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 (8): 728-732, 2009
ISSN 1682-8356

Evaluation of Commercial Disinfectants Against Bacterial Pathogens Isolated from Broiler Farms

Essam S. Soliman[1], Mohamed A.A. Sobeih[2], Z.H. Ahmad[2], M.M. Hussein[2] and H.A.A. Moneim[3]
[1]Department of Pathobiology, College of Veterinary Medicine,
Nursing and Allied Health, Tuskegee University, Tuskegee, Alabama 36088, USA
[2]Department of Animal Hygiene, Zoonoses and Animal Behavior,
College of Veterinary Medicine, Suez Canal University, Ismailia, Egypt
[3]Department of Clinical Pathology, College of Medicine, Suez Canal University, Ismailia, Egypt

Abstract: Five disinfectants [TH4® (combination of quaternary ammonium compounds and gluteraldhyde), Microzal® (combination of quaternary ammonium compounds and gluteraldhyde), Incospect IC 22XA (combination of quaternary ammonium compounds, gluteraldhyde and formalin), Povidone Iodine® (iodophore) and Formalin® (commercial formaldehyde 37%) were tested in a laboratory trials against four bacterial isolates (*Staphylococcus aureus*, *Escherchia coli*, *Klebsiella oxytoca* and *Pseudomonas aureuginosa*) at concentration of (~10^5) isolated during epidemiological surveillance. The trials were carried in the presence and absence of organic matter (dried yeast 5%) using MIC use-dilution test. Minutely samples were collected for the bacterial counts. In the absence of organic matter, TH4® achieved the 100% killing efficacy against *Staphylococcus aureus*, *Escherchia coli*, *Klebsiella oxytoca* and *Pseudomonas aureuginosa* after 5, 5, 10 and 5 min ($p \leq 0.0001$) respectively, Microzal® and Incospect IC 22XA achieved the 100% killing efficacy against the four Micro-organisms after 5 min ($p \leq 0.0001$), Povidone Iodine® achieved the 100% killing efficacy after 5, 5, 5 and 10 min ($p \leq 0.0001$) respectively and Formalin® achieved the 100% killing efficacy after 5, 5, 5 and 20 min ($p \leq 0.0001$) respectively. In the presence of organic matter, TH4® achieved the 100% killing efficacy against *Staphylococcus aureus*, *Escherchia coli*, *Klebsiella oxytoca* and *Pseudomonas aureuginosa* after 10, 5, 20 and 20 min ($p \leq 0.0001$) respectively, Microzal® achieved the 100% killing efficacy after 5, 5, 5 and 10 min ($p \leq 0.0001$) respectively, Incospect IC 22XA achieved the 100% killing efficacy after 5, 5, 10 and 5 min ($p \leq 0.0001$) respectively, Povidone Iodine® achieved the 100% killing efficacy against the four micro-organisms after 30 min ($p \leq 0.0001$) and Formalin® achieved the 100% killing efficacy after 30, 5, 20 and 20 min ($p \leq 0.0001$) respectively. The results revealed that quaternary ammonium-gluteraldhyde combination (TH4®, Microzal® and Incospect IC 22XA) although they are not proven to be environmentally safe; they are the most powerful disinfectants because of the synergistic action of the quaternary ammonium and gluteraldhyde bases.

Key words: Disinfectants, evaluation, bacterial pathogens, broiler

INTRODUCTION

Disinfection of a building implies the elimination of all micro-organisms that are capable of multiplication and causing diseases from the poultry houses. It is poor economy to leave poultry houses empty in an attempt to eliminate the micro-organisms, if such a period was to have any meaning it would need to be very long indeed, and even might be a waste of time. So the procedures are to carry out cleansing and disinfection that might be considered safe to place birds back into the building, (Sainsbury, 1982).

It must be stressed that a total cleaning is an essential prior to disinfection in order to remove the organic matter and biofilm that have a great power to reduce the effectiveness of disinfectants. 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).

Each chemical disinfectant has its own germicidal power under optimal conditions relating to several parameters influences the in-use activity of disinfectants, these parameters included the concentration of the agents, the number, type and location of the micro-organisms, temperature and pH of the treatment as well as the presence of the other materials (organic matter). Unfortunately, poor sanitation procedures and/or increased soil moisture levels have been linked to increased or sustained bacteria levels, (Rudolfs *et al*., 1950; Pepper *et al*., 1993).

