Evaluation of disinfectants commonly used by the commercial poultry industry under simulated field conditions

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ABSTRACT The correct usage of disinfectants is an important component of a successful biosecurity program. The objective of this study was to determine the effect of time, temperature, and organic matter (OM) on disinfectant efficacy. *Staphylococcus aureus* and *Salmonella* Typhimurium were used to represent gram-negative and gram-positive bacteria commonly found in commercial poultry housing. The first study evaluated the effect of temperature (4, 20, 32, or 43°C) and time (1, 2, 3, 4, 6, 8, 12, 16, 20, 24, and 30 wk) on the efficacy of disinfectants diluted to working concentrations. The second study determined the effect of OM on the efficacy of working concentrations of freshly prepared disinfectants against the bacteria. For the third study, we compared the bactericidal properties of freshly prepared disinfectants and 30-wk-old disinfectants in the presence of OM. Quaternary ammonium-, chlorhexidine-, phenolic-, and binary ammonium-based solutions represented disinfectants commonly used within the poultry industry. In the first study, all of the disinfectants were effective against *S. aureus* and *Salmonella* Typhimurium regardless of treatment. However, the phenolic compound had reduced ($P \leq 0.05$) efficacy against *Salmonella* Typhimurium after 6 wk of storage at the highest temperature of 43°C and after 16 wk at the second highest temperature of 32°C. All of the disinfectants were effective against *S. aureus* regardless of temperature treatment. In the second study, the addition of sterile chicken litter had deleterious effects on all 4 classes of disinfectants against *Salmonella* Typhimurium. Of the disinfectants tested, the phenolic compound retained efficacy against *S. aureus.* In the third study, the presence of OM significantly reduced ($P \leq 0.05$) the efficacy of the 30-wk-old quaternary ammonium and phenolic compound against *Salmonella*. The fresh quaternary ammonium and binary compound achieved a greater kill ($P \leq 0.05$) of *Staphylococcus*, relative to the 30-wk-old disinfectant. These results emphasize the need to use fresh disinfectants and that OM should be removed before disinfection.

Key words: disinfectant, poultry, *Salmonella*, *Staphylococcus*, phenol

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INTRODUCTION

Poultry diseases are costly to the poultry industry and are difficult to control (Fussell, 1998). During the last 30 yr, avian influenza has been introduced into the United States multiple times (Halvorson et al., 2003). After outbreaks, poultry facilities are exceptionally difficult and expensive to clean, sanitize, and disinfect. *Staphylococcus aureus* infections increase morbidity and mortality from yolk sac infections and secondary infections affecting the bones, tendon sheaths, and leg joints (Moya, 1986). It is estimated that 1.4 million humans contract salmonellosis at a cost of $3 billion annually (Williams, 1988).

Disease prevention and control largely rely on biosecurity. Biosecurity includes protocols and procedures taken to prevent pathogens from infecting a farm and to prevent the transmission of disease by humans, insects, wild birds, or other animals (Poss, 1998). Physical barriers, shower facilities, and on-farm labor are examples of procedures employed in a biosecurity program to minimize disease exposure (Dekich, 1998). Tire wash stations and foot baths are also used to reduce pathogen transmission (Davison et al., 1999).

Disinfectants are important components of a biosecurity program. Classes of disinfectants include phenolics, quaternary ammonium compounds (**QAC**), halogens, oxidizing agents, chlorhexidine compounds, and alcohols (Smith and June, 1999; Dvorak, 2005). The objec-

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tive of disinfection is to reduce microbial populations (Eckman, 1994). Disinfectants act on microorganisms at several target sites resulting in membrane disruption, metabolic inhibition, and lysis of the cell (Denyer and Stewart, 1998; Maillard, 2002). In the field, disinfectants are often mixed with water in portable sprayers and exposed to extreme environmental conditions before their actual application. Disinfectants may have a limited lifespan after their initial dilution and it is possible that heat, sunlight, time, organic matter (**OM**), and adulterants may reduce their efficacy. Quaternary ammonium, binary, phenolic, and chlorhexidine compounds are commonly used in agricultural settings. In this experiment, we used *Salmonella* Typhimurium and *S. aureus* in a use-dilution assay (Robison et al., 1988) to compare the effects of time, temperature, and OM on representative disinfectants.

