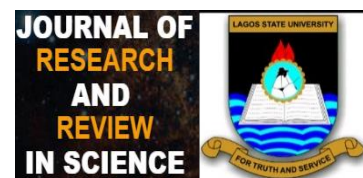


ORIGINAL RESEARCH



Evaluation of some chemical disinfectant formulations against *Pseudomonas* and *Staphylococcus* species from poultry and poultry environment

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Abstract:

Introduction: Diskol, Iodasteryl and Morigad are disinfectant formulations commonly used in the poultry industry in Nigeria to prevent infections of birds.

Aims: The formulations were evaluated for effectiveness in controlling the growth of bacterial isolates from poultry and poultry environment.

Materials and Methods: The bacteria were isolated from water, feed, litter materials, and cloacae swabs, and were identified using the Analytical Profile Index kits. The disinfectant evaluation was carried out using the kill kinetics method at 0.5X, 1X and 1.5X the manufacturer's recommended dilutions.

Results: The pseudomonads had percentage occurrence of *Pseudomonas putida* (25) > *P. mendocina* (20) > *P. aeruginosa* (15), *P. stutzeri* (15), *P. fluorescens* (15) > *P. shigelliodes* (10), while the staphylococci had *Staphylococcus lentus* (78.26) > *S. sciuri* (13.04) > *S. aureus* (8.70). Diskol and Morigad recorded 8 log₁₀ reduction (i.e. 100% kill) at the 3 concentrations against all the *Pseudomonas* isolates at 10 min contact time but were only able to achieve same at 1X and 1.5X against the staphylococci. At 0.5X, however, both Diskol and Morigad were only able to achieve 8 log₁₀ reduction (100% kill) of all *Staphylococcus* sp. at 30 min except against *S. sciuri* SN3 where Diskol achieved same record at 10 min. The best performances of Iodasteryl were less than 100% kill even at 8log 10 reduction against *P. putida* FaF12, *P. mendocina* FaW48, *P. stutzeri* FaM35, and *P. shigelliodes* FbM36 at 1.5X and 60 min and 7.99log 10 reduction at 1.5X and 60 min against *S. lentus* SN1, *S. lentus* SN2, *S. sciuri* SN3, *S. sciuri* SN4 and *S. aureus* SM2. All disinfectants demonstrated increasing activity with increase in time and concentration.

Conclusion: The study indicated the potential of the disinfectants in elimination of some bacteria of public health significance thus justifying their use in the control of poultry infections.

Keywords: Diskol, Morigad, Iodasteryl, *Pseudomonas*, *Staphylococcus*, disinfectants, Poultry.

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1. INTRODUCTION

Poultry farming is one of the important means of supplying the fast-growing global population with high quality protein and also providing incomes to farmers [1]. Poultry farming is one of the ways Africa has engaged to increase its production of animal protein. It offers the best yield in conversion of vegetable calories to high yield animal protein [2]. The world is experiencing an increasing incidence of food poisoning caused by the contamination of food by pathogenic microorganisms with the attendant adverse effects on health and socio - economic life of the people [3, 4]. There is, therefore, an unprecedented concern for food safety by the general public (the consumers) and the food industry [5]. Of major Importance among sources of microbial infection and contamination of poultry birds, poultry products and environment are the faeces, feed, water, water troughs, air, and floor of poultry house.

Birds that enter the processing line already contaminated with microorganisms will provide a veritable source of contamination of the final products. Live birds may shed microorganisms into feed, water, floor, transport containers and in faeces, thereby causing re-infection of other birds [5]. If poultry houses and transport containers are not properly cleaned and disinfected, they may act as continuous culture system where the microorganisms deposited in previous trips will reside and multiply to contaminate subsequent flocks or batches housed in the same farm or transported in the same unclean container [5, 6]. Once chickens are exposed to infection, the poultry flock is colonized quickly. The infected chickens may lead to meat contamination during slaughtering. Therefore, a farm-level disinfection program is needed to eliminate microbial infection and poultry food poisoning.

