isolates to 9 antibacterial drugs (azithromycin, ciprofloxacin, erythromycin, gentamycin, tetracycline, florfenicol, nalidixic acid, telithromycin, clindamycin).

Disinfectants

Different formulas with different ratios of citric acid, lactic acid, and SDS were pre-screened to determine their effectiveness.

Concentrated solutions of citric acid (4%), lactic acid (5%), and SDS (1%) were prepared separately. For example, 4 g citric acid solid was dissolved in 96 g water to obtain a 4% solution. Lactic acid (5%), and SDS (1%) solutions were prepared in the same way. Then, 1.5 g citric acid (4%), 1.6 g lactic acid (5%), 2 g SDS (1%), and 94.9 g water were mixed with stirring to make a total

Table 1. Different disinfectant combinations tested.

Different disinfectants	w/w (%)		
	CA	LA	SDS
1	0.04	0.05	0.01
2	0.06	0.08	0.02
3	0.02	0.025	0.0125
4	0.03	0.04	0.01
5	0.01	0.0125	0.006
6	0.015	0.019	0.009
7	-	0.08	0.005
8	-	0.08	0.01
9	-	0.08	0.02
10	-	0.08	0.03
11	-	0.08	0.04
12	-	0.10	0.02
13	-	0.12	0.02
14	-	0.14	0.02
15	-	0.16	0.02

Abbreviations: CA, citric acid; LA, lactic acid; SDS, sodium dodecyl sulfate.

weight of 100 g. The same method was used to produce the disinfectant formula of CA-0.06%, LA-0.08%, SDS-0.02%, as well as formulas with other ratios of the 3 constituents (Table 1).

Suspension Quantitative Tests

Suspension quantitative tests were carried out to evaluate the efficacy of the disinfectant formulas. Before starting the tests, all reagents were equilibrated to 20°C in a water-bath. Turbidity was measured with a 0.5 MacFarland turbidimetric tube, and the concentration of the modulated bacterial suspensions was about 1×10^7 to 1×10^8 CFU/mL. An aliquot of each bacterial suspension (0.5 mL) was added to 4.5 mL disinfectant solution, and the mixture was mixed by vortexing. After a defined reaction time (15 s, 30 s, or 1 to 30 min), 0.5 mL mixture was removed and added to 4.5 mL neutralization medium. After a neutralization time of 10 min, further decimal dilutions (1−100 times) were made with an appropriate diluent and 0.1 mL of the mixture was inoculated onto duplicate plates of an appropriate medium. The plates were incubated at 37°C for 24 h to culture *Salmonella* and at 42°C for 24 to 48 h under micro-aerobic conditions to culture *Campylobacter*. In the control group, saline was used instead of disinfectant. The viable bacteria were quantified by counting the colonies after incubation.

The reduction rate at each time point was calculated based on the number of viable bacteria in the control group and the test group before and after disinfection. The reduction rate was calculated as follows: $(P_t) = [(n_0 - n_t)/ n_0] \times 100$ %, where t is the disinfection time, n_0 is the number of bacteria disinfection or in the control group, and n_t is the number of bacteria after disinfection or in the test group.

Neutralization Medium

Neutralization medium was prepared by adding 0.5 mL NaOH solution (1 mol/L) to 200 mL phosphate buffer

Table 2. Neutralization test of disinfectant.

Recovery of bacteria number in each group (CFU/mL)						Error rate of colony number among Groups 3, 4, and 5 (%)
1	2	3	4	5	6	2.8
183	260	5.6×10^5	5.3×10^5	5.5×10^5	0	

Group 1: disinfectant + bacterial suspension; Group 2: (disinfectant + bacterial suspension) + neutralizer; Group 3: neutralizer + bacterial suspension; Group 4: (disinfectant + neutralizer) + bacterial suspension; Group 5: diluent + bacterial suspension; Group 6: (diluent + neutralizer) + culture medium.
Composition of disinfectant used in neutralization test: CA-0.04%, LA-0.05%, SDS-0.01%; reaction time: 2 min.

