Original Research Article

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Evaluation of the efficiency of the most common commercial disinfectants against some pathogens isolated from New-valley poultry farms "in-vitro studies."

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Abstract

This study was conducted to compare the efficiency of three commercially available disinfectants (Virkon S, T.H5 and Np50) against 3 bacterial isolates from New-valley broiler farms (Salmonella typhimurium, E. coli and Staph. aureus) in vitro. The evaluation was conducted at three consequent contact times 5, 15, 30 min. All tested disinfectants were diluted with sterile distilled water and applied at three different concentrations. The concentrations used were the manufacturer's instruction beside a higher and lower one. At the recommended concentration by the manufacturer, the most powerful disinfectant against all tested organisms was TH5 followed by Virkon S. On the other hand, NP50 was the weakest disinfectant. At 5 minutes, the reduction log showed by TH5 on S. typhimurium, E. coli, and S. aureus were, 3.6, 2.55, 3.54, respectively. Moreover, the reduction log for Virkon-S were 3.02, 2.21, and 3.37 on S. typhimurium, E. coli, and S. aureus, respectively. On the other hand, the reduction log induced by NP50 were,1.76, 1.91, 3.37 on S. typhimurium, E. coli, and S. aureus, respectively. At 15 minutes, the reduction log showed by TH5 on S. typhimurium, E. coli, and S. aureus were, 4.97, 3.59, 4.11, respectively. Moreover, the reduction log for Virkon-S were 4.97, 3.31, and 5.19 on S. typhimurium, E. coli, and S. aureus, respectively. On the other hand, the reduction log induced by NP50 were,2.82, 1.82, 4.27 on S. typhimurium, E. coli, and S. aureus, respectively. After 30 minutes, no growth was observed for S. aureus with 100% inhibition for TH5 while the reduction log for S. typhimurium, E. coli were, 7.32 and 7.16, respectively. Concerning Virkon-S the reduction log was, 6.5, 4.67 for S. typhimurium, E. coli, respectively while, S. aureus were completely inhibited with 100% inhibition. On the other hand, the reduction log induced by NP50 after 30 minutes were, 5.93, 3.4, 7.35 for S. typhimurium, E. coli, and S. aureus, respectively.

Keywords: Broiler farms, Disinfectants, E. coli, S. aureus, S. typhimurium.

Introduction

Disinfection process is the destruction of disease producing microorganisms on the inanimate objects and usually involve chemicals, heat or ultraviolet light. The nature of chemical disinfection varies with the type of product used (CDC, 2016). Poultry house sanitation plays a crucial role in the control and prevention of poultry diseases. A good sanitation program increases bird performance and minimize the incidence of contaminated flocks. (Corrier et al., 1992; Davies and Wray, 1995). The principles of disease prevention and control within the poultry industry are based on flock management, biosecurity, preventive vaccination and sanitation (Zander, 1997). Biosecurity which regularly includes cleaning and disinfection is the best method to reduce the microbial load particularly 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 (Gasparini et al., 1995). The efficiency of Virkon.S was assessed in vitro against S. aureus and E. coli. The results showed that Virkon.S has a high concentration coefficient and a wide range of action and low concentrations of it can inactivate all studied bacteria (Ruano et al., 2001). Many of the

commercially available disinfectant when tested in the absence of organic matter were effective at the manufacturer's recommended level within 10 min of contact time (Abdulghaffar and El Bahgy, 2016). Consequently, the objective of this study was to evaluate the efficacy of some available disinfectants against some bacterial isolates obtained from commercial poultry facilities.

Materials and Methods

The disinfectants: Three concentrations of each tested disinfectant were used including lower, higher as well as the recommended concentration by manufacturer. Three different disinfectants commonly used in veterinary practice were used in the present study (Table 1).

Table (1): The used chemical disinfectants and the used dilutions

Disinfectant	Dilution			Active ingredients	Manufacturer
	Low conc.	Recommended Conc.	High conc.		
Virkon S	0.50%	1*%	2%	potassium peroxymonosulfate and sodium chloride	LANXESS AG
TH$_5$	1%	2*%	4%	Alkyl dimethyl benzyl, ammonium chloride, glutaraldehyde	THESEO
NP50	2%	4*%	6%	Potassium monopersulphonate, sodium hexmate phosphate	SOLVEDA, EGYPT

Microorganisms: E. coli S. typhimurium and S. aureus were used in the current study. These organisms were isolated from poultry farms in the New-Valley governorate during the period between May 2018 and April 2019

Preparation of bacterial isolates: A loopful from 24 h. nutrient agar slope was transferred to 10 ml nutrient broth and incubated at 37oc for 18-24 h. The total colony count was determined by the plating technique (Cruickshank, 1980; Tuncan, 1993).