Disinfection in poultry farms and by poultry workers is essential in order to reduce financial losses due to disease outbreaks. Proper disinfection reduces the likelihood of infection of flocks and workers. Disinfection is an essential component of the biosecurity program for the poultry industry [7]. Mounting concern over the potential for microbial contamination and infection risks in the food and general consumer markets have also led to increased usage of antiseptics and disinfectants by the general public [5].

The increasing reports of food borne diseases and nosocomial infections have not only suggested an exaggerated belief in the effectiveness of disinfection procedures [8] but more importantly have increased interest in the evaluation of effectiveness of disinfectants in destroying pathogens and microbial contaminants [9]. Members of the genera; *Pseudomonas* and *Staphylococcus* have been implicated in poultry infections in other climes of the world, but there is dearth of information in literature, in Nigeria, on the distribution of *Pseudomonas* and *Staphylococcus* in poultry and poultry environment. In

addition, the standardization of disinfection program for poultry industry is practically non – existent in Nigeria. Therefore, the present study evaluated three disinfectant formulations, commonly used in the poultry industry in Nigeria, for their effectiveness in controlling the growth of *Pseudomonas* and *Staphylococcus* species.

2. MATERIAL AND METHODS

2.1 Preparation of test concentrations of the chemical disinfectants

Diskol is a synergistic blend of a quaternary ammonium compound and two aldehydes to give the formulation; Benzalkonium chloride 5%, glutaraldehyde 7.5% and formaldehyde 7%. The recommended concentration (1X) was 2:100 (2 ml of Diskol solution was added to 98 ml of distilled water, pH 8.7). The 50% below recommended concentration (0.5X) was prepared by adding 1 ml of Diskol solution to 99 ml of distilled water and shaken to homogenize. The 50% above recommended dilution (1.5X) was prepared by adding 3 ml of Diskol solution to 97 ml distilled water.

Morigad contains 32% Phenol v/v and 1X was 1:111 (1 ml of Morigad solution was added to 110 ml of distilled water,). To obtain the 0.5X, 0.5 ml Morigad solution was added to 110.5 ml distilled water. For the 1.5X, 1.5 ml Morigad solution was added to 109.5 ml distilled water.

Iodasteryl is a solution of active iodine 55mg/100 ml and 1X was 0.1:100 (0.1 ml of Iodasteryl solution was added to 99.9 ml of distilled water,). The 0.5X was prepared by adding 0.05 ml Iodasteryl solution to 99.95 ml distilled water. For the 1.5X, 0.15 ml Iodasteryl solution was added to 99.85 ml distilled water.

2.1.1 Collection of samples

The Aiyedooto Poultry Farm Settlement (comprising 30 poultry farms) in Ojo, Lagos State, Southwestern Nigeria was used as the sampling site. Triplicate samples each of water, feed (layer's feed), litter material (which consisted of wood shavings), and cloacae swabs (of layer birds) were taken from six different farms in the settlement. The water samples were aseptically collected from the tits, troughs, and the reservoir tank. Cloacae swabs were collected using sterile swab sticks pre – moistened in peptone water. Both the water and cloacae swab samples were placed in an insulating foam box containing ice. All the samples were immediately transported to the laboratory within one hour for analysis.

2.2 Bacterial isolation and identification

Ten grams each of the feed and litter material was separately homogenized with 90 ml sterile water in a sterilized blender to make the 10^{-1} dilution. This was serially diluted to 10^{-6} dilution and then 1 ml of appropriate dilution was separately inoculated on mannitol salt agar, nutrient agar and pseudomonas CN agar. The water samples (1ml) were directly pour-plated on each of the three media. The swabs were cultured by spread plate method onto the three media. All cultured plates were aerobically incubated at 37°C for 24 h. distinct colonies were subcultured twice to obtain pure cultures, which were stored as stock cultures in nutrient agar slants in the refrigerator at 4°C till further analysis. The isolates were identified with the method of [10] on colonial and cellular morphology. Isolates from the mannitol salt agar and nutrient agar that were Gram positive cocci in clusters and catalase positive were selected as presumable *Staphylococcus* strains and were further characterized using the analytical profile index (API) kits of API STAPH (BioMerieux) while, isolates from the pseudomonas CN agar and nutrient agar that were Gram negative bacilli, oxidase positive and catalase positive were selected as presumable *Pseudomonas* strains and were further identified using API 20NE (BioMerieux).