(pH 7.2, consisting of Na_2HPO_4, KH_2PO_4 and NaCl at 0.02, 0.01, and 0.15 mol/L, respectively). The neutralization medium analysis was carried out according to the *Technical Standard for Disinfection* issued by the Ministry of Health of the People's Republic of China in 2002. As shown in Table 2, 6 groups were tested, separately: Group 1: disinfectant + bacterial suspension; Group 2: (disinfectant + bacterial suspension) + neutralizer; Group 3: neutralizer + bacterial suspension; Group 4: (disinfectant + neutralizer) + bacterial suspension; Group 5: diluent + bacterial suspension; Group 6: (diluent + neutralizer) + nutrient medium. The concentration of the modulated bacterial suspension was about $1 \times 10^7 \sim 1 \times 10^8$ CFU/mL. The formula of the disinfectant used in these tests was CA-0.04%, LA-0.05%, SDS-0.01%. After a defined reaction time, 0.1 mL mixture was inoculated onto duplicate plates as described for the quantitative suspension tests. Saline solution was used as the diluent.

Organic Protection Experiment

In these analyses, FBS was used as the organic interfering substance. According to the Technical Specification for Identification of Veterinary Disinfectants issued by Ministry of Agriculture of the People's Republic of China in 1992, FBS was added to the bacterial suspension to reach final concentrations of 10, 15, and 20%. The control consisted of the bacterial suspension without FBS. The organic protection experiment was carried out in the same way as the quantitative suspension tests, with bacterial suspensions containing FBS (0.5 mL) added to the 4.5 mL disinfectant solution.

Stability Test

The stability of the disinfectants was tested in 2 ways: temperature-accelerated testing and long-term storage. The disinfectant was stored at 54°C for 14 d, or stored at room temperature for 12 mo. The bactericidal effects of the disinfectant were determined by quantitative suspension tests.

On-Site Disinfection

Three chicken slaughterhouses in Anhui and Hebei provinces in China were selected for in vivo treatments. In one slaughterhouse, swab samples were taken after pre-cooling, and *Salmonella* was isolated. In the test

group, 30 chicken carcasses were soaked in a pre-cooling tank (about 50 L) for disinfection with the compound disinfectant (CA-0.06%, LA-0.08%, SDS-0.02%) for 15 min or 60 min, and then swab samples were taken from carcasses without drying. The control group was not subjected to disinfection treatments before swab samples were taken. In another 2 slaughterhouses, 60 or 40 swab samples were taken from segmentation tools after the pre-cooling process, and *Salmonella* and *Campylobacter* strains were isolated. In the test group, the cutting tools and gloves used to process carcasses were changed and soaked in the compound disinfectant (CA-0.06%, LA-0.08%, SDS-0.02%) for 1 min. In the control group, the cutting tools and gloves were not changed or subjected to disinfection treatments. Swab samples were taken from cutting tools and gloves after each disinfection in the test group, or after each carcass was segmented in the control group.

The percentage decrease in separation rate was calculated as follows: (separation rate without disinfectant − separation rate with disinfectant) / separation rate without disinfectant × 100%.

RESULTS

Neutralization Medium Analyses

The results of the neutralization medium analyses are shown in Table 2. Only a small number of colonies grew in Group 1. There were slightly more colonies in Group 2. Groups 3, 4, and 5 had the largest number of colonies, and a similar amount of bacteria grew. The error rate of colony number among Group 3, 4, 5 was 2.8%. Group 6 grew aseptically. These results show that the neutralization agent was able to effectively neutralize the organic acid disinfectant.