Disinfectant efficacy is often tested against laboratory bacterial suspensions (Parkinson, 1981; Bloomfield *et al*., 1991). However, this approach may not always prove to simulate commercial production conditions, thus, making it difficult to determine the true effectiveness of the disinfectant. Disinfectants that are effective against bacterial suspensions may have a reduced effect

against bacteria that adhere to surfaces, (Mosteller and Bishop, 1993).

The aim of this study was to compare the effect of the five disinfectants in relation to time against bacterial isolates (*Staphylococcus aureus, Escherchia coli, Klebsiella oxytoca* and *Pseudomonas aureuginosa*) in the presence and absence of organic matter as an extra-challenge to the action of the disinfectants.

MATERIALS AND METHODS

Preparation of recommended concentration of the chemical disinfectants: TH4® is a combination of four quaternary ammonium compounds and gluteraldhydes as well as plant extract (pine oil and turpine oil) were added to obtain a pleasant perfume. It contains gluteraldhyde as an acid solution and activated by sodium bicarbonate to alkaline pH. One liter contains gluteraldhyde (62.50 g), didecyle dimethyl ammonium chloride (18.75 g), dioctyl dimethyl ammonium chloride (18.75 g), octyl decyl dimethyl ammonium chloride (37.50 g), alkyl dimethyl benzyl ammonium chloride (50 g), Pine oil (20 g), Terpine oil (50 g). The recommended concentration was 1:100 (1 ml of TH4® solution was added to 100 ml distilled water, pH 8.7).

Microzal® is synergistic blend of gluteraldhyde (hydrophilic biocide) and four exclusive Quaternary ammonium compounds (lipophilic biocide) with proven efficacy on all viruses responsible for major animal diseases, bacteria, fungi and Mycoplasma with recommended concentration was 1:100 (1 ml of Microzal® solution was added to 100 ml distilled water, pH 8.4).

Incospect IC 22XA is a combination of Bardac (22) didecyldimethyl ammonium chloride (100 g-20%), gluteraldhyde (80 g-16%) and formalin (32 g-9%) and used at recommended concentration of 0.5% (0.5 ml of Incospect IC 22XA was added to 100 ml distilled water, pH 9.6).

Povidone iodine® is iodophore compound that have a characteristic odor and used by recommended concentration of 7.5% (7.5 ml of Povidone iodine® was added to 100 ml distilled water, pH 7.8).

Formaline® 37% is the most active chemical disinfectant against most types of micro-organisms as bacteria and their spores, fungi, viruses however; it is usually recommended at concentration of 2.5% (2.5 ml of Formaline® 37% was added to 100 ml distilled water, pH 7.9).

Propagation of the bacterial isolates: The bacterial isolates (*Staphylococcus aureus, Escherchia coli, Klebsiella oxytoca* and *Pseudomonas aureuginosa*) were propagated using pour plate method, (Cruickshank *et al*., 1980). A loopful was transferred from all bacterial strains that was stored onto nutrient slopes into 10 ml nutrient broth and incubated at 37°C for 20-24 h. Tenfold serial dilutions were carried out into tubes containing 9ml phosphate buffered saline, one ml from each dilution was transferred into a sterile peridish then 10 ml of standard plate count agar melted and cooled at 45°C were aseptically poured into each petridish. After thoroughly mixing the plates were left to solidify, incubated at 37°C for 20-24 h, The calculation was carried out using the following formula: Log (average CFU/drop vol.) (dilution factor) (Vol. scrapped into/surface area) (Zelver *et al*., 1999; Herigstad *et al*., 2001).

Preparation of source of organic matter: 5% stock solution of yeast suspension (5 g of dried yeast was added to 100 ml of sterile distilled water); the yeast suspension was dispensed into 5 ml tubes, sterilized by autoclaving for 20 min at 121°C.

Evaluation of the efficacy of chemical disinfectants against the bacterial pathogens: The laboratory evaluation of the efficacy of the chemical disinfectants was carried out using modified use-dilution test (Robinson *et al*., 1988). The test was repeated twice; once in the presence of organic matter and the second time in the absence of the organic matter.