MATERIALS AND METHODS

Bacterial Culture

Isolates of *S. aureus* (American Type Culture Collection no. 12600) and a primary poultry isolate of *Salmonella* Typhimurium were obtained from the USDA, Agriculture Research Service, Southern Plains Agricultural Research Center (College Station, TX).

Staphylococcus aureus was cultured in tryptic soy broth (Difco Laboratories, Detroit, MI) for 12 h. Cells were washed 3 times with Butterfield's solution by centrifugation (3,000 × *g*) and the approximate concentration of the stock solution was determined spectrophotometrically (625 nm). The bacterial stock solution was serially diluted and confirmed by colony counts of 3 replicate samples (0.1 mL per replicate) that were spread-plated on mannitol salt agar (Becton, Dickinson and Co., Sparks, MD) plates.

Salmonella Typhimurium was cultured in tryptic soy broth (Difco Laboratories) for 8 h. The cells were washed 3 times with Butterfield's solution by centrifugation (3,000 × *g*) and the approximate concentration of the stock solution was determined spectrophotometrically (625 nm). A series of 10-fold dilutions of the stock solution were performed and confirmed by colony counts of 3 replicate samples (0.1 mL per replicate) that were spread-plated on brilliant green agar (Becton, Dickinson and Co.) plates containing 25 ug/mL of novobiocin (Sigma Chemical Co., St. Louis, MO) to inhibit the growth of contaminated organisms.

Disinfectants

Four commercial disinfectants were diluted to the manufacturers' recommended working concentrations with distilled water. The QAC (RXVeterinary Products, Grapevine, TX) consisted of 10% alkyl dimethyl ammonium chloride and 10% dimethyl ethyl benzyl ammonium chloride (working concentration; 8.8 mL/3.8 L). The phenolic compound (Biosentry Inc., Stone Mountain, GA) contained 7.92% *o*-phenylphenol, 9.97% *o*-benzyl-*p*-chlorophenol, and 1.95% *p*-tert-amylphenol (14.7 mL/3.8 L). The chlorhexidine compound (Fort Dodge Animal Health, Fort Dodge, IA) consisted of 2% chlorhexidine diacetate (29.5 mL/3.8 L). The binary compound (Wilson Manufacturing Company Inc., Jefferson, GA) consisted of 13.02% didecyl dimethyl ammonium chloride, and 8.68% alkyl dimethyl benzyl ammonium chloride (7.3 mL/3.8 L). All cultures were carried out in 15-mL glass tubes in triplicate. Disinfectant activity was determined by comparing bacteria survival in each disinfectant evaluated. Three independent experiments were conducted to evaluate the effect of time, temperature, and OM on disinfectant efficacy.

Disinfectant Evaluation

We used a modified use-dilution test (no. 955.15) provided by the Association of Official Analytical Chemists (1984) and Robinson et al. (1988) to evaluate disinfectant efficacy. In each experiment, 0.5 mL of 10^8 cfu/mL of the test organism was added to separate 15-mL glass tubes, each containing 4.5 mL of each of the diluted disinfectant (in experiments 2 and 3, disinfectants were supplemented with chicken litter). Then, each tube of disinfectant was briefly vortexed at the lowest setting for 3 s. After a 10-min incubation at room temperature, the treatment tube was vortexed and serially diluted into 4 subsequent dilution tubes containing 4.5 mL of Butterfield's solution per disinfectant sample. One hundred microliters of each dilution tube was then spread-plated onto selective agar. In experiment 2, Dey-Engley agar (Difco Laboratories) was used for both organisms, incubated at 37°C for 24 h, and the number of colony-forming units were recorded. Dey-Engley neutralizing agar contains ingredients that neutralize residual activity of disinfectants. In preliminary studies (data not shown), we found that there were no differences in bacterial growth on Dey-Engley agar when compared with mannitol salt and brilliant green agar.