2.2.1 Evaluation of the chemical disinfectants against the bacterial isolates

Evaluation of disinfectant was carried out using Suspension kill kinetics method of [11] with few modifications. Four milliliters of a 16 - 24 h tryptone soy broth culture of each test organism was added to 96 ml phosphate buffered saline (PBS, 0.33 M NaCl, 3 mM KCl, 8.4 mM Na_2HPO_4 , 1.6 mM KH_2PO_4 , pH 7.2) and the bacterial cell density was spectrophotometrically adjusted with PBS to 10^8 cfu/ml. Aliquot amount of 2.5 ml was taken from each test organism suspension and mixed with 2.5 ml of each concentration of disinfectant solution to make a 5 ml organism – disinfectant mixture. At intervals of 10, 20, 30, and 60 minutes, 1 ml of the test mixture was transferred to 9 ml of 3% Tween 80 solution (to neutralize the disinfectant) and allowed to stand for 5 min. Aliquot amount of 1 ml was taken from each test mixture – neutralizer tube and inoculated onto standard plate count agar by pour plate method and incubated at 37°C for 24 h to determine the population of surviving viable cells in cfu/ ml.

2.2.2 Statistical analysis

Statistical analysis of the log reduction values of population of surviving cells was performed using the analysis of variance (ANOVA) 2 factor without replication at the significance level 0.05. Statistical difference was detected as $P < 0.05$.

3. RESULTS

3.1 Occurrence and distribution of Pseudomonads and Staphylococci

The API 20NE results indicated the following degree of homology (% ID), *P. putida* (97.9), *P. mendocina* (97.6), *P. fluorescens* (98.9), *P. stutzeri* (97.7), *P. aeruginosa* (99.8) and *P. shigelliodes* (96.5) while API STAPH indicated the following; *S. lentus* (99.2), *S. aureus* (97.8) and *S. sciuri* (99.9). Table 1 show that *Pseudomonas putida* had the highest percentage occurrence of 25 among the isolated Pseudomonads followed by *P. mendocina* (20), *P. aeruginosa* (15), *P. stutzeri* (15). The lowest % occurrence among the pseudomonads was 10 for *P. shigelliodes*. For the staphylococci, *Staphylococcus lentus* had the highest percentage occurrence of 78.26, followed by *S. sciuri* (13.04) and the least % occurrence was 8.70 for *S. aureus*. All the samples, except litter material, contained *P. putida* and *P. mendocina*. *Pseudomonas stutzeri* was found only found in the feed and litter material while *P. aeruginosa* was present in all the samples except the feed. Litter material is the only sample with *P. shigelliodes*. All the samples contained *S. lentus* but the feed had the highest distribution of the organism at 10. *Staphylococcus sciuri* was found in all the samples except cloacae swab and *S. aureus* was not present in drinking water and cloacae swabs.

