ISSN 1682-8356
ansinet.org/ijps

INTERNATIONAL JOURNAL OF

POULTRY SCIENCE

ANSInet
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan
Mob: +92 300 3008585, Fax: +92 41 8815544
E-mail: editorijps@gmail.com

International Journal of Poultry Science 8 (3): 237-241, 2009
ISSN 1682-8356

In vitro Efficacy Comparisons of Disinfectants Used in the Commercial Poultry Farms

Z. Moustafa Gehan[1], W. Anwer[1], H.M. Amer[2], I.M. EL-Sabagh[2], A. Rezk[1] and E.M. Badawy[1]
[1]Department of Veterinary Hygiene and Management, [2]Department of Virology,
Faculty of Veterinary Medicine, Cairo University, Giza, Egypt

Abstract: Studies have indicated variations in the degree of efficacy of the commercial disinfectants commonly used in poultry production facilities. An adequate method of *in vitro* testing was used to compare the efficacy of some of these disinfectants while testing them in conditions similar to those of the poultry facilities. Five commercially available disinfectants were tested against 7 selected bacterial, fungal and viral isolates. The obtained results indicated that, most of the tested disinfectant products were effective at the manufacturer recommended level within 30 min contact time when tested in the absence of organic matter. However, when organic matter was present longer contact times were needed to demonstrate the effectiveness. *Pseudomonas aeruginosa*, *Fusarium* species and Newcastle disease virus showed variable degrees of resistance to some of the tested disinfectant products in the presence of organic matter. Conclusively, monitoring program should be adopted regularly in poultry facilities to test the problematic microbes individually for their resistance against commercial disinfectants.

Key words: Disinfectants, monitoring poultry farms, bacterial and fungal resistance, viral resistance, *in vitro* evaluation of disinfectants

INTRODUCTION

Sanitation programs include complex and critical issues that are designed to control pathogenic microorganisms. The principles of disease prevention and control within the poultry industry are based on flock management, bio-security, preventive vaccination and sanitation (Zander *et al*., 1997). Bio-security which regularly includes cleaning and disinfection is one of the best methods used to reduce the microbial load generally and the level of pathogens in particular in poultry farms. In general, a sanitation program should include safe and easy procedures outlining the correct application of detergents and disinfectants, proper use of application equipment and an efficient monitoring system (Spielholz, 1998). Studies had shown variations in the degree of efficiency of commercial disinfectants used in poultry facilities. Resistance to commercially available disinfectants involves bacteria that infect newly hatched chicks, through the yolk sac (Willinghan *et al*., 1996). *Pseudomonas aeruginosa* can invade fertile eggs causing death of embryos and newly hatched chicks. Virulent strains of this bacterium can cause diarrhea, dehydration, septicemia, dyspnea and death in young chicks (Walker and Sander, 2004). Reportedly, Pseudomonas species is present in 10% of yolk sac of infected chicks (Sarma *et al*., 1985). Walker *et al*., 2002 indicated that Pseudomonas species were more resistant to germicides than other bacteria and the concentration of disinfectants effective against Salmonella and Staphylococcus species were lower than those successful against Pseudomonas species. Microbial resistance can be either a natural property of an organism (intrinsic) or acquired by mutation or acquisition of plasmids (self-replicating, extra chromosomal DNA). Intrinsic (innate) resistance is thus a natural, chromosomally controlled property of a bacterial cell that enables it to circumvent the action of an antiseptic or disinfectant. Gram-negative bacteria tend to be more resistant than gram-positive organisms, such as staphylococci (McDonnell and Russell, 2001). Molds are generally more resistant than yeasts and considerably more resistant than non-sporulating bacteria as the cell wall composition in molds confers a high level of intrinsic resistance (Russell and Furr, 1996). Mechanisms of viral resistance include Multiplicity reactivation (Young and Sharp, 1985), viral aggregation (McDonnell and Russell, 2001) and the possibility of viral adaptation to new environmental conditions (Bates *et al*., 1977).