Enrichment

Staphylococcus broth (Difco Laboratories) and tetrathionate broth (Difco Laboratories) were used as enrichment for *S. aureus* and *Salmonella* Typhimurium, respectively. Dey-Engley broth (Difco Laboratories) was used for both organisms in experiment 2. One hundred microliters of solution was collected from the initial incubation tube containing *Salmonella* or *Staphylococcus* and was inoculated into their respective enrichment broth. After 24-h incubation of the enrichment broth, 100 μL of each culture in experiments 1 and 3 were struck for isolation onto agar plates and incubated at 37°C for 24 h. In experiment 2, after the 24-h incubation in enrichment broth, a color change of the broth indicated the presence of viable bacteria. Samples that did not have any bacterial growth at the 1:100 dilutions but produced growth after culture in enrichment broth

were assigned an arbitrary value of 1.50 $\log_{10}$ of indicator organism according to Corrier et al. (1993).

Experimental Design

Experiment 1. Working concentrations of disinfectants (n = 3 replicates per treatment) were stored at 4, 20, 32, and 43°C. Samples of diluted disinfectant were collected at 1, 2, 3, 4, 6, 8, 12, 16, 20, 24, and 30 wk of storage.

Experiment 2. Two trials (n = 3 replicates per treatment) were conducted to evaluate the effect of OM on disinfectant efficacy of freshly made disinfectants stored at room temperature. Chicken litter was used as the source of OM. The litter was dried, finely ground, and sterilized before use. The OM was added to an incubation tube at concentrations of 0, 0.75, 1.5, or 3%.

Experiment 3. Two trials (n = 3) were conducted to determine the effect of time and OM on 30-wk-old and freshly made disinfectants stored at room temperature. Sterilized OM was added to each incubation tube at 1.5%.

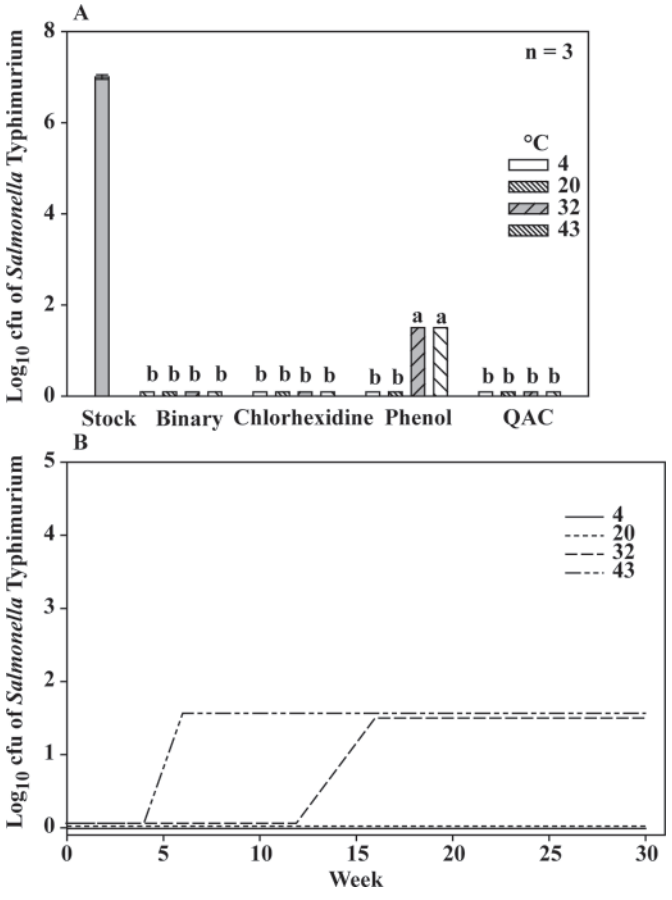

Figure 1. (A) Disinfectant efficacy after 30 wk of storage at treatment temperatures against *Salmonella* Typhimurium. QAC = quaternary ammonium compounds. [a,b]Means with different letters differ significantly ($P \leq 0.05$). (B) Timeline illustrating when reduced effectiveness of the phenolic compound against *Salmonella* Typhimurium was observed.

