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N-halamine incorporated antimicrobial nonwoven fabrics for use against avian influenza virus

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ABSTRACT

Airborne pathogens are one of the most common avenues leading to poultry diseases. Preventing the avian influenza (AI) virus from entering the chicken hatchery house is critical for reducing the spread and transmission of AI disease. Many studies have investigated the incorporation of antimicrobials into air filters to prevent viruses from entering the indoor environment. N-halamines are one of the most effective antimicrobial agents against a broad spectrum of microorganisms. In this study, 1-chloro-2,2,5,5-tetramethyl-4-imidazolidinone (MC, a variety of N-halamine) was coated on nonwoven fabrics to give the fabric antimicrobial activity against the AI virus. Results showed that MC exhibited potent antiviral activity either in suspension or in the air. Higher concentrations of MC completely inactivated AI viruses and disrupted their RNA, preventing them from being detected by the real time reverse transcriptase-polymerase chain reaction (RT-PCR) assay. Coating the fabrics with MC resulted in remarkably reduced presence of AI virus on the MC-treated fabric in a short period of time. Furthermore, aerosolized AI viruses were completely inactivated when they passed through filters coated with the MC compound. In addition, MC is not volatile and does not release any gaseous chlorine. The active chlorine in the MC compound is stable, and the coating procedure is straightforward and inexpensive. Therefore, this study validates a novel approach to reducing airborne pathogens in the poultry production environment.

1. Introduction

The avian influenza (AI) virus has caused severe poultry disease outbreaks around the world. The outbreaks of both highly pathogenic and low pathogenic AI virus have caused significant economic losses, including the financial losses associated with depopulation, disinfection, declining poultry exports, declining income, and increasing consumer costs (Swayne and Halvorson et al., 2008). Moreover, AI viruses such as H5N1 and H7N9 have exhibited interspecies transmission from poultry to humans, causing infection and death in humans. The World Health Organization (WHO) reported that since Feburary 2013 there have been 859 laboratory-confirmed cases of human infection from the A H5N1 avian influenza virus (including 453 deaths) and 1584 cases associated with the H7N9 virus (including 612 deaths) (WHO, 2017). Therefore, controlling the disease in animal sources is critical to decreasing the health risk to animals and humans.

The spread of the AI virus on poultry farms may easily occur as a result of direct contact between infected and susceptible birds, or through indirect contact including exposure to aerosol droplets. Therefore, biosecurity actions are important in preventing the spread of infection with the AI virus in poultry flocks. Many studies have reported that major disinfectants such as household bleach could effectively inactivate the AI virus if used properly (Prince et al., 2001). However, when the virus was shed in feces or feed with a significant amount of organic matter, the antiviral activity of aqueous chlorine was impaired. Additionally, although viruses in poultry are eliminated at the beginning of disinfection, viruses introduced later from outside the farm will continue to spread and be transmitted once disinfectants evaporate. The AI virus can be introduced onto poultry farms by various routes, including by infected birds, contaminated water and clothing, delivery vehicles, farm workers, etc. (Swayne and Halvorson et al., 2008). Therefore, preventing the introduction of the AI virus onto poultry farms is critical to eliminating or reducing AI disease.

Filter are commonly used in the ventilation system of an enclosed chicken hatchery to prevent the transmission of pathogenic bioaerosol from outside of the house. The pristine untreated filter can block some viruses from entering broiler houses but cannot inactivate them. Airborne viruses and pathogens that have accumulated on the surface of the filters may survive for weeks or even months, and then contaminate birds and workers if they are not handled and disposed of properly.

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Therefore, the development of air filters with antimicrobial functions is necessary.

For indoor air quality and safety, antimicrobial air filtration technology to control bioaerosols has been widely studied. Previous research has investigated various antimicrobial agent coatings, including silver (Sharma et al., 2009; Yoon et al., 2008), silver nitrate (Miaśkiewicz-Peska and Łebkowska, 2011), copper, carbon nanotubes (CNTs) (Jung et al., 2011a), N-halamines (Demir et al., 2015; Qian and Sun, 2003; Zhu et al., 2012), and natural antimicrobials such as silk sericin (Magaraphan et al., 2003), plant extracts (Choi et al., 2015; Jung et al., 2013, 2011b), and chitosan (Desai et al., 2009). Most of them were investigated for their antimicrobial activity against aerosolized bacteria; few of these materials were evaluated for their antiviral properties, particularly against the AI virus. In addition, these materials require sophisticated processes to manufacture and did not show significant antimicrobial activity. Copper-based antimicrobials have been investigated primarily for their antiviral effects. Borkow et al. (Borkow et al., 2007), however, showed that copper-oxide-based polypropylene filters did not cause a large reduction in human H3N2 virus. The same research group introduced copper oxide into a respiratory face mask containing a polypropylene layer, and the human H1N1 and avian H9N2 virus were not recovered after 30 min of exposure to aerosolized viruses (Borkow et al., 2010). Coating textiles with copper oxide is complicated and requires the use of high-risk chemicals such as formaldehyde (Borkow and Gabbay, 2004). Because of problems with all these methods, there is a need to identify materials that can inactivate the AI virus rapidly and effectively, and that can be manufactured using a simple and inexpensive technology.

