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Application of slightly acidic electrolyzed water for decontamination of stainless steel surfaces in animal transport vehicles

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ABSTRACT

The effectiveness of slightly acidic electrolyzed water (SAEW) in reducing *Escherichia coli*, *Salmonella typhimurim*, *Staphylococcus aureus* or bacterial mixtures on stainless steel surfaces was evaluated and compared its efficacy with composite phenol solution for reducing total aerobic bacteria in animal transport vehicles. Stainless steel surfaces were inoculated with these strains individually or in a mixture, and sprayed with SAEW, composite phenol, or alkaline electrolyzed water for 0.5, 1, 1.5 and 2 min. The bactericidal activity of SAEW increased with increasing available chlorine concentration and spraying duration. The SAEW solution of 50 mg l^{-1} of available chlorine concentration showed significantly higher effectiveness than composite phenol in reducing the pathogens on stainless steel surfaces ($P < 0.05$). Complete inactivation of pathogens on stainless steel surfaces were observed after treatment with alkaline electrolyzed water followed by SAEW at 50 mg l^{-1} of available chlorine concentration for 2 min or alkaline electrolyzed water treatment followed by SAEW treatment at 90 mg l^{-1} of available chlorine concentration for 0.5 min. The efficacy of SAEW in reducing total aerobic bacteria in animal transport vehicles was also determined. Vehicles in the disinfection booth were sprayed with the same SAEW, alkaline electrolyzed water and composite phenol solutions using the automatic disinfection system. Samples from vehicle surfaces were collected with sterile cotton swabs before and after each treatment. No significant differences in bactericidal efficiency were observed between SAEW and composite phenol for reducing total aerobic bacteria in the vehicles ($P > 0.05$). SAEW was also found to be more effective when used in conjunction with alkaline electrolyzed water. Results suggest that the bactericidal efficiency of SAEW was higher than or equivalent to that of composite phenol and SAEW may be used as effective alternative for reducing microbial contamination of animal transport vehicles.

1. Introduction

Contaminated poultry and livestock transport vehicles have been reported to be sources of many disease pathogens, including *Salmonella*, *Campylobacter*, *Escherichia coli*, *Streptococcus suis*, *Mycoplasma hyopneumoniae*, *Actinobacillus pleuropneumoniae*, classic swine fever virus, porcine epidemic diarrhea virus, porcine circovirus, and porcine reproductive and respiratory syndrome virus under field and experimental conditions (Dee and Corey, 1993; Fedorka-Cray et al., 1997; Fussing et al., 1998; Rajkowski et al., 1998; Gebreyes et al., 2004; Dee and Deen, 2006; Amass et al., 2007; Patterson et al., 2011; Hernández et al., 2013; Lowe et al., 2014). Pathogens-contaminated transport vehicles have the potential to infect other farms, abattoirs, and other animals if the vehicles were not cleaned and disinfected between trips (Fedorka-Cray et al., 1995; Rajkowski et al., 1998). Gebreyes et al. (2004) isolated the same serovars in the trucks as those were isolated from lymph nodes and cecal contents of slaughtered pigs. In another study, Mannion et al. (2008) reported that *Salmonella* isolates from the trucks were indistinguishable from those isolated on the farm, indicating the possibility of contamination during transport. Therefore, decontamination of animal transport vehicles have been shown to be an important component of an effective biosecurity program for lowering the potential in cross-contaminating other animals and farms. For example, cleaning and disinfection can reduce microbial contamination of poultry transport crates and decrease the prevalence of *Salmonella* spp. and *Escherichia coli* found in trucks (El-Assaad et al., 1995; Rajkowski et al., 1998; Allen et al., 2008).

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Chemical disinfectants like sodium hypochlorite, quaternary ammonium, phenol, and iodine have been used to disinfect transport cages and vehicles (El-Assaad et al., 1995; Ramesh et al., 2002, 2004; Berrang and Northcutt, 2005). However, sodium hypochlorite is highly unstable and inactivated by organic matter, may cause corrosion due to its oxidation potential, and can form potentially carcinogenic and teratogenic trihalomethanes, haloacetic acids, haloketones and chloropicrin (Allende et al., 2009; Gil et al., 2009; Keskinen et al., 2009). Povidone iodine has the disadvantage of hypersensitivity, discoloration of skin, and bacterial resistance (Zamora, 1986). An environmentally friendly method, electrolyzed water, has been introduced as an alternative and a novel sanitizer in the poultry and livestock industry (Hao et al., 2013a,b, 2014; Zheng et al., 2013).