Consequently, the objective of this study was to evaluate the efficacy of some available disinfectants against some problematic bacterial, fungal and viral strains obtained from commercial poultry facilities and so to investigate the possible microbial resistance against these disinfectants.

MATERIALS AND METHODS

The used disinfectants: Five different disinfectants commonly used for disinfection in different poultry facilities were used in the present study (Table 1).

Table 1: The used chemical disinfectants and the used dilutions

Disinfectant	Dilution	Supplier	Active ingredients
Perasan®	1%	Henkel (Germany)	Peracetic acid 5%, H_2O_2 20% and Acetic acid 10%
H_2O_2®	3%	Henkel (Germany)	H_2O_2 50% and Dihydroxybenzole 100 ppm
Aldekol®	1%	EWABO Chemkalien, Gmbh	Glutaraldehyde Quaternary ammonium compound Formalin
Quatovet®	1%	EWABO Chemkalien, Gmbh	Quaternary ammonium compound
Virkon-S®	1%	Antec International LTD UK	potassium peroxymonosulfate and sodium chloride

The tested microorganisms: Microbial isolates from clinical and research cases were obtained from Department of Microbiology, Faculty of Veterinary Medicine, Cairo University. Bacterial and fungal isolates included *Pseudomonas aeruginosa* (Pa), *Escherichia coli* (Ec), *Salmonella typhimurium* (St), *Staphylococcus aureus* (Sa), *Aspergillus fumigatus* (Af) and *Fusarium species* (Fs). The Lasota strain of Newcastle Disease Virus (NDV) was obtained from [Intervet-International B.V., Boxmeer, Holland].

Chicken embryos: Nine-days-old Specific-pathogen-free (SPF) embryonated eggs were obtained from SPF Eggs production Farm, Agriculture Research Center, Ministry of Agriculture, Egypt.

Preparation of bacterial and fungal isolates: Bacterial isolates were grown in the nutrient broth at 37°C for 18-24 h. Fungal isolates were previously grown on a slant of Sabouraud dextrose agar and set at room temperature for 5 days to induce sporulation and then the spore suspension was prepared by adding 10 mL of Sabouraud dextrose broth into the tube and rubbing the slant surface with a cotton swab.

Preparation of viral inoculants: Virus strains were propagated in the allantoic cavity of SPF embryonated eggs. Harvested virus preparation was titrated using a standard plate haemagglutination assay.

Evaluation of disinfectants against bacteria and fungus: Tubes containing 9.5 mL of nutrient broth supplemented with 10% calf serum were inoculated with 0.5 mL of each of microbial strains to test the disinfectant effectiveness in the presence of organic matter. Similar sets containing normal saline solution were used to prepare the microbial- disinfectant mixture without organic matter. The disinfectants were added to the tubes at the manufacturer's recommended level (Table 1). Subcultures were performed at 10, 30 and 60 minutes contact time which included the transfer of 0.5 mL of the sample mixture into new sets of tubes containing 5 mL of nutrient broth. Those tubes were incubated at 37°C for 24 h. Microbial growth was determined by turbidity or precipitation at the bottom of the tubes. Disinfectant efficacy was determined by the absence of microbial growth (Pilotto *et al*., 2007).

Evaluation of disinfectants against Newcastle disease virus: To test effectiveness of the different disinfectant products on Newcastle disease virus, 1 mL of the virus stock was mixed with 19 mL of Phosphate Buffered Saline (PBS). Disinfectants were added to 1 mL aliquots of the virus-saline mixture. Samples were prepared in replicates and incubated at room temperature for 1, 10, 30 and 60 min. The activity of disinfectants was halted by addition of 5µ of Tween-80. In order to avoid embryo toxicity, the virus-disinfectant mixtures were diluted 1/10 in PBS and then inoculated in sets of 3 SPF embryonated eggs via the allantoic route. Following 5 days of incubation at 37°C, the allantoic fluid of the inoculated eggs was harvested, clarified and tested for the haemagglutination activity of NDV using rapid and plate haemagglutination tests. The virus titer was expressed as the reciprocal of the highest virus dilution showing complete haemagglutination. Separate aliquots without disinfectant were incubated for the same periods and were tested for NDV as virus controls. Similar sets of the virus diluted in PBS supplemented with 10 % fetal bovine serum were used to test the disinfectant activity in the presence of organic matter (Miguel Ruano *et al*., 2001).