N-halamines are a group of compounds containing one or more nitrogen-halogen covalent bonds. N-halamines are increasingly drawing the public's attention because of their superior antimicrobial efficiency, and they have been reviewed intensively (Dong et al., 2017). Many N-halamine compounds have been reported to possess potent antimicrobial activity (Hui and Debiemme-Chouvy, 2013). The antimicrobial mechanism involves the direct transfer of oxidative halogens to a cell after contact. The transferred halogens oxidize the amino acids in the cell membrane, inactivating the microorganism (Kenawy et al., 2007; Worley et al., 1988). N-halamine molecules are stable and do not release free oxidative halogen until they come into contact with microorganisms (Kenawy et al., 2007; Worley et al., 1988). Among N-halamine compounds, 1-chloro-2,2,5,5-tetramethyl-4-imidazolidinone (MC, Fig. 1) has been proven to have high antimicrobial activity and can be used to treat edible eggs, fish tanks, water, and equipment surfaces in chicken houses to kill microorganisms (Lauten et al., 1992; Ren, 2015; Worley et al., 1992). In addition, MC showed potent and long-lasting antibacterial effect when introduced to nonwoven fabrics (Demir et al., 2015). MC is considered a low toxic N-halamine compound. Kern et al. (2000) reported that the acute oral LD50 of MC was 338 mg/kg, which indicates MC has a potential application in food-related areas.

In this study, the antiviral activity of MC compounds was determined. We coated nonwoven fabrics with MC to test the antiviral activity against AI viruses in suspension, as well as antiviral activity against the aerosolized viruses that went through the pre-treated air filters. The damage to the AI virus' RNA and the chlorine loadings of MC in the filters were determined.

2. Materials and methods

2.1. MC treated filter

Nonwoven fabric from the US National Institute for Occupational Safety and Health (NIOSH) certified N95 respirator (3 M, 1860) was used as the MC carrier material in this study. Because of the small pore size, the nonwoven fabric layer is key to preventing the passage of pathogens or particles through the fabric. The materials were cut into round coupons with a diameter of 2.54 cm, and these coupons were sterilized by UV light overnight before coating. The coupons were then soaked for 1 min in two MC solutions, one at 0.1% and one at 1% (w/v) concentrations in 95% ethanol. Finally, the treated coupons were air-dried at room temperature for 48 h. Coupons treated with 95% ethanol served as the control.

2.2. Active chlorine loadings

The coating efficiency of MC on these nonwoven treated fabrics was verified by Fourier transform infrared spectroscopy (FT-IR) (Nicolet 6700 FT-IR Spectrometer, Thermo Scientific, Madison, WI). Since the chlorine bonded to N-halamines provides antimicrobial activity, active chlorine loadings in the MC-coated coupons were also measured using the modified iodometric/thiosulfate titration method (Worley et al., 2005).

2.3. Virus propagation

This study used AI H1N1 virus (A/blue-winged teal/AL/167/2007) isolated from hunter-killed waterfowl from a wildlife refuge in Alabama (Dormitorio et al., 2009). The virus was propagated in allantoic fluid (AF) from 9-day-old specific-pathogen-free (SPF) embryonated chicken eggs. After 48 h post-inoculation, AF containing the viruses was harvested and stored at -80 °C until use. The 50% embryo infectious dose per mL (EID_{50}/mL) titer of virus was determined based on the Spearman-Karber method (Reed and Muench, 1938). Stock virus was thawed quickly and diluted to the desired concentration with phosphate buffer saline (PBS), and kept on ice during use.

2.4. In vitro antiviral efficacy evaluation of MC and sodium hypochlorite

MC and a commonly used disinfectant, sodium hypochlorite, were used to test antiviral activity, following the test method of Zou et al. (2013). MC stock solution at 5% (w/v) was diluted with sterile distilled water to achieve concentrations of 0.005%, 0.05%, and 0.5%, producing 10, 100, and 1000 ppm of oxidative chlorine, respectively. Sodium hypochlorite was diluted to 0.2%, which generated 1000 ppm of active

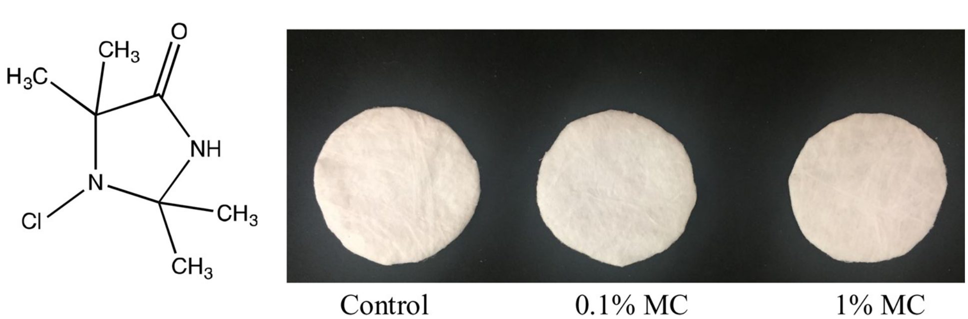

Fig. 1. Nonwoven fabrics coated with 0, 0.1% and 1% MC (1-chloro-2,2,5,5-tetramethyl-4-imidazolidinone).