Evaluation of the efficacy of chemical disinfectants in the absence of organic matter: Bacterial suspension was prepared and propagated. Ten ml of the tested chemical disinfectant were poured into a sterile test tubes, 0.1 ml of the bacterial suspension (~10^5) was added and shaken thoroughly to give the chance for micro-organism to come in contact with the disinfectant. At time interval 5, 10, 20 and 30 min from original zero time 1 ml of disinfectant-bacterial mixture were taken into tube containing 9 ml of in-activator (Tween 80 3%) in nutrient broth, mix thoroughly. One ml from in-activator tubes was used for the bacterial count using pour plate method (Cruickshank *et al*., 1980). The numbers of survival bacteria on each plate were counted. The calculation was carried out using the following formula: Log (average CFU/ drop vol.) (dilution factor) (Vol. scrapped into/ surface area) (Zelver *et al*., 1999; Herigstad *et al*., 2001).

Evaluation of the efficacy of chemical disinfectants in the presence of organic matter: A suspension of bacterial yeast extract mixture was prepared by adding 4.5 ml Yeast extract 5% to 0.5 ml of the bacterial suspension (~10^5) and mixed gently. Nine ml of tested chemical disinfectant concentration were poured in a sterile test tubes, 1 ml of bacterial yeast extract mixture was added and shaken thoroughly to give the chance for micro-organism to come in contact with the disinfectant. At time interval 5, 10, 20 and 30 min. from original zero time 1 ml. of disinfectant bacterial yeast extract mixture

from each tube were taken to the corresponding tube containing 9 ml of in-activator (Tween 80 3%) in nutrient broth, mix thoroughly. One ml from in-activator tubes was used for the bacterial count using pour plate method, (Cruickshank *et al*., 1980). The numbers of survival bacteria on each plate were counted. The calculation was carried out using the following formula: Log (average CFU/ drop vol.) (dilution factor) (Vol. scrapped into/ surface area) (Zelver *et al*., 1999; Herigstad *et al*., 2001).

Statistical analysis: The statistical analysis was carried out by performing analysis of variance (ANOVA, GLM, MIXED) using SAS 9.2.0 software.

RESULTS AND DISCUSSION

The objective of this study was to evaluate the efficacy of some commercial disinfectants that was not proven to be environmentally safe and if it is possible to be used in poultry houses while the birds are still present.

In the absence of organic matter, TH4®, Microzal®, Incospect IC 22XA, Povidone Iodine® and Formalin® achieved 100% efficacy against *Staphyococcus aureus* after 5 min ($p \leq 0.0001$) Table 1.

In the presence of organic matter, Microzal® and Incospect IC 22XA achieved the 100% efficacy after 5 min ($p \leq 0.0001$). TH4® starting to show high efficacy against *Staphylococcus aureus* after 5 min ($p \leq 0.0001$) with killing efficacy (99.98%) and showed the 100% efficacy after 10 min ($p \leq 0.0001$). Povidone Iodine® and Formalin® starting to show high efficacy after 5 min ($p \leq 0.001$) with killing efficacy (99.96%) and (99.95%) respectively and showed the 100% efficacy after 30 min ($p \leq 0.0001$) Table 1.

In the absence of organic matter, TH4®, Microzal®, Incospect IC 22XA, Povidone Iodine® and Formalin® achieved 100% efficacy against *Escherichia coli* after 5 min ($p \leq 0.0001$) Table 2.

In the presence of organic matter, TH4®, Microzal®, Incospect IC 22XA and Formalin® achieved the 100% efficacy after 5 min ($p \leq 0.0001$). Povidone Iodine® starting to show high efficacy after 5 min ($p \leq 0.001$) with killing efficacy (99.96%) and showed 100% efficacy after 30 min ($p \leq 0.0001$) Table 2.

In the absence of organic matter, Microzal®, Incospect IC 22XA, Povidone Iodine® and Formalin® achieved 100% efficacy against *Klebsiella oxytoca* after 5 min ($p \leq 0.0001$). TH4® starting to show high efficacy against *Klebsiella oxytoca* after 5 min ($p \leq 0.0001$) with killing efficacy (99.99%) and achieved the 100% efficacy after 10 min ($p \leq 0.0001$) Table 3.