RESULTS

The recorded results in (Table 2-4) showed that most of the selected bacteria and fungi were resistant to H_2O_2, quatovet (QAC) and virkon-s within the first 10 minutes of the contact time. However, Perasan (PAA) was able to kill most of these microbes, during that period of time, but when 10% of yeast extract (organic matter) was added, the product was not able to retain its effectiveness. On the other hand, Aldekol-03 (Glutaradehyde + QAC) exhibited a high level of antimicrobial activity even in the presence of organic matter within such short contact time.

After 30 min contact time (Fs) was still resistant to PAA and H_2O_2 and quatovet in the presence of organic matter. Also (Ec) (Ps) (St) and (Sa) were resistant to quatovet in presence of organic matter.

After 60 min contact time (Pa) (Ec) (St) and (Fs) were still resistant to QAC in the presence of organic matter. Also, most of the selected bacteria and fungi were still resistant to virkon-s except (Sa) and (Af) in absence of organic matter.

Results recorded in Table 5 illustrated that most of the disinfectant products utilized were effective against Newcastle disease virus at the used concentrations along the different contact periods. PAA, aldekol-03 and QAC completely inactivate the virus growth and

Table 2: Disinfectant efficacy against the selected bacteria and fungi after 10 min contact time

	Disinfectant Efficacy											
	Without organic matter						In the presence of organic matter					
Product	Pa	Ec	St	Sa	Af	Fs	Pa	Ec	St	Sa	Af	Fs
Perasan®	_	_	_	_	_	+	+	+	+	+	_	+
H_2O_2®	+	+	+	+	+	+	+	+	+	+	+	+
Aldekol®	_	_	_	_	_	_	_	_	_	_	_	_
Quatovet®	+	+	+	+	+	+	+	+	+	+	+	+
Virkon-S®	+	+	+	+	+	+	+	+	+	+	+	+

(Pa): *Pseudomonas aeruginosa* (Ec): *Escherichia coli* (St): *Salmonella typhimurium* (Sa): *Staphylococcus aureus* (Af): *Aspergillus fumigatus* and (Fs): *Fusarium species*. NB :(-) is equivalent to no microbial growth and (+) is equivalent to microbial growth.

Table 3: Disinfectant efficacy against selected bacteria and fungi after 30 minutes contact time

	Disinfectant Efficacy											
	Without organic matter						In the presence of organic matter					
Product	Pa	Ec	St	Sa	Af	Fs	Pa	Ec	St	Sa	Af	Fs
Perasan®	_	_	_	_	_	_	_	_	_	_	_	+
H_2O_2®	_	_	_	_	_	_	_	_	_	_	_	+
Aldekol®	_	_	_	_	_	_	_	_	_	_	_	_
Quatovet®	_	_	-	-	_	_	+	+	+	+	_	+
Virkon-S®	+	+	+	-	_	+	+	+	+	+	+	+

NB: (-) is equivalent to no microbial growth and (+) is equivalent to microbial growth

Table 4: Disinfectant efficacy against selected microbial after 60 minutes of contact time

	Disinfectant Efficacy											
	Without organic matter						In the presence of organic matter					
Product	Pa	Ec	St	Sa	Af	Fs	Pa	Ec	St	Sa	Af	Fs
Perasan®	_	_	_	_	_	_	_	_	_	_	_	_
H_2O_2®	_	_	_	_	_	_	_	_	_	_	_	_
Aldekol®	_	_	_	_	_	_	_	_	_	_	_	_
Quatovet®	_	_	_	_	_	_	+	+	+	_	_	+
Virkon-S®	+	+	+	_	_	+	+	+	+	+	+	+

NB: (-) is equivalent to no microbial growth and (+) is equivalent to microbial growth

multiplication in embryonating eggs as shown by plate haemagglutination test. The antiviral activity of these disinfectants was determined so early after 1 min of virus-disinfectant contact time. On the other hand, H_2O_2 and virkon-s did not show significant antiviral activity against NDV. However, their effect increased to a certain limit by extending the virus-disinfectant contact time.