Reduction of Salmonella and Campylobacter In Vitro

We investigated the bactericidal effects of disinfectants at different concentrations using a quantitative method. As shown in Figure 1, for *Salmonella*, when the concentrations of citric acid, lactic acid, and SDS were 0.03, 0.04, and 0.01%, respectively (formula 3: CA-0.03%, LA-0.04%, SDS-0.01%), the reduction rate of the disinfectant was 100% (no visible colonies on the nutrient agar plate without dilution) after 20 min of treatment. Formula 2, which had the same concentrations of
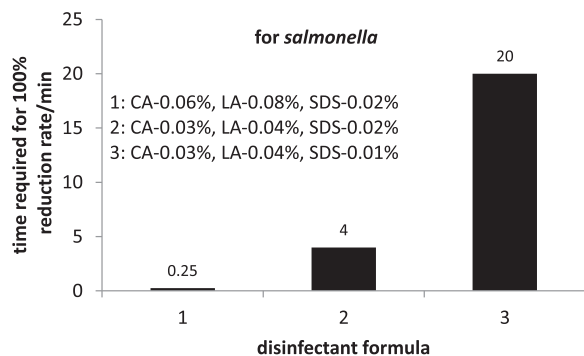
Figure 1. Time required for disinfectants to reach 100% reduction rate against *Salmonella. Salmonella* strain was multidrug resistant to ampicillin and sulfafurazole, and was isolated from a chicken slaughterhouse in Shandong Province.

citric acid and lactic acid as formula 3 but double the SDS concentration (formula 2: CA-0.03%, LA-0.04%, SDS-0.02%) showed a 100% reduction rate after 4 min. Formula 1 (CA-0.06%, LA-0.08%, SDS-0.02%), which had twice the concentrations of citric acid, lactic acid, and SDS as formula 3, showed a greatly improved reduction rate (100% in 15 s).

Next, the bactericidal effects of the disinfectants at different concentrations against *Campylobacter* were investigated (Figure 2). Formula 3 (CA-0.01%, LA-0.0125%, SDS-0.006%) had a reduction rate of 100% after 25 min. Formula 2 (CA-0.015%, LA-0.019%, SDS-0.009%), which had 1.5 times the concentrations of citric acid, lactic acid, and SDS as formula 3, had a 100% reduction rate after 3 min. Formula 1 (CA-0.02%, LA-0.025%, SDS-0.0125%), which had double the concentrations of components compared with formula 3, had a reduction rate of 100% in 15 s.

Influence of Active Ingredients on Disinfection Effect

The effect of the concentration of organic acids and SDS on the disinfection effect was investigated. First, the concentration of lactic acid was kept constant (0.08%) while the concentration of SDS was increased, and quantitative suspension tests were conducted
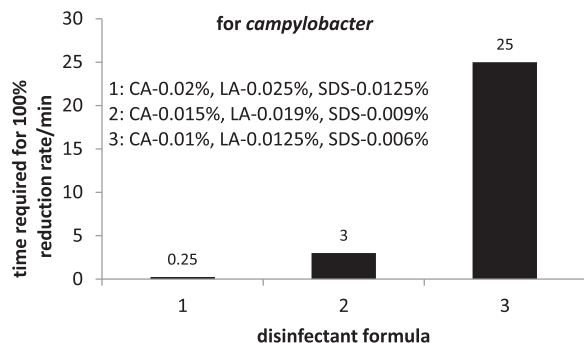

Figure 2. Time required for disinfectants to reach 100% reduction rate against *campylobacter. Campylobacter* strain was multidrug resistant to nalidixic acid, ciprofloxacin, and tetracycline, and was isolated from a chicken slaughterhouse in Hebei Province.
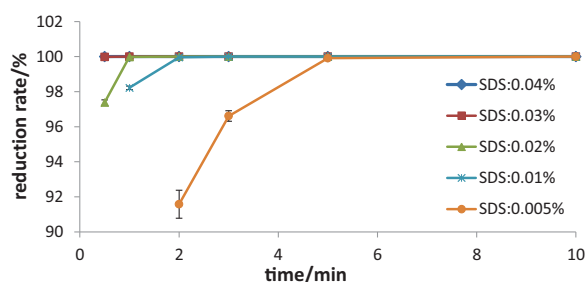

Figure 3. Effect of SDS concentration on bactericidal activity of disinfectant against *Salmonella* (all disinfectants contained 0.08% lactic acid).