In the presence of organic matter, Microzal® only achieved the 100% efficacy after 5 min ($p \leq 0.0001$). Incospect IC 22XA both starting to show high efficacy against *Klebsiella oxytoca* after 5 min ($p \leq 0.0001$) with killing efficacy (99.99%) and achieved the 100% efficacy after 10 min ($p \leq 0.0001$). TH4® and Formalin® stating to show high efficacy against *Klebsiella oxytoca* after 5 min ($p \leq 0.0001$) with killing efficacy (99.94%) and (99.95%) respectively and showed the 100% efficacy after 20 min ($p \leq 0.0001$). Povidone Iodine® starting to show high efficacy after 5 min ($p \leq 0.001$) with killing efficacy (99.96%) and showed 100% efficacy after 30 min ($p \leq 0.0001$) Table 3.

In the absence of organic matter, TH4®, Microzal® and Incospect IC 22XA achieved 100% efficacy against *Pseudomonas aureuginosa* after 5 min ($p \leq 0.0001$). Povidone Iodine® starting to show high efficacy against *Pseudomonas aureuginosa* after 5 min ($p \leq 0.0001$) with killing efficacy (99.96%) and achieved 100% efficacy after 10 min ($p \leq 0.0001$). Formalin® starting to show high efficacy against *Pseudomonas aureuginosa* after 5 min ($p \leq 0.0001$) with killing efficacy (99.95%) and achieved the 100% efficacy after 20 min ($p \leq 0.0001$), Table 4.

In the presence of organic matter, Incospect IC 22XA achieved the 100% efficacy after 5 min ($p \leq 0.0001$). Microzal® starting to show high efficacy against *Pseudomonas aureuginosa* after 5 min ($p \leq 0.0001$) with killing efficacy (99.95%) and achieved the 100% efficacy after 10 min ($p \leq 0.0001$). TH4® starting to show high

Table 1: Efficacy of the five chemical disinfectants against *Staphylococcus aureus* in the absence and presence of organic matter

		Absence of organic matter				Presence of organic matter			
		Time/min				Time/min			
Disinfectants	Parameter	5	10	20	30	5	10	20	30
TH4® (1%)	Log_{10} count	0^{a**}	0^{a}	0^{a}	0^{a}	3.91^{a**}	0^{b**}	0^{b}	0^{b}
	Killing %	100	100	100	100	99.98	100	100	100
Microzal® (1%)	Log_{10} count	0^{a**}	0^{a}	0^{a}	0^{a}	0^{a**}	0^{a}	0^{a}	0^{a}
	Killing %	100	100	100	100	100	100	100	100
Incospect IC 22XA (0.5%)	Log_{10} count	0^{a**}	0^{a}	0^{a}	0^{a}	0^{a**}	0^{a}	0^{a}	0^{a}
	Killing %	100	100	100	100	100	100	100	100
Povidone Iodine® (7.5%)	Log_{10} count	0^{a**}	0^{a}	0^{a}	0^{a}	3.84^{a*}	3.59^{b*}	3.49^{b}	0^{c**}
	Killing %	100	100	100	100	99.96	99.99	99.99	100
Formalin® (2.5%)	Log_{10} count	0^{a**}	0^{a}	0^{a}	0^{a}	4.07^{a*}	3.79^{b*}	3.49^{c*}	0^{d**}
	Killing %	100	100	100	100	99.95	99.97	99.99	100

^{a,b,c,d}represented the significance between the different sampling time within each disinfectant.

*represents values with significance at $p \leq 0.001$, **represents values with highly significance at $p \leq 0.0001$

Table 2: Efficacy of the five chemical disinfectants against *Escherichia coli* in the absence and presence of organic matter

		Absence of organic matter				Presence of organic matter			
		Time/min				Time/min			
Disinfectants	Parameter	5	10	20	30	5	10	20	30
TH4® (1%)	Log_{10} count	0^{a**}	0^{a}	0^{a}	0^{a}	0^{a**}	0^{a}	0^{a}	0^{a}
	Killing %	100	100	100	100	100	100	100	100
Microzal® (1%)	Log_{10} count	0^{a**}	0^{a}	0^{a}	0^{a}	0^{a**}	0^{a}	0^{a}	0^{a}
	Killing %	100	100	100	100	100	100	100	100
Incospect IC 22XA (0.5%)	Log_{10} count	0^{a**}	0^{a}	0^{a}	0^{a}	0^{a**}	0^{a}	0^{a}	0^{a}
	Killing %	100	100	100	100	100	100	100	100
Povidone Iodine® (7.5%)	Log_{10} count	0^{a**}	0^{a}	0^{a}	0^{a}	3.84^{a**}	3.70^{b**}	3.47^{c*}	0^{d**}
	Killing %	100	100	100	100	99.96	99.97	99.99	100
Formalin® (2.5%)	Log_{10} count	0^{a**}	0^{a}	0^{a}	0^{a}	0^{a**}	0^{a}	0^{a}	0^{a}
	Killing %	100	100	100	100	100	100	100	100