Statistical Analysis

Statistical analyses were completed with SPSS statistical software package version 11.0 for Windows (SPSS Inc., Chicago, IL). Data in all experiments were analyzed via a 1-way ANOVA using the GLM procedure due to the presence of significant interactions. Differences were deemed significant at $P \leq 0.05$ and means were separated using a Duncan's multiple range test. Significant interactions were as follows: experiment 1 – disinfectant and temperature, experiment 2 – disinfectant and OM, experiment 3 – disinfectant and storage time.

RESULTS

In experiment 1, the effects of time and temperature on disinfectant efficacy were evaluated. After long-term storage, all disinfectants remained bactericidal against *Salmonella* Typhimurium. However, only a reduction ($P \leq 0.05$) of *Salmonella* Typhimurium was observed with the phenolic compound after 6 wk of incubation at 43°C, and after 16 wk of incubation at 32°C compared with our stock concentration of *Salmonella* Typhimurium at 10^7 cfu/mL (Figure 1A and 1B). All disinfectants remained effective against *S. aureus* regardless of temperature or storage treatment (Figure 2).

In experiment 2, decreases ($P \leq 0.05$) in disinfectant efficacy were observed in response to increasing levels of OM (Figure 3). At 0% OM, all disinfectants reduced the stock concentration of the stock *Salmonella* Typhimurium to undetectable limits. After the addition of OM, we observed reductions in efficacy on all disinfectants in a dose-dependent manner. The phenolic compound was the most resistant disinfectant, whereas the chlorhexidine was the most susceptible to *Salmonella* Typhimurium.

All disinfectants were effective at a 0% ($P \leq 0.05$) concentration of OM against *S. aureus* (Figure 4). The

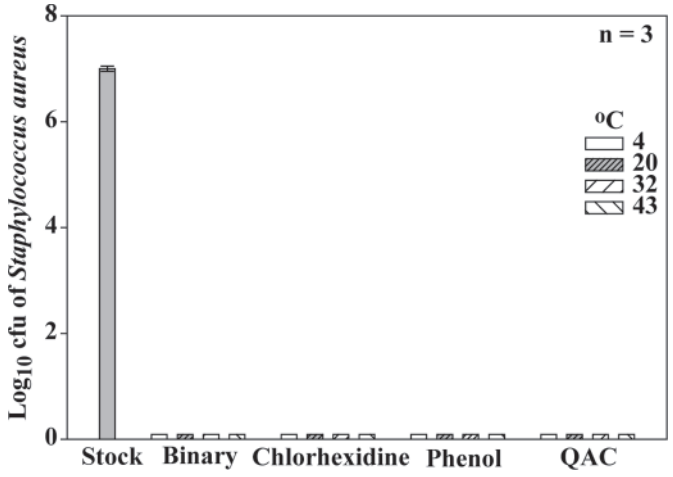

Figure 2. Disinfectant efficacy after 30 wk of storage at treatment temperatures against *Staphylococcus aureus*. QAC = quaternary ammonium compounds.

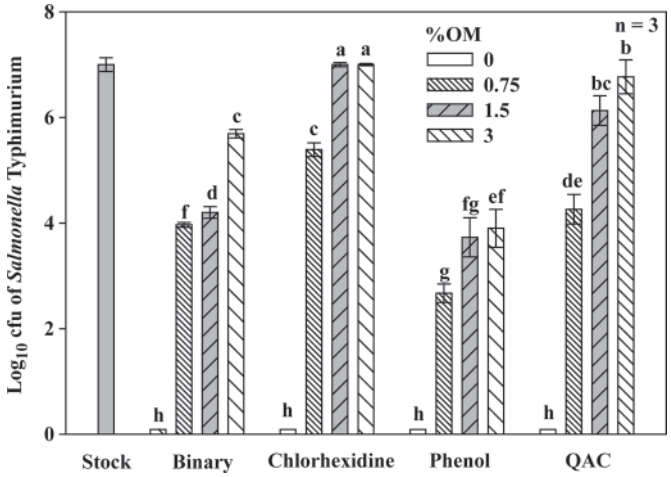

Figure 3. Effect of of organic matter (OM) on fresh disinfectants at room temperature against *Salmonella* Typhimurium. QAC = quaternary ammonium compounds. [a–h]Means with different letters differ significantly ($P \leq 0.05$).