(Figure 3). When the SDS concentration was 0.005, 0.01, and 0.02%, the reduction rate of the disinfectant for *Salmonella* was 100% after 5 min, 2 min, and 1 min, respectively. When the concentration of SDS increased to 0.03%, the reduction rate of the disinfectant for *Salmonella* reached 100% in 30 s.

Next, the influence of the lactic acid concentration on bactericidal activity was investigated. The concentration of SDS was kept constant (0.02%) while the concentration of lactic acid changed, and quantitative suspension tests were conducted (Figure 4). When the concentration of lactic acid was 0.08%, the reduction rate of the disinfectant for *Salmonella* was 100% after 2 min. As the lactic acid concentration increased, the time for the disinfectant to reach 100% reduction rate gradually decreased. When the concentration of lactic acid was 0.14%, the reduction rate of the disinfectant was 100% after 30 s.

Thus, the concentrations of organic acids and SDS in the disinfectant strongly affected its bactericidal activity.

Organic Protection Experiment

There are many kinds of organic substances that interfere with the activity of disinfectants in vivo. Therefore, we tested the bactericidal activity of the disinfectant in the presence of FBS at a range of concentrations. Different concentrations of FBS were used in tests with the disinfectant and *Salmonella* and *Campylobacter* (Table 3). In these tests, 10 or 15% FBS showed negligible interference, but 20% FBS weakly interfered with the bactericidal activity of the disinfectant (reduction rate of 100% in 3 min).
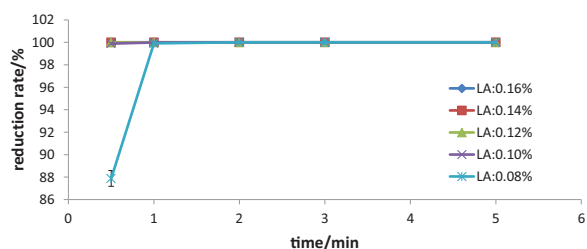

Figure 4. Effect of lactic acid concentration on bactericidal activity of disinfectant against *Salmonella* (all disinfectants contained 0.02% SDS).

Table 3. Bactericidal activity of disinfectant in the presence of FBS.

	Reduction rates at different times %									
	Salmonella [1]					*Campylobacter* [2]				
FBS	1 min	3 min	5 min	10 min	15 min	1 min	3 min	5 min	10 min	15 min
0%	100	100	100	100	100	100	100	100	100	100
10%	100	100	100	100	100	100	100	100	100	100
15%	100	100	100	100	100	99.98	100	100	100	100
20%	99.89	100	100	100	100	99.58	100	100	100	100

[1]Disinfectant composition: CA-0.06%, LA-0.08%, SDS-0.02%.
[2]Disinfectant composition: CA-0.02%, LA-0.025%, SDS-0.0125%.

Table 4. Bactericidal activity of disinfectant before and after temperature-accelerated test.

	Reduction rates at different times %									
	Salmonella [1]					*Campylobacter* [2]				
Acceleration time	1 min	3 min	5 min	7 min	10 min	1 min	3 min	5 min	7 min	10 min
0 d	100	100	100	100	100	100	100	100	100	100
14 d	100	100	100	100	100	100	100	100	100	100

[1]Disinfectant composition: CA-0.06%, LA-0.08%, SDS-0.02%.
[2]Disinfectant composition: CA-0.02%, LA-0.025%, SDS-0.0125%.

Stability Test

For practical applications, it is very important that a disinfectant is stable. Therefore, we tested the stability of the disinfectant at high temperatures and during long storage. As shown in Table 4, the disinfectant showed no decrease in bactericidal activity against *Campylobacter* and *Salmonella* in both the accelerated temperature test and the long-term stability test. Thus, the disinfectant has good thermal stability and long-term stability.