^{a,b,c,d}represented the significance between the different sampling time within each disinfectant.
*represents values with significance at $p \leq 0.001$, **represents values with highly significance at $p \leq 0.0001$

Table 3: Efficacy of the five chemical disinfectants against *Klebsiella oxyfoca* in the absence and presence of organic matter

		Absence of organic matter				Presence of organic matter			
		Time/min				Time/min			
Disinfectants	Parameter	5	10	20	30	5	10	20	30
TH4® (1%)	Log_{10} count	3.49^{a**}	0^{b**}	0^{b}	0^{b}	4.12^{a**}	4.07^{b**}	0^{c**}	0^{c}
	Killing %	99.99	100	100	100	99.94	99.95	100	100
Microzal® (1%)	Log_{10} count	0^{a**}	0^{a}	0^{a}	0^{a}	0^{a**}	0^{a}	0^{a}	0^{a}
	Killing %	100	100	100	100	100	100	100	100
Incospect IC 22XA (0.5%)	Log_{10} count	0^{a**}	0^{a}	0^{a}	0^{a}	3.50^{a**}	0^{b**}	0^{b}	0^{b}
	Killing %	100	100	100	100	99.99	100	100	100
Povidone Iodine® (7.5%)	Log_{10} count	0^{a**}	0^{a}	0^{a}	0^{a}	3.90^{a**}	3.84^{b**}	3.47^{c**}	0^{d**}
	Killing %	100	100	100	100	99.96	99.96	99.99	100
Formalin® (2.5%)	Log_{10} count	0^{a**}	0^{a}	0^{a}	0^{a}	4.09^{a**}	3.69^{b*}	0^{c**}	0^{c}
	Killing %	100	100	100	100	99.95	99.98	100	100

^{a,b,c,d}represented the significance between the different sampling time within each disinfectant.
*represents values with significance at $p \leq 0.001$, **represents values with highly significance at $p \leq 0.0001$

Table 4: Efficacy of the five chemical disinfectants against *Pseudomonas aureuginosa* in the absence and presence of organic matter

		Absence of organic matter				Presence of organic matter			
		Time/min				Time/min			
Disinfectants	Parameter	5	10	20	30	5	10	20	30
TH4® (1%)	Log_{10} count	0^{a**}	0^{a}	0^{a}	0^{a}	4.13^{a**}	3.77^{b**}	0^{c**}	0^{c}
	Killing %	100	100	100	100	99.94	99.95	100	100
Microzal® (1%)	Log_{10} count	0^{a**}	0^{a}	0^{a}	0^{a}	4.0^{a**}	0^{b**}	0^{b}	0^{b}
	Killing %	100	100	100	100	99.95	100	100	100
Incospect IC 22XA (0.5%)	Log_{10} count	0^{a**}	0^{a}	0^{a}	0^{a}	0^{a**}	0^{a}	0^{a}	0^{a}
	Killing %	100	100	100	100	100	100	100	100
Povidone Iodine® (7.5%)	Log_{10} count	3.95^{a**}	0^{b**}	0^{b}	0^{b}	3.90^{a**}	3.70^{b**}	3.47^{c**}	0^{d**}
	Killing %	99.96	100	100	100	99.96	99.98	99.99	100
Formalin® (2.5%)	Log_{10} count	4.07^{a**}	3.77^{b**}	0^{c**}	0^{c}	3.91^{a**}	3.83^{b*}	0^{c**}	0^{c}
	Killing %	99.95	99.97	100	100	99.96	99.97	100	100

^{a,b,c,d}represented the significance between the different sampling time within each disinfectant.
*represents values with significance at $p \leq 0.001$, **represents values with highly significance at $p \leq 0.0001$

efficacy against *Pseudomonas aureuginosa* after 5 min ($p \leq 0.0001$) with killing efficacy (99.94%) and showed the 100% efficacy after 20 min ($p \leq 0.0001$). Formalin® starting to show high efficacy against *Pseudomonas aureuginosa* after 5 min ($p \leq 0.0001$) with killing efficacy (99.96%) and achieved the 100% efficacy after 20 min ($p \leq 0.0001$). Povidone Iodine® starting to show high efficacy after 5 min ($p \leq 0.001$) with killing efficacy (99.96%) and showed 100% efficacy after 30 min ($p \leq 0.0001$) Table 4.