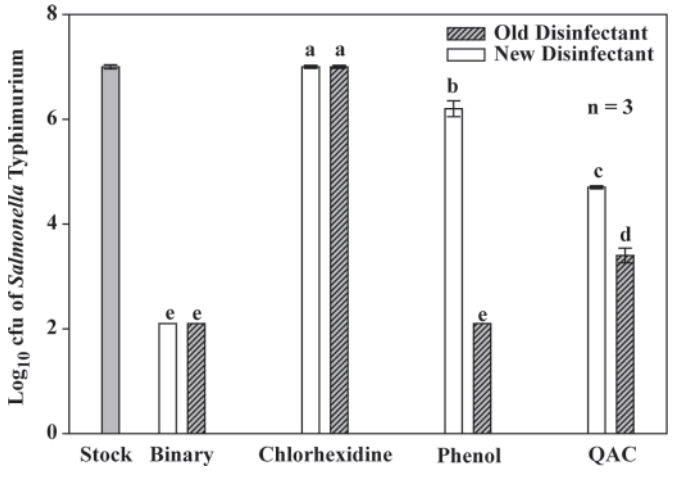

Figure 5. Comparison of 30-wk-old disinfectants to fresh disinfectants against *Salmonella* Typhimurium. QAC = quaternary ammonium compounds. [a–e]Means with different letters differ significantly ($P \leq 0.05$).

binary and phenolic compounds reduced ($P \leq 0.05$) the total *S. aureus* population at 0.75% and 1.5% OM, when compared with QAC and chlorhexidine compound. At 3% OM, the phenolic compound reduced ($P \leq 0.05$) the total *S. aureus* population and the chlorhexidine compound was ineffective. The QAC and binary compound were able to reduce colony-forming units of the stock solution of *S. aureus*.

In experiment 3, the consequences of long-term storage on disinfectant efficacy in the presence of OM against *Salmonella* Typhimurium and *S. aureus* were evaluated (Figure 5 and 6). The efficacy of the 30-wk-old QAC was significantly reduced when compared with freshly prepared disinfectant against *Salmonella* Typhimurium (Figure 5). The 30-wk-old phenolic compounds were significantly reduced in efficacy when compared with freshly prepared disinfectant against *Salmonella* Typhimurium. There were no significant differences between the fresh and 30-wk-old solutions for the chlorhexidine and binary treatments against *Salmonella* Typhimurium. The fresh and 30-wk-old chlorhexidine compound was ineffective against *Salmonella* Typhimurium.

A significant decrease was observed in the efficacy of the 30-wk-old QAC and binary compound against *S. aureus* (Figure 6), relative to freshly prepared solutions of the disinfectant. There were no differences between fresh and 30-wk-old phenolic and chlorhexidine compounds.

DISCUSSION

Once disinfectants are diluted, they are often stored and exposed to less than optimal conditions, such as UV light or extreme temperatures, before their actual application. This study demonstrates that working concentrations of disinfectants may be stored for up to 30