Applications of Organic Acid Disinfectant

Disinfection Effects Against Different Strains To further investigate the bactericidal effect of the organic acid disinfectant, it was tested against several isolated drug-resistant strains. As shown in Table 5, 36 strains were tested, including 23 strains of *Salmonella* (including 2 genetic drug-resistant strains and 10 phenotypic drug-resistant strains) and 13 strains of *Campylobacter* (11 phenotypic drug-resistant strains).

After treatment with the disinfectant for 15 s, the reduction rate was as high as 100%. As shown in Table 6, the abundance of all the investigated strains was reduced by more than 4.7 log CFU mL^{-1}, compared with their positive controls. Therefore, the organic acid disinfectant was able to kill food-borne bacteria quickly and efficiently, including 23 strains of drug-resistant bacteria (genetic and phenotypic).

On-Site Disinfection in Three Slaughterhouses The pre-cooling and segmentation steps are the key points at which cross-contamination of food-borne pathogens in slaughterhouses can be controlled. Thus, we evaluated

Table 5. Resistance of different *Salmonella* and *Campylobacter* strains

Strain number	Antibiotic resistance pattern	Strain number	Antibiotic resistance pattern	Strain number	Antibiotic resistance pattern
1[a]	tert(D)A B G T, sul2 4, dfrA3, TEM-1, AAC(6')-ly, aadA17, mcr-4 mcr-3.11	13	s	25	s
2	s	14	s	26[b]	CIP-TET-NAL
3[a]	tert(D)A B G T, sul2 4, dfrA3, AAC(6')-ly, aadA17, mcr-4 mcr-3.11	15	s	27[b]	CIP-TET-NAL-GEN-CLI-ERY-AZI-TEL
4[b]	CEF	16[b]	AMP-SUL	28[b]	CIP-TET-NAL-GEN-CLI-ERY-AZI-TEL
5	s	17[b]	AMP-SUL	29[b]	CIP-TET-NAL-GEN-CLI-ERY-AZI-TEL
6	s	18[b]	AMP-SUL	30[b]	CIP-TET-NAL-GEN-CLI-ERY-AZI-TEL
7[b]	COL	19[b]	AMP-MER-SUL	31[b]	CIP-TET-NAL-GEN-CLI-ERY-AZI-TEL
8[b]	COL	20[b]	AMP-MER-SUL	32[b]	CIP-TET-NAL-GEN-CLI-ERY-AZI-TEL
9[b]	CEF	21	s	33[b]	CIP-TET-NAL-GEN-CLI-ERY-AZI-TEL
10	s	22	s	34[b]	CIP-TET-NAL-GEN-CLI-ERY-AZI-TEL
11	s	23[b]	AMP-SUL	35[b]	CIP-TET-NAL
12	s	24	s	36[b]	CIP-TET-NAL

Abbreviations: AMP, ampicillin; AZI, azithromycin; CEF, ceftiofur; CIP, ciprofloxacin; COL, colimycin; CLI, clindamycin; ERY, erythromycin; GEN, gentamicin; MER, meropenem; NAL, nalidixic acid; SUL, sulfafurazole; TEL, telithromycin; TET, tetracycline.
1-23, *Salmonella*. 1-15, *pullorum* (1978-2015). 16-23, *enteritidis* (2018-2019), isolated from chicken slaughterhouses in Shandong, Henan, and Hebei. 24-36, *Campylobacter*. 24-25, *coli* (2017). 26-36, *jejuni* (2019).
[a]Bacteria with drug resistance genes.
[b]Drug-resistant bacteria. s, sensitive.

Table 6. Numbers of bacteria before and after treatment with organic acid disinfectant (log CFU mL^{-1}).