Conclusion: From the trial we concluded that quaternary ammonium compounds and gluteraldhyde although they are non environmentally safe compounds; when exist in one compound, it resulted in a synergistic action that enables the elimination of the microorganisms as easy task even in the presence of organic matter and this was clear in case of TH4® and Microzal®, both of them was able to eliminate the bacterial load and suppress its growth with 5-10 min after application. Formalin is one of the most powerful compounds that

have been used against the bacterial load in poultry houses, but it is non-environmentally safe compound. When Formalin is used alone it required the removal of the organic matter that can retard it action especially against highly powerful organisms like *Pseudomonas aureuginosa* (achieved the action after 20 min). On the contrary when it is added to other compounds like a mixture of quaternary ammonium compound and gluteraldhyde it gave the ultimate compound and this was clear in the results of Incospect IC 22XA.

Povidone Iodine® when applied to the poultry houses, it is required to remove the organic matter from the surfaces to prevent the retardation in action, as the organic matter prevent the Povidone Iodine® to reach the target organism.

REFERENCES

Bloomfield, S.F., M. Arther, E. Looney, K. Begun and H. Patel, 1991. Comparative testing of disinfectant and antiseptic products using proposed European suspension testing methods. Lett. Appl. Microbiol., 13: 233-237.

Cruickshank, R., J.P. Duguid, B.P. Marimion and R.H. Swain, 1980. Medical microbiology. E.L.B.S. 12th Edn., vol. 11, reprinted Churchill Livingstone and Robert Stevenso. Edinburgh, EHI, 3AF.

Denyer, S.P. and G.S.A.B. Stewart, 1998. Mechanisms of action of disinfectants. Int. Biodeterior. Biodegradation, 41: 261-268.

Herigstad, B., M. Hamilton and J. Heersink, 2001. How to optimize the drop plate method for enumerating bacteria. J. Microbiol. Meth., 44: 121-129.

Maillard, J.Y., 2002. Bacterial target sites for biocide action. J. Appl. Microbiol., 92 (Suppl.) :16S-27S.

Mosteller, T.M. and J.R. Bishop, 1993. Sanitizer efficacy against attached bacteria in a milk biofilm. J. Food Prot., 56: 34-41.

Parkinson, E., 1981. Testing of disinfectants for veterinary and agricultural use. Pages 33-36 in Disinfectants: Their use and evaluation of effectiveness. C.H. Collins, M.C. Allwood, S.F. Bloomfield and A. Fox, Eds. Acad. Press, London.

Pepper, I.L., K.L. Josephson, R.L. Bailey, M.D. Burr and C.P. Gerba, 1993. Survival of indicator organisms in Sonoran Dessert soil amended with sewage sludge. J. Environ. Sci. Health, A28: 1287-1302.

Robinson, R.A., H.L. Bodily, D.F. Robinson and R.P. Christensen, 1988. A suspension methods to determine reuse life of chemical disinfectants during clinical use. Appl. Environ. Microbiol., 54: 158-164.

Rudolfs, W., L.L. Falk and R.A. Rgotzki, 1950. Literature review of the occurrence and survival of enteric pathogenic and relative organisms in soil, water sewage and sludge and on vegetation. Sewage and Industrial Wastes, 22: 1261-1281.

Sainsbury, D., 1982. The disinfection of poultry houses. In poultry health and management. Granada, London, Toronto, Sydney, New York.

Zelver, N., M. Hamilton, B. Pitts, D. Goeres, D. Walker, P. Sturman and J. Heersink, 1999. Measuring antimicrobial effects on biofilm bacteria: in RJ Doyle *et al.* (Eds), biofilm: methods in enzymology, Academic Press, San Diego, CA, pp: 608-628.