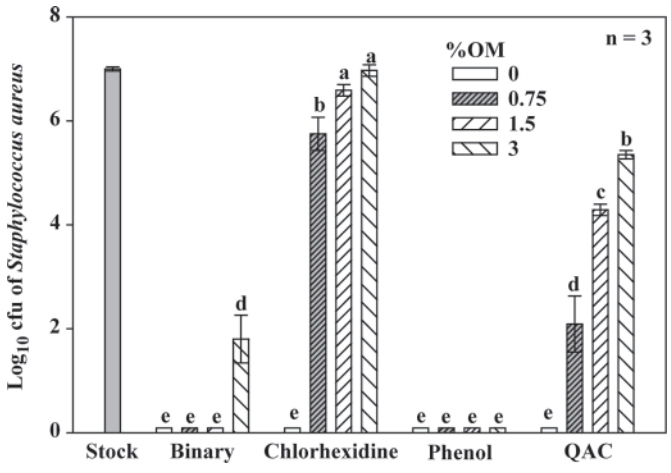

Figure 4. Effect of of organic matter (OM) on fresh disinfectants at room temperature against *Staphylococcus aureus*. QAC = quaternary ammonium compounds. [a–e]Means with different letters differ significantly ($P \leq 0.05$).

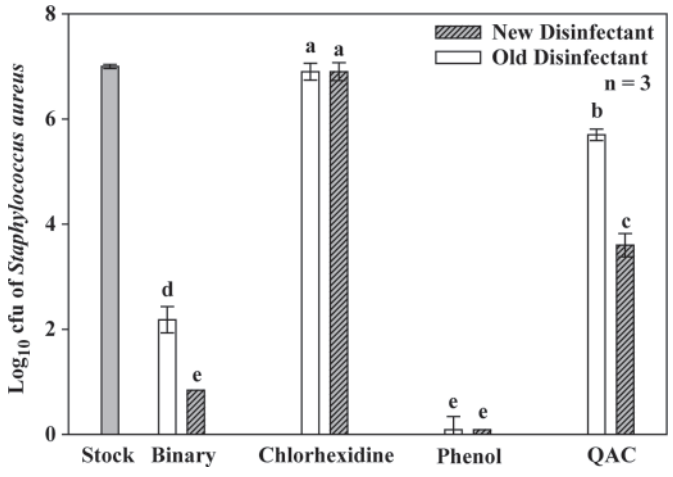

Figure 6. Comparison of 30-wk-old disinfectants to fresh disinfectants against *Staphylococcus aureus*. QAC = quaternary ammonium compounds. [a–e]Means with different letters differ significantly ($P \leq 0.05$).

wk at less than optimal temperatures without loss of effectiveness against *Salmonella* Typhimurium and *S. aureus* (Figure 1A and 1B). However, in the presence of OM, long-term storage of diluted disinfectants may have a detrimental effect on efficacy.

Disinfectants should be used after cleaning and removal of OM (blood, fecal matter, litter, fat, and hatchery fluff). Organic matter provides a physical barrier that protects microorganisms from contact with the disinfectant (Dvorak, 2005). Quinn and Markey (2001) suggest, similar to our observations (Figure 3 and 4), that phenolic compounds should be used for any application where excessive OM may be present, due to increased efficacy in the presence of OM.

Commercially available disinfectants are not all classified as broad-spectrum agents. Multiple factors should be considered when a disinfectant is chosen, such as OM on the surface to be treated, presence of OM in the dilutent, quality of water, corrosiveness or toxicity of the product, application method, temperature, porosity of the surface being treated, length of contact time, infectious organisms targeted, susceptibility of the infectious organisms, and correct dilution (Prince et al., 1991; Quinn and Markey, 2001; Dvorak, 2005; Payne et al., 2005).

In conclusion, long-term storage of disinfectants at 4, 20, 32, or 43°C did not reduce efficacy in the absence of OM against *S. aureus*. However, a reduction in efficacy was observed over time with the phenolic compound against *Salmonella* Typhimurium. After the inclusion of OM, reduced efficacy was observed in a dose-dependent manner against both organisms, with the exception of the phenolic compound against *S. aureus*. Freshly prepared disinfectant performed better in the presence of OM than 30-wk-old disinfectant. These results emphasize the need to use fresh disinfectants and that OM should be removed before disinfection. The appropriate use of disinfectants should be considered as an important intervention strategy to control avian diseases in poultry. Biosecurity and an effective disinfectant program will reduce foodborne pathogens, immunosuppressive viruses, reportable diseases, and opportunistic infections.

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