For testing the impact of organic matter on the efficacy of the different disinfectant products, 10% fetal bovine serum was added to all reactants. Although PAA retained its full effect on NDV in the presence of organic matter, the other disinfectant products showed variable degrees of anti-viral activities. However, Aldekol was the least product affected by the presence of organic matter for the extent that incubation of the virus-disinfectant mixture for 10 min is sufficient to completely inactivate the virus, QAC showed a dramatic decrease in its anti-viral activity under the same conditions. On the other hand, virkon-s and H_2O_2 could not demonstrate satisfactory results.

DISCUSSION

The inclusion of 10% yeast extract in the media or 10% fetal bovine serum in viral testing was utilized in order to simulate organic matter present in field conditions because the interference of organic matter on disinfectant efficacy has a negative impact (North and Bell, 1990). Most microbials needed increased contact times with the disinfectant or may need higher disinfectant concentration. Under these considerations, it is clear that disinfectants should be used subsequent to the cleaning and removal of the maximum organic material on the surfaces subjected to sanitation. In addition, disinfectant preparations and concentrations need to be carefully scrutinized (Miguel Ruano *et al*., 2001). For example, peroxides preparations (virkon-s) commercially available for the poultry industry are more frequently recommended and used at a concentration of 1%. Unfortunately, this concentration had led to unsatisfactory results in the present study and when this

Table 5: Disinfectant efficacy against Newcastle disease virus following different contact times

		NDV plate HA titer*(log_2)							
		Without organic matter				In the presence of organic matter			
Product	Dilution	**1min	10min	30min	60min	1min	10min	30min	60min
Perasan®	1.5	0	0	0	0	0	0	0	0
H_2O_2®	0.5	8	7.7	6.7	6	9	8	7	7
Aldekol®	0.5	0	0	0	0	1.3	0	0	0
Quatovet®	0.5	0	0	0	0	9	7.5	7	7
Virkon-S®	0.5	9	8.7	7	7	10	10	10	10
***Saline	-	10	10	10	10	10	10	10	10

**Virus-disinfectant contact time. ***Used for virus control

product was used as a disinfectant under field conditions as reported by Spielholz, 1998. However, Gasparini *et al*., 1995 found that virkon-s is effective against Pa and Ec. Most of the selected bacteria and fungi were found to be resistant to Quatovet in the presence of organic matter. This is not surprising because QAC has been used in poultry industry for many years. Prolonged use of the disinfectant may have selected resistant populations. This finding coincides with those of Tennet *et al*., 1985; Gillespie *et al*., 1986; Russell and Chopra, 1996; Willinghan *et al*., 1996; Sidhu *et al*., 2002; Moustafa *et al*., 2004 and Gilinsky, 2006. All of them reported different level of bacterial resistance to QAC specifically for Salmonella typhimurium, Staphylococcus aureus, *E. coli* and Pseudomonas aeruginosa.

Hydrogen peroxide and PAA had satisfactory antimicrobial activity in the presence of organic matter. These findings are in agreement with Bailey *et al*., 1996, Sander and Wilson 1999 and Rodgers *et al*., 2001, who recommended the use of H_2O_2 as a hatchery disinfectant. Miguel Ruano *et al*., 2001 found that H_2O_2 at 2% concentration had excellent antimicrobial activity in the presence of organic matter. However, Blakistone *et al*., 1999 and Thamlikitkul *et al*., 2001 proved that PAA is an effective bactericide, Block, 1991, stated that PAA is considered a more potent biocide than hydrogen peroxide, being sporicidal, bactericidal, virucidal and fungicidal at low concentrations(<0.3%). Malchesky, 1993 mentioned that PAA decompose to safe by-products (acetic acid and oxygen) but has the added advantages of being free from decomposition by peroxidases, unlike H_2O_2 and remaining active in the presence of organic loads. While Rodgers *et al*., 2001 reported that PAA was the most potent bactericide against Staphylococcus species in hatcheries. On the other hand, Rossoni and Gaylarde, 2000 mentioned that PAA could not recommend as the sanitizing agent of choice for chicken processing equipment. Aldekol-03 had satisfactory antimicrobial activity even in the presence of organic matter, a finding which is agreeable with Qayyum *et al*., 1999 who recorded that aldekol 0.5% was able to inactivate NDV, McDonnell and Russell, 2001 who stated that glutaradehyde has a broad spectrum activity against bacteria and their spores, fungi and viruses and Youseif *et al*., 2001 who found that aldekol was effective on Salmonella typhimurium and Staphylococcus aureus.