Alkaline electrolyzed water (AlEW) with high pH value is produced from the cathode compartment in the electrolytic cell with a diaphragm by electrolyzing 0.1% sodium chloride solution, while acidic electrolyzed water is produced from the anode compartment (Ding et al., 2010; Fujiwara et al., 2011). AlEW is reported to have antioxidative effects on highly unsaturated fats and oils and have superoxide dismutase-like and catalase-like activities (Shirahata et al., 1997; Miyashita et al., 1999). Slightly acidic electrolyzed water (SAEW) with pH values of 5.0–6.5 is generated by electrolysis of dilute hydrochloric acid or sodium chloride solution or both in the electrolytic cell without a diaphragm separation (Nan et al., 2010; Ni et al., 2015). SAEW at a near-neutral pH has the advantage of being less corrosive for equipment and irritating for hands, and minimizing human health and safety issues from Cl_2 off-gassing. SAEW is also easy to transport and store as it can be produced onsite (Abadias et al., 2008; Guentzel et al., 2008; Cao et al., 2009). The principal ingredient of SAEW is the hypochlorous acid which has strong antimicrobial activity (Cao et al., 2009; Koide et al., 2009). Previous studies have shown that SAEW has strong bactericidal effect on *Salmonella*, *E. coli*, *S. aureus*, porcine reproductive and respiratory syndrome viruses, and pseudorabies virus (Issa-Zacharia et al., 2010; Nan et al., 2010; Hao et al., 2013c). SAEW is also effective in reducing or eliminating *S. enteritidis*, *E. coli* O157:H7, *S. aureus* and indigenous microbiota on the surfaces of egg shells (Cao et al., 2009; Ni et al., 2014), and in reducing the populations of *Listeria monocytogenes*, *Salmonella Typhimurium* and natural microflora in fresh chicken breast meat (Rahman et al., 2012). SAEW has also been proved effective in reducing microbes on the equipment surfaces and airborne bacteria in laying-hen houses and swine barns (Hao et al., 2013a,b, 2014; Zheng et al., 2013), and in removing biofilms and nosocomial pathogens in dental facility water systems (Komachiya et al., 2014). However, few studies have reported the effectiveness of slightly acidic electrolyzed water in reducing microbial contamination in animal transport vehicles. Therefore, the objective of this research was to evaluate the efficacy of SAEW in reducing populations of *E. coli*, *Salmonella typhimurium*, *S. aureus* or bacterial mixtures on stainless steel surfaces typically used in transport vehicles, and to compare its efficacy with composite phenol solution for reducing total aerobic bacteria in animal transport vehicles.

2. Materials and methods

2.1. Bacterial cultures

Escherichia coli (ATCC25922) and *Staphylococcus aureus* (ATCC6538) were obtained from the China Veterinary Culture Collection (CVCC, Beijing, China), and the strain of *Salmonella typhimurium* isolated from pigs was obtained from Jiangsu Academy of Agricultural Sciences (Jiangsu, China). All strains of *Escherichia coli*, *Salmonella typhimurium*, or *Staphylococcus aureus* were grown in 9 ml Tryptic Soy Broth (TSB, AOBOX Biotechnology Co. Ltd., Beijing, China) at 37 °C for 24 h. Following incubation, 10 ml of each culture was centrifuged at 3000 × *g* for 10 min at 4 °C (3K15, Sigma, Germany), washed twice with 0.1% peptone water and resuspended in 10 ml of the same solution to obtain a final cell concentration of about 10 $\log_{10}$ CFU ml^{-1}. The bacterial population in each culture was confirmed by plating 0.1 ml of appropriate dilutions of the suspension on Tryptic Soy Agar plates (TSA, AOBOX Biotechnology Co. Ltd., Beijing, China) and incubating the plates at 37 °C for 24 h. A pig fecal slurry was prepared by dissolving one part pig manure in five parts of deionized water to produce an organic load that would resemble the load during the actual cleaning of transport containers (Ramesh et al., 2002). One ml each of the bacterial culture and five ml of the sterilized pig fecal slurry solution were mixed to yield an organic suspension containing approximately 9 $\log_{10}$ CFU ml^{-1}.

2.2. Preparation and inoculation of surfaces

The test surface used in this study was 5 cm × 5 cm coupons of stainless steel representative of interior surfaces of typical animal transport vehicles. Before inoculation, each test was washed with detergent (Shenzhen Pukeyuan Huanbao Keji Co. Ltd, Shenzhen, China), rinsed in deionized water, and then autoclaved at 121 °C for 20 min. 0.1 ml of bacterial culture (9 $\log_{10}$ CFU ml^{-1}) in 20% pig fecal slurry was applied and spread evenly on a marked area of nine cm^2 on the tested surface with a sterile inoculating loop. After inoculation, the surfaces were air dried under a biosafety hood for one h at room temperature of 24 ± 1 °C.

2.3. Preparation of slightly acidic electrolyzed water and chemical disinfectant

SAEW at available chlorine concentration (ACC) of 30, 50, 70 and 90 mg l^{-1} were generated by electrolyzing 10% sodium chloride solution using a SAEW generator (Beijing Zhouji Ziyuan Huanbao Technology Co. Ltd., Beijing, China) set at five, seven, nine and 11 A, respectively. AlEW was prepared from the cathode compartment by electrolysis of 0.1% sodium chloride solution using an acidic electrolyzed water generator (Beijing Zhouji Ziyuan Huanbao Technology Co. Ltd., Beijing, China), which contained an electrolytic cell where anode and cathode electrodes were separated by a diaphragm (part of the product formed at the anode was redirected into the cathode chamber and mixed with the product formed at the cathode). Composite phenol (CP) solution was prepared by diluting with deionized water to obtain the final concentration of 1367 mg l^{-1}. Deionized water was used as control for this experiment. The ACC of SAEW and AlEW was determined by a colorimetric method using a digital chlorine test kit (RC-3F, Kasahara Chemical Instruments Corp., Saitama, Japan), which had a detection limit of 0–300 mg l^{-1}. The pH and oxidation-reduction potential (ORP) of all solutions were measured using a dual scale pH/ORP meter (ORP-421, Shanghai Kangyi Technology Co. Ltd., Shanghai, China) with pH and ORP electrodes. The accuracy of pH and ORP measurement were 0.01% and 0.1%, respectively, in the range of 0.00 to 14.00 for pH and −1999 to +1999 for ORP.

2.4. Effect of SAEW on E. coli, S. typhimurium or S. aureus on stainless steel surfaces

The surface disinfection test was based on EN 13697:2001 (EN13697, 2001), with the following modifications: Stainless steel surfaces inoculated with *E. coli*, *S. typhimurium* or *S. aureus* were randomly divided into nine treatment groups, each consisting of three samples. Inoculated samples (test coupons of stainless steel) were placed in petri dishes and sprayed with different disinfec-