Evaluation of disinfectants: Tubes containing 9.5 mL of saline were inoculated with 0.5 mL of each of microbial strains to be tested. At time intervals, 5, 15, and 30 minutes, 1 ml was taken into tubes containing 9 ml. saline and the colony count was conducted by the pour plate technique (Pilotto et al., 2007). Each disinfectant was tested against the disinfectants at three different concentrations including the recommended concentrations as well as a higher and lower concentration (Table 1).

Results

Results reported in tables (2-5) revealed that, at 5 minutes, the reduction log showed by TH5 on S. typhimurium, E. coli, and S. aureus were, 3.6, 2.55, 3.54, respectively. Moreover, the reduction log for Virkon-S were 3.02, 2.21, and 3.37 on S. typhimurium, E. coli, and S. aureus, respectively. On the other hand, the reduction log induced by NP50 were,1.76, 1.91, 3.37 on S. typhimurium, E. coli, and S. aureus, respectively. At 15 minutes, the reduction log showed by TH5 on S. typhimurium, E. coli, and S. aureus were, 4.97, 3.59, 4.11, respectively. Moreover, the reduction log for Virkon-S were 4.97, 3.31, and 5.19 on S. typhimurium, E. coli, and S. aureus, respectively. On the other hand, the reduction log induced by NP50 were,2.82, 1.82, 4.27 on S. typhimurium, E. coli, and S. aureus, respectively.

After 30 minutes, no growth was observed for S. aureus with 100% inhibition induced by TH5 and Virkon-S. On the other hand, TH5 showed reduction log as 7.32, and 7.16 for S. typhimurium, E. coli, respectively (tables 4-5) while Virkon-S showed reduction log by 6.5 and 4.67 on S. typhimurium, E. coli, respectively (tables 4-5). Moreover, NP50 induced 5.39, 3.4, and 7.35 reduction log for S. typhimurium, E. coli, and S. aureus, respectively (tables 4-5).

Table (2): Disinfectant efficiency against selected bacteria after 5 minutes contact time

Disinfectants	Concentration	*S. Typhimurium*	Reduction/Log	*E. Coli*	Reduction /Log	S. aureus	Reduction /Log
Virkon-s	0.5%	1.3×10^{7}	1.32	2.63×10^{7}	0.69	4.5×10^{5}	2
	1*%	2.6×10^{5}	3.02	0.81×10^{6}	2.21	1.9×10^{4}	3.37
	1.5%	0.3×10^{3}	5.95	5.9×10^{4}	3.34	0.8×10^{3}	4.75
T.H4	½%	2.4×10^{6}	2.05	1.97×10^{6}	1.82	0.75×10^{5}	2.78
	1*%	6.8×10^{4}	3.6	3.7×10^{5}	2.55	1.30×10^{4}	3.54
	2%	1.2×10^{2}	6.35	1.8×10^{4}	3.86	5.51×10^{3}	3.91
NP50	2%	2.91×10^{7}	0.97	4.92×10^{7}	0.42	0.33×10^{5}	3.13
	4*%	4.72×10^{6}	1.76	1.61×10^{6}	1.91	1.94×10^{4}	3.37
	6%	1.21×10^{4}	4.35	1.01×10^{5}	3.11	9.8×10^{3}	3.66

*Initial count for *S. typhimurium* was (2.7×10^{8}), *E. coli* (1.3×10^{8}), and *S. aureus* (4.5×10^{7}).

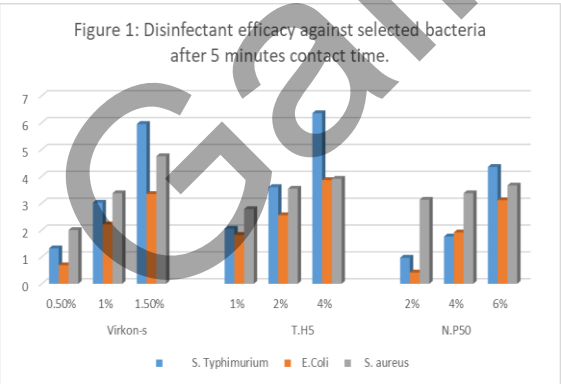

Table (3): Disinfectant efficacy against selected bacteria after 15 minutes contact time