Conclusion: In conclusion, simple identification of the bacteria causing problems in poultry facilities dose not appears to be sufficient for disinfectant selection as individual bacteria of the same genus and species may have variations in sensitivity to the commonly used disinfectants. Moreover, disinfectants with similar but not identical chemical formulations will have very different degrees of efficacy against bacteria of field origin when evaluated by a method that closely recreates actual industry settings. Also, it was clear that disinfectants should be used subsequent to the cleaning and removal of most of organic material on the surfaces subjected to sanitation. It was suggested that, monitoring program should be adopted regularly in poultry facilities to test the problematic microbes individually for their resistance against the available commercial disinfectants.

REFERENCES

Bailey, J.S., R.J. Buhr, N.A. Cox and M.E. Berrang, 1996. Effect of hatching cabinet sanitation treatments on salmonella cross-contamination and hatchability of broiler eggs. Poult. Sci., 75: 191-196.

Bates, R.C., P.T.B. Schaffer and S.M. Sutherland, 1977. Development of poliovirus having increased resistance to chlorine inactivation. Appl. Environ. Microbiol., 3: 849-853.

Blakistone, B., R. Chuyate, D. Jr. Kautter, J. Charbonneau and K. Suit, 1999. Efficacy of oxania active against selected spore former. J. Food Prot. Mar., 62: 262-267.

Block, S.S., 1991. Peroxygen compounds. In: Block S.S., Ed. Disinfection, sterilization and preservation. 4th Ed. Philadelphia, Pa: Lea and Febiger, pp: 167-181.

Gasparini, R., T. Pozzi, R. Magnelli, D. Fatighenti, E. Giotti, G. Polise Pratelli, R. Severini, P. Bonanni and L. De Feo, 1995. Evaluation of *in vitro* efficacy of the disinfectant virkon. European J. Epidemiology, 11: 193-197.

Gilinsky Ellen, 2006. Biosecurity procedures for poultry farm visits. http://www.deq.virginia.gov/water.

Gillespie, M.T., J.W. May and R.A. Skurray, 1986. Plasmid-encoded resistance to acriflavin and quaternary ammonium compound in methicillin-resistant staphylococcus aureus. FEMS Microbiology, 34: 47-51.

Malchesky, P.S., 1993. Peracetic acid and its application to medical instrument sterilization. Artif Organs, 17: 147-152.

McDonnell, G. and A.D. Russell, 2001. Antiseptics and Disinfectants: Activity, Action and Resistance. Clinical Microbiology Reviews, January, 14: 227.

Miguel Ruano, J. El-Attrache and Pedrovillegas, 2001. Efficacy comparisons of disinfectants used by the commercial poultry industry. Av. Dis., 45: 972-977.

Moustafa Gehan, Z., W. Anwer and S.T. Moubarak, 2004. Evaluation of some chemical disinfectants used in commercial poultry hatcheries. J. Egypt. Vet. Med. Assoc., 64: 221-233.

North, M.O. and D.B. Bell, 1990. Commercial chicken production manual. 4th Ed. Van Nortrand Reitihold, New.

Pilotto, F., L.B. Rodrigues, L.R. Santos, W.A. Klein, F.M. Colussi and V.P. Nascimento, 2007. Antibacterial efficacy of commercial disinfectants on dirt floor used in poultry breeder houses. Rev. Bras. Cienc Avic. 9, Apr./June.