Table 1: occurrence of *pseudomonas* and *staphylococcus* species in different poultry and poultry sources

Sample	Species occurrence
<i>Pseudomonas</i> sp.	
Drinking water	<i>P. putida</i> (4), <i>P. mendocina</i> (3), <i>P. aeruginosa</i> (1)
Feed	<i>P. putida</i> (4), <i>P. mendocina</i> (3), <i>P. stutzeri</i> (3)
Litter material	<i>P. aeruginosa</i> (3), <i>P. stutzeri</i> (3), <i>P. shigelliodes</i> (4)
Cloacae swab	<i>P. putida</i> (2), <i>P. aeruginosa</i> (2), <i>P. fluorescens</i> (6), <i>P. mendocina</i> (2)
<i>Staphylococcus</i> sp.	
Drinking water	<i>S. lentus</i> (4), <i>S. sciuri</i> (1)
Feed	<i>S. lentus</i> (10), <i>S. aureus</i> (1), <i>S. sciuri</i> (1)
Litter material	<i>S. lentus</i> (2), <i>S. aureus</i> (1), <i>S. sciuri</i> (1)
Cloacae swab	<i>S. lentus</i> (2)

3.1.1 Effects of disinfectants on the *Pseudomonas* and *Staphylococcus* isolates

The results in Fig 1 – 6 show that Diskol and Morigad recorded 8 log₁₀ reduction (i.e. 100% kill) at the 3 concentrations against all the *Pseudomonas* isolates at 10 min contact time but were only able to achieve same at 1X and 1.5X against the staphylococci. At 0.5X, however, both Diskol and Morigad were only able to achieve 8 log₁₀ reduction (100% kill) of all *Staphylococcus* sp. at 30 min except against *S. sciuri* SN3 where Diskol achieved same record at 10 min. The best performance of Iodasteryl against the pseudomonads was less than 100% kill even at 8log 10 reduction against *P. putida* FaF12, *P. mendocina* FaW48, *P. stutzeri* FaM35, and *P. shigelliodes* FbM36 at 1.5X and 60 min. At this concentration and time, Iodasteryl was least effective against *P. aeruginosa* FbW30 with 7.99log₁₀ reduction.. Against the staphylococci, Iodasteryl's best was 7.99log 10 reduction at 1.5X and 60 min against *S. lentus* SN1, *S. lentus* SN2, *S. sciuri* SN3, *S. sciuri* SN4 and *S.*

The predominance of *P. putida*, *P. mendocina* and *S. lentus* in the different samples from layer birds and poultry environment (Table 1) is in conformity with the reports of [13] who isolated *Staphylococcus* sp. from poultry litter and [14] who isolated *P. fluorescens*, *P. aeruginosa*, *S. aureus* and *Staphylococcus* sp. from poultry faeces. However, *P. mendocina*, *P. stutzeri*, *P. shigelliodes*, *S. lentus* and *S. sciuri* were isolated in this study but not reported by [14]. This difference may be due to the fact that [14] limited their samples to only faeces. In this study, *P. aeruginosa* was not found in feed but [15] reported the organism in feed. Differences in feed quality, feed storage and handling are possible explanation for such difference. The presence of *P. aeruginosa* and *S. aureus* among the isolates recorded in this study underscores the importance of proper disinfection in the poultry industry since these two organisms are potential opportunistic pathogens of man.

Diskol and Morigad showed high degree of efficacy against all the test bacteria. The outcomes of this study accord with [16] who reported that glutaraldehyde and Benzalkonium chloride respectively yielded effective disinfectant activity against *Pseudomonas* sp. in less than 10 min of exposure. Glutaraldehyde was also reported to exert significant antimicrobial activity against planktonic bacteria [17]. The result is also consistent with the report of [18] that Microzal (combination of quaternary ammonium compound and glutaraldehyde) and Incospect (combination of quaternary ammonium compound, glutaraldehyde and formalin) achieved 100% kill against *Pseudomonas aeruginosa* after 5 min. Diskol proved the most effective of the three disinfectant formulations used in this study. The bacterial population reductions by Diskol and Morigad were significantly ($P < 0.05$) greater than that of Iodasteryl but there was no significant difference between the bacterial population reductions by Diskol

aureus SM2. It was least effective at 0.5X and 10 min against *S. aureus* SM2 with 7.72log₁₀ reduction. All disinfectants demonstrated increasing activity with increase in time and concentration.