Strain number	Treatment		Reduction	Strain number	Treatment		Reduction	Strain number	Treatment		Reduction
	Before	After			Before	After			Before	After	
1	7.08	<2	5.08	13	6.60	<2	4.60	25	7.16	<2	5.16
2	7.08	<2	5.08	14	6.83	<2	4.83	26	7.12	<2	5.12
3	7.03	<2	5.03	15	6.84	<2	4.84	27	6.81	<2	4.81
4	7.33	<2	5.33	16	6.85	<2	4.85	28	7.40	<2	5.40
5	7.22	<2	5.32	17	7.25	<2	5.25	29	7.38	<2	5.38
6	7.10	<2	5.10	18	7.28	<2	5.28	30	7.23	<2	5.23
7	7.13	<2	5.13	19	7.5	<2	5.50	31	6.62	<2	4.62
8	7.05	<2	5.05	20	7.34	<2	5.34	32	6.54	<2	4.54
9	6.93	<2	4.93	21	7.39	<2	5.39	33	7.38	<2	5.38
10	6.61	<2	4.61	22	7.39	<2	5.39	34	7.38	<2	5.38
11	6.86	<2	4.86	23	7.22	<2	5.22	35	7.15	<2	5.15
12	7.04	<2	5.04	24	6.68	<2	4.68	36	7.08	<2	5.08

Detection limit of quantitative suspension tests, <2.
Disinfectant composition: CA-0.06%, LA-0.08%, SDS-0.02%.

the disinfection effect of the organic acid compound disinfectant at broiler slaughterhouses. Compared with the control group (no disinfection treatment), the test group showed markedly decreased bacterial contamination. As shown in Table 7, the separation rate of *Salmonella* from chicken carcasses was reduced by 80 and 100% in slaughterhouse I after disinfection treatments for 15 min and 60 min, respectively, at the pre-cooling stage. The total separation rate of bacteria from gloves and cutting tools was reduced by 78 and 67%, respectively, in slaughterhouse II, and by 64 and 67%, respectively, in slaughterhouse III. Thus, the organic acid disinfectant was able to reduce the separation rate at the pre-cooling stage, and to reduce secondary cross-infection caused by the spread of pathogenic bacteria through gloves, cutting tools, and other equipment. The gloves were more easily contaminated than were the cutting tools.

DISCUSSION

To develop a safe and effective disinfectant for use in chicken slaughterhouses, we selected the ingredients carefully. Lactic acid and citric acid are known to have good bactericidal effects and comply with food standards (Dibner and Buttin, 2002; Mroz, 2005; GB 1886.173-2016, 2016; GB 1886.235-2016, 2016), while SDS is a permitted ingredient in a food disinfectant (The Ministry of Health of China, 2009). Therefore, citric acid, lactic acid, and SDS were selected as the components of the compound disinfectant.

Previous studies have shown that the combination of a surfactant and organic acids can enhance the

bactericidal activity of organic acids, probably because of a synergistic effect between them (Hamdy et al., 2015). For example, SDS was found to enhance the bactericidal activity of levulinic acid against *Salmonella* derived from chicken and cantaloupes (Zhao et al., 2011; Webb et al., 2013). Similarly, in this study, we observed a synergistic interaction among citric acid, lactic acid, and SDS. When the concentrations of citric acid and lactic acid remained constant and the SDS concentration was increased, the bactericidal activity of the disinfectant against *Salmonella* and *Campylobacter* increased. Similarly, increasing concentrations of organic acids while the SDS concentration remained constant increased the bactericidal activity of the disinfectant.

The synergistic effect between organic acids and SDS is related to the mechanism of the compound disinfectant. Previous studies have shown that organic acids play an antibacterial role by reducing the intracellular pH (Davidson, 2001; Ricke, 2003; Maurer et al., 2005), and permeating the outer membrane of bacteria (Young, and Foegeding, 1993). As an anionic surfactant, SDS lyses membranes, resulting in their disintegration (Ward et al., 1998; Caspar et al., 2017). The synergistic effect between organic acids and SDS is probably because SDS weakens or destroys membranes, allowing H$^+$ to enter the bacterial cells (Hamdy et al., 2015).