Qayyum, R., M. Naeem and K. Muhammad, 1999. Effect of physico-chemical factors on survival of Newcastle disease virus. Int. J. Agric. Biol., 1: 42-44.

Rodgers, J.D., J.J. McCullagh, P.T. McNamee, J.A. Smyth and H.J. Ball, 2001. An investigation into the efficacy of hatchery disinfectants against strains of staphylococcus aureus associated with poultry industry. Vet. Microbiology, Sept., 20: 82: 131-140.

Rossoni, E.M. and C.C. Gaylarde, 2000. Comparison of sodium hypochlorite and peracetic acid as sanitizing agents for stainless steel food processing surfaces using epifluorescence microscopy. Int. J. Food Microbiol. Oct; 61: 81-85.

Russell, A.D. and I. Chopra, 1996. Understanding antibacterial action and resistance. 2nd Ed. Chichester, England: Ellis Horwood.

Russell, A.D. and J.R. Furr, 1996. Biocides: mechanisms of antifungal action and fungal resistance. Sci. Prog., 79: 27-48.

Sander, J.E. and J.L. Wilson, 1999. Effect of H_2O_2 disinfection during incubation of chicken egg on microbial levels and productivity. Av. Dis., 43: 227-233.

Sarma, D.R., L. Char, N.L. Rao, D.J. Khan and G. Narayna, 1985. A comprehensive study on bacterial flora isolated from yolk sac infection (omphalities) in chicks. In. J. Poult. Sci., 20: 262-266. (Cited by Walker and Sander, 2004).

Sidhu, M.S., H. Sorum and A. Holck, 2002. Resistance to quaternary ammonium compound in food related bacteria. Microbiology drug resistance, 8: 393-399.

Spielholz, B., 1998. Properties of hatchery disinfectants. World Poult., 14: 50-51.

Tennet, J.M., B.R. Lyon, M.T. Gillespie, J.W. May and R.A. Skurray, 1985. Cloning and expression of staphylococcus aureus plasmid-mediated quaternary ammonium resistance in E.coli. Antimicrobial agents' chemotherapy, 27: 79-83.

Thamlikitkul, V., S. Trakulsomboon, S. Louisiriro-tchanakul, A. Chaiprasert, S. Foongladda, K. Thipsuvan, W. Arjratanakool, R. Kunyok, C. Wassi, S. Santiprasitkul and S. Danchaivijitr, 2001. Microbial killing activity of peracetic acid. J. Vet. Med. Assoc., Thai. Oct., 84: 1375-1382.

Walker, S.E., J.E. Sander, I. Hsincheng and R.E. Wooley, 2002. The *in vitro* efficacy of quaternary ammonium compound disinfectant and/or Ethylenediam-inetetraacetic Acid-Tris against commercial broiler hatchery isolates of *Pseudomonas aeruginosa*. Avian Dis., 46: 826-830.

Walker, S.E. and J.E. Sander, 2004. Effect of Biosentry 904 and Ethylenediaminetetraacetic Acid-Tris disinfecting during incubation of chicken eggs on microbial levels and productivity of poultry. Avian Dis., 48: 238-234.

Willinghan, E.M., J.E. Sander, S.G. Thayer and J.L. Wilson, 1996. Investigation of bacterial resistance to hatchery disinfectant.

Young, D.C. and D.C. Sharp, 1985. Virion conformational forms and the complex inactivation kinetics of echovirus by chlorine in water. Appl. Environ. Microbiol., 49: 359-364.

Youseif, H.M.Z., Z.A.W. Hassanin and M. Bader Jihan, 2001. The sanitizing effect of glutaraldehyde-quaternary ammonium combination (Aldekol GDA0 on bacterial infection in hatching eggs (laboratory and field trials). Vet. Med. J. Giza, 49: 531-541.

Zander, D.V., A.J. Bermudez and E.T. Mallinson, 1997. Principles of disease prevention: diagnosis and control. In: Diseases of poultry, 10th Ed. B.W. Canek and H.J.Barnes, C.W. Beard, L.Mac Dougold and Y.M. Saif, eds. Iowa state University Press, Ames, IA., pp: 369-413.