Disinfectants	Concentration	S. typhimurium	Reduction /Log	E. coli	Reduction /Log	S. aureus	Reduction /Log
Virkon-s	0.5%	5.4×10^{5}	2.7	8.2×10^{6}	1.2	1.7×10^{4}	3.42
	1*%	2.9×10^{3}	4.97	6.4×10^{4}	3.31	2.9×10^{2}	5.19
	1.5%	4.1×10^{2}	5.82	0.91×10^{3}	5.15	0.09×10^{2}	6.7
T.H5	1%	1.32×10^{5}	3.31	4.90×10^{5}	2.42	1.77×10^{4}	3.41
	2*%	2.92×10^{3}	4.97	3.32×10^{4}	3.59	3.51×10^{3}	4.11
	4%	0.87×10^{2}	6.49	1.75×10^{3}	4.87	1.93×10^{2}	5.37
NP50	2%	1.47×10^{6}	2.46	3.84×10^{6}	1.53	1.53×10^{4}	3.47
	4*%	4.1×10^{5}	2.82	1.99×10^{5}	1.82	2.41×10^{3}	4.27
	6%	1.93×10^{3}	5.15	0.55×10^{4}	4.37	2.62×10^{2}	5.23

*Recommended concentration by the manufacturer.

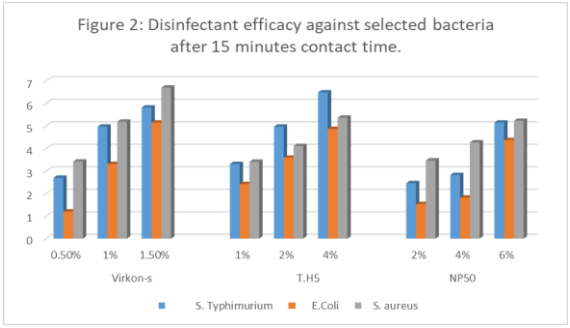

Table (4): Disinfectant efficacy against selected bacteria after 30 minutes contact time

Disinfectants	Concentration	S. typhimurium	Reduction /Log	E. coli	Reduction /Log	S. aureus	Reduction /Log
Virkon-s	0.5%	9.7×10^{3}	4.44	4.9×10^{4}	3.42	0.30×10^{2}	6.18
	1%	0.85×10^{2}	6.5	2.8×10^{3}	4.67	No growth	10
	1.5%	0.03×10^{2}	7.95	0.7×10^{2}	6.27	No growth	10
T.H5	1%	1.88×10^{3}	5.16	1.75×10^{2}	5.87	0.12×10^{2}	6.57
	2%	0.13×10^{2}	7.32	0.09×10^{2}	7.16	No growth	10
	4%	No growth	10	No growth	10	No growth	10
NP50	2%	1.53×10^{4}	4.25	2.22×10^{5}	2.77	1.33×10^{2}	5.53
	4%	1.11×10^{3}	5.39	5.2×10^{4}	3.4	0.02×10^{2}	7.35
	6%	0.15×10^{2}	7.26	1.12×10^{2}	6.05	No growth	10

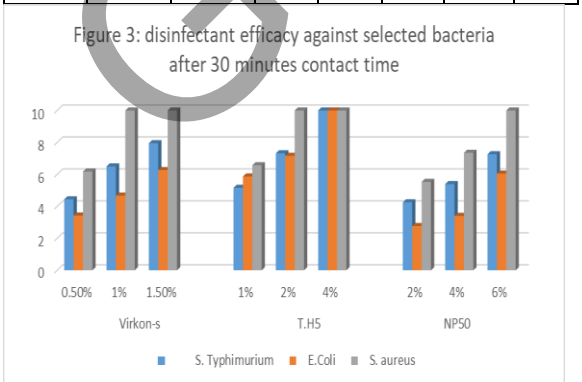

Table (5): Reduction log for the organisms used by the selected disinfectants under test at the recommended concentrations.