Although previous studies have shown that SDS can enhance the bactericidal activity of organic acids (levulinic acid, lactic acid, and acetic acid) against some intestinal pathogens (such as *Salmonella* and pathogenic *Escherichia coli* O157H7) (Zhao et al., 2009; Hamdy et al., 2015), no previous studies have focused on

Table 7. Bacterial contamination during pre-cooling and segmentation process without (−) and with (+) organic acid compound disinfectant treatment.

	Pre-cooling process				Segmentation process							
	Slaughterhouse I				Slaughterhouse II				Slaughterhouse III			
	15 min		60 min		Gloves		Cutting tools		Gloves		Cutting tools	
	No.	SR[1]	No.	SR[1]	No.	SR[2]	No.	SR[2]	No.	SR[2]	No.	SR[2]
−	5/30	16.7%	5/30	16.7%	14/60	23.3%	9/60	15%	11/40	27.5%	6/40	15%
+	1/30	3.3%	0/30	0%	3/60	5%	3/60	5%	4/40	10%	2/40	5%

[1]Separation rate of *Salmonella* from chicken carcasses.
[2]Total separation rate of bacteria, including *Salmonella* and *Campylobacter*. Disinfectant composition: CA-0.06%, LA-0.08%, SDS-0.02%.

the combination of SDS and citric acid. In addition, drug resistance is becoming an increasingly serious problem, and disinfectants must be able to kill drug-resistant bacteria to ensure food safety and protect public health. The drug resistance mechanisms of many intestinal pathogens are due to changes in their surface structure and function (Allen et al., 2010; Blair et al., 2015). It is possible that these changes may also affect their sensitivity to disinfectants. However, few previous studies have tested the sterilization effect of organic acid disinfectants against drug-resistant intestinal bacteria. To investigate the bactericidal effect of the organic acid disinfectant, several drug-resistant strains were used in the disinfection tests. The organic acid disinfectant showed excellent bactericidal activity against drug-resistant bacteria obtained in different years, from a range of sources, of different serotypes (*Salmonella: enteritidis* and *pullorum; Campylobacter: jejuni* and *coli*), and with different types of resistance (genetic and phenotypic).

We also determined whether the disinfectant retained its bactericidal activity in the presence of interfering organic substances, in this case, FBS. This experiment mimicked the environment of slaughtering lines, where many organic substances are present. Previous studies have shown that the disinfection effects of a single organic acid are dramatically reduced in the presence of various residues (Christian et al., 2020), and this may limit the usefulness of those disinfectants in slaughterhouses. The compound disinfectant in this study remained effective even in the presence of 15% FBS, and showed advantages over a single organic acid when used in slaughterhouses.

The organic acid compound disinfectant showed an excellent bactericidal effect during on-site disinfection in slaughterhouses. To our knowledge, this is the first report of the bactericidal effect of an organic acid compound disinfectant against drug-resistant bacteria during chicken processing. Our results show that the developed disinfectant formula has great potential to control cross-contamination with foodborne pathogens during the slaughtering process and to reduce the spread of pathogens (including drug-resistant bacteria) in chicken products supplied to the retail market.

CONCLUSIONS

We developed a compound disinfectant consisting of organic acids and SDS that can quickly and efficiently kill different food-borne pathogens such as *Salmonella* and *Campylobacter,* including drug-resistant strains. The disinfectant was found to be effective in the presence of an organic interfering compound and stable under high temperatures and over time. When used during the pre-cooling process and segmentation of chicken carcasses in slaughterhouses, the compound disinfectant (CA-0.06%, LA-0.08%, SDS-0.02%) reduced the separation rate of *Salmonella* and *Campylobacter*. This new organic acid disinfectant has potential uses in the prevention and control of food-borne pathogens during poultry slaughter and processing.

DISCLOSURES

The authors have no conflicts of interest to report.

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