Disinfectant	*S. typhimurium*			*E. coli*			*S. aureus*		
	5 min	15 min	30 min	5 min	15 min	30 min	5 min	15 min	30 min
Virkon S (1%)	3.02	4.97	6.5	2.21	3.31	4.67	3.37	5.19	10
TH5 (2%) *	3.6	4.97	7.32	2.55	3.59	7.16	3.54	4.11	10
NP50(4%) *	1.76	2.82	5.39	1.91	1.82	3.4	3.37	4.27	7.35

Discussion

Protection of poultry environment against infection spreading has become a major concern all over the world. In the present study various compounds were used covering a wide range of the most common disinfectants in the veterinary field including Virkon-S, TH5 and NP50 (Table 1) The bactericidal effects of disinfectants varied widely between different types of disinfectants, so the disinfectants should be evaluated before their using (Moretro et al., 2013). The optimum concentration (as recommended by the producers) and the time of contact between the disinfectant and the organisms should be considered. The results in tables (2-5) showed the most sensitive organism to the used disinfectant is S. aureus. The growth of the organism was completely inhibited by the recommended concentration of Virkon-S, and TH5 within 30 minutes. On the other hand, using of NP50 at the recommended concentration showed weak effect against S. aureus while its effect on S typhimurium, E. coli was non-significant. Moreover, E. coli showed more or less resistant against all the used organisms. No noticeable log reduction in all bacteria strains under test were recorded after 5 minutes contact time all tested concentrations for all disinfectants against S.

typhimurium, E. coli and S. aureus were resistant to virkon-S, TH5 and Np50 within the first 5 minutes of the contact time at the three levels of concentration. These results more or less similar to those reported by Spielholz (1998) and McLaren et al. (2011) who found that, Virkon-S failed to kill S. typhimurium in the first 10 minutes of contact in the absence of organic matter. Moreover, Kamal et al. (2019) showed that some microorganisms as E. coli and Salmonella were able to survive and detected in the environmental samples after disinfection. Results in tables (2-5) revealed that E. coli is the most resistant organism against all the tested disinfectants Martínez-Martínez et al. (2016) stated that Virkon-S is not able to inactivate E. coli. On the other hand, Gasparini, et al. (1995) found that, Virkon-S is effective against E. coli. After 15 minutes of the contact time, S. typhimurium and E. coli still resistant to Virkon-S and NP50, while TH5 had a good effect on all the tested bacteria. The addition of glutaraldehyde to quaternary ammonium compounds (QACs) resulting in high antibacterial effectiveness. These results are in consistent with the results reported by McDonnell and Russell (2001) who found that QACs have complete effectiveness against gram positive and gram-negative bacteria. Similar results were recorded by Chima et al. (2013) and Noha et al. (2017). Moreover, S. aureus is highly sensitive reduced by 6.7 log at the high level of concentration String fellow et al. (2009). However, Soliman et al. (2009) found that TH4 showed high efficacy against S. aureus after 5 min ($p<0.0001$) with killing efficacy (99.98%) and 100% efficacy after 10 min ($p<0.0001$) in the absence of organic matter. After 30 minutes contact time, all disinfects showed a considerable effect on all tested organisms. S. aureus are completely inhibited by TH5 and Virkon-S. Moreover, NP50 is strongly affect on E. coli and made reduction log of 7.35. On the other hand, S. typhimurium showed a moderate sensitivity to the used disinfectant and its reduction log were ranged from 5.3-6.5 (table 5). On the other hands, E.coli showed some resistant to the used disinfectant and its reduction log were ranged from 3.4 and 4.67 for NP50 and Virkon-S, respectively while TH5 exert reduction log of 7.16 (table 5). The effectiveness of TH5 against all tested organisms may be attributed to their glutaraldehyde (González-Fandos et al., 2005). Glutaraldehyde-based disinfectants are able to reduce the bacterial population by five to six logs after 10 minutes of contact (Ruano, et al., 2001; Ahmed and Sotohy, 2003) Under these considerations, disinfectant preparations and concentrations should be carefully considered (Ruano, et al., 2001). The miss uses of disinfectants, including lower doses, lack of change, and other factors lead to the development of disinfectant microbial resistance (Davies et al., 2019). Generally, the efficiency of disinfectants depends on the concentration and exposure time.

Conflict of interest

The authors declare that they have no competing interest.

References

Abdulghaffar TA and El Bahgy HE. (2016). Effect of level of biosecurity measures on prevalence of some pathogenic microorganisms in poultry farm. Benha Veterinary Medical Journal. 31 (1): 147-153.

Ahmed MM and Sotohy AS. (2003). In-vitro study of some selected disinfectants on some pathogens of hygienic significance in veterinary practice. Bull. Environ. Res. 6 (1): 13-25.

CDC. (2016). Centre for disease control division of oral health- infection control glossary.

Chima IU, Uchegbu M, Okoli I, Ezema C and Wehke S. (2013). Evaluation of the efficiency of disinfectants used against bacterial isolates from intensive poultry farming environments in imo state, nigeria. Journal of Biological Sciences. 13 (5): 349-356.

Corrier DE, Hinton Jr A, Hargis B and DeLoach JR. (1992). Effect of used litter from floor pens of adult broilers on salmonella colonization of broiler chicks. J Avian Diseases. 897-902.

Cruickshank R, Duguid Jp, Marmion Bp. And Swain (1980). Medical microbiology 12th edition, vol. 11, . Book title. 12 ed. reprinted Churchill Livingstone and Robert Steveson Edinburgh; p.

Davies R, Wales AJCrifs and safety f. (2019). Antimicrobial resistance on farms: A review including biosecurity and the potential role of disinfectants in resistance selection. 18 (3): 753-774.

Davies RH and Wray C. (1995). Observations on disinfection regimens used on salmonella enteritidis infected poultry units. J Poultry science. 74 (4): 638-647.

Gasparini R, Pozzi T, Magnelli R, Fatighenti D, Giotti E, Poliseno G, Pratelli M, Severini R, Bonanni P and De Feo LJEjoe. (1995). Evaluation of in vitro efficacy of the disinfectant virkon. 11 (2): 193-197.

González-Fandos E, Sanz J, Garcia-Fernandez M and Garcia-Arias M. (2005). Effectiveness of disinfectants used in the food industry on microorganisms of sanitary interest. J Acta Alimentaria. 34 (3): 253-258.

Kamal MA, Khalaf MA, Ahmed ZAM and El Jakee J. (2019). Evaluation of the efficacy of commonly used disinfectants against isolated chlorine-resistant strains from drinking water used in egyptian cattle farms. Veterinary world. 12 (12): 2025.

Martínez-Martínez S, Yubero-Delgado S, Rodríguez-Ferri E-F, Frandoloso R, Álvarez-Estrada Á and Gutiérrez-Martín C-B. (2016). In vitro efficacy of several disinfectants against salmonella enterica serovar enteritidis and escherichia coli strains from poultry. J Ciência Rural. 46 (8): 1438-1442.

McDonnell G and Russell AD. (2001). Antiseptics and disinfectants: Activity, action, and resistance. Clinical microbiology reviews. 14 (1): 227.

McLaren I, Wales A, Breslin M and Davies R. (2011). Evaluation of commonly-used farm disinfectants in wet and dry models of salmonella farm contamination. J Avian Pathology. 40 (1): 33-42.

Moretro T, Langsrud S and Heir E. (2013). Bacteria on meat abattoir process surfaces after sanitation: Characterisation of survival properties of listeria monocytogenes and the commensal bacterial flora. Advances in Microbiology. 3 (3): 255-264.

Noha A, Hayfa Ismail and Mohammed A. (2017). Laboratory evaluation of some disinfectants used in poultry farms against some bacteria isolates. Journal of Applied Science And Research,. 5 (6): 32-41.

Pilotto F, Rodrigues L, Santos L, Klein W, Colussi F and Nascimento VPd. (2007). Antibacterial efficacy of commercial disinfectants on dirt floor used in poultry breeder houses. Brazilian Journal of Poultry Science. 9 (2): 127-131.

Ruano M, El-Attrache J and Villegas P. (2001). Efficacy comparisons of disinfectants used by the commercial poultry industry. J Avian Diseases. 972-977.

Soliman ES, Sobeih M, Ahmad Z, Hussein M and Moneim H. (2009). Evaluation of commercial disinfectants against bacterial pathogens isolated from broiler farms. J Int J Poult Sci. 8: 728-732.

Spielholz B. (1998). Properties of hatchery disinfectants. J World Poult. 14: 50-51.

Stringfellow K, Anderson P, Caldwell D, Lee J, Byrd J, McReynolds J, Carey J, Nisbet D and Farnell M. (2009). Evaluation of disinfectants commonly used by the commercial poultry industry under simulated field conditions. J Poultry science. 88 (6): 1151-1155.

Tuncan EU. (1993). Effect of cold temperature on germicidal efficacy of quaternary ammonium compound, lodophor, and chlorine on listeria. Journal of food protection. 56 (12): 1029-1033.

Zander D. (1997). Principles of disease prevention: Diagnosis and control. J Diseases of Poultry. 3-45.

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