3.2 DISCUSSION

Disinfectants are widely used in several fields for disease prevention and control [12] and they have become an integral part of modern livestock and poultry farms [7]. The efficacy of disinfectants is affected by disinfectant type, formulation, mode of application, exposure time, concentration, natural microbial population, surfaces, and temperature. This study provides insight into the occurrence and distribution of *Pseudomonas* and *Staphylococcus* species in poultry (Layer chicken) and poultry environments in a farm settlement in Lagos state and the effectiveness of three chemical disinfectants (commonly used by poultry farmers in the area) in controlling the growth of the bacteria under different conditions of concentration and contact times (10, 20, 30, and 60 min).

and Morigad except at the 0.5X. This promising disinfecting ability of Diskol is most probably the result of its unique formulation, comprising a synergistic combination of three compounds; Benzalkonium chloride 5%, glutaraldehyde 7.5% and formaldehyde 7%. The use of two or more active ingredients in commercial disinfectants is expected to increase the antibacterial effect of these products [19] and this explains why products containing quaternary ammonium and glutaraldehyde (and / or another aldehyde) have been the most widely used for disinfection of poultry houses.

Morigad (32% phenol v/v) also proved to be very effective against the test bacteria in this study. The bacterial population reductions by Morigad were significantly greater than those for Iodasteryl. Two phenolic formulations, termed Phenol 1 and Phenol 2 were reported with antibacterial activity against *E. coli* and coliforms isolated from poultry floor dirt [19]. Phenol has the ability to cause leakage of intracellular materials when used at low concentrations and coagulation of both enzymatic and structural proteins (including cell membranes) at higher concentrations (20; 21).

Iodasteryl (active iodine 55mg/100ml) was the least effective of the three disinfectants studied. The best performance of Iodasteryl against the pseudomonads was less than 100% kill even at 8log 10 reduction against *P. putida* FaF12, *P. mendocina* FaW48, *P. stutzeri* FaM35, and *P. shigelliodes* FbM36 at 1.5X and 60 min. Against the staphylococci, Iodasteryl did not achieve 8log 10 reduction. Against all the test bacteria, Iodasteryl could not achieve 100% kill. The performance of Iodasteryl in this study was consistent with the report of [22] that iodine was unable to achieve 100% efficiency against *Pseudomonas* sp.

[19] reported poor disinfecting ability of iodine (1:320) against total and fecal coliforms. Also [18] reported lower activity of povidone iodine against bacteria.

However, Iodasteryl activity in this present study was better than that reported by [19]. Apart from the different dilutions and test bacteria, experimental conditions may also be responsible for this difference.

Diskol, Morigad and Iodasteryl demonstrated significantly increasing inhibitory activity with increase in time and concentration. This is further supported by earlier reports [8, 23, 24] showing that the antimicrobial activities of disinfectants are concentration dependent.

The *Pseudomonas* isolates demonstrated more susceptibility to all the test disinfectants than the *Staphylococcus* species. This is at variance with many reports that Gram negative bacteria are generally less susceptible to disinfectants than Gram positive bacteria. However, [17] has reported that *P. fluorescens* biofilms were more susceptible to glutaraldehyde than *Bacillus cereus* biofilms. This

present result may be explained by the fact that, different microbial strains are influenced by different external factors, such as transfer of resistance genes, physiological injuries due to prolonged use, previous exposure to underdosage of disinfectants which may lead to the development of varying levels of resistance. Care and caution is necessary in the handling of disinfectants. Benzalkonium chloride, which is generally non – irritating, was reported to cause irritation of the middle ear tissue [25]. Phenol and its vapours are corrosive to the eyes, skin, and the respiratory tract but, it is not known to be carcinogenic [26]. Formaldehyde is not acutely toxic but, could be irritating to the eyes and mucous membrane on long term exposure. Glutaraldehyde is toxic and a strong irritant but, there is no evidence of carcinogenic activity [27]. The use of gloves, nose masks, goggles, proper handling and avoidance of undue exposure are measures to ensure safety in handling of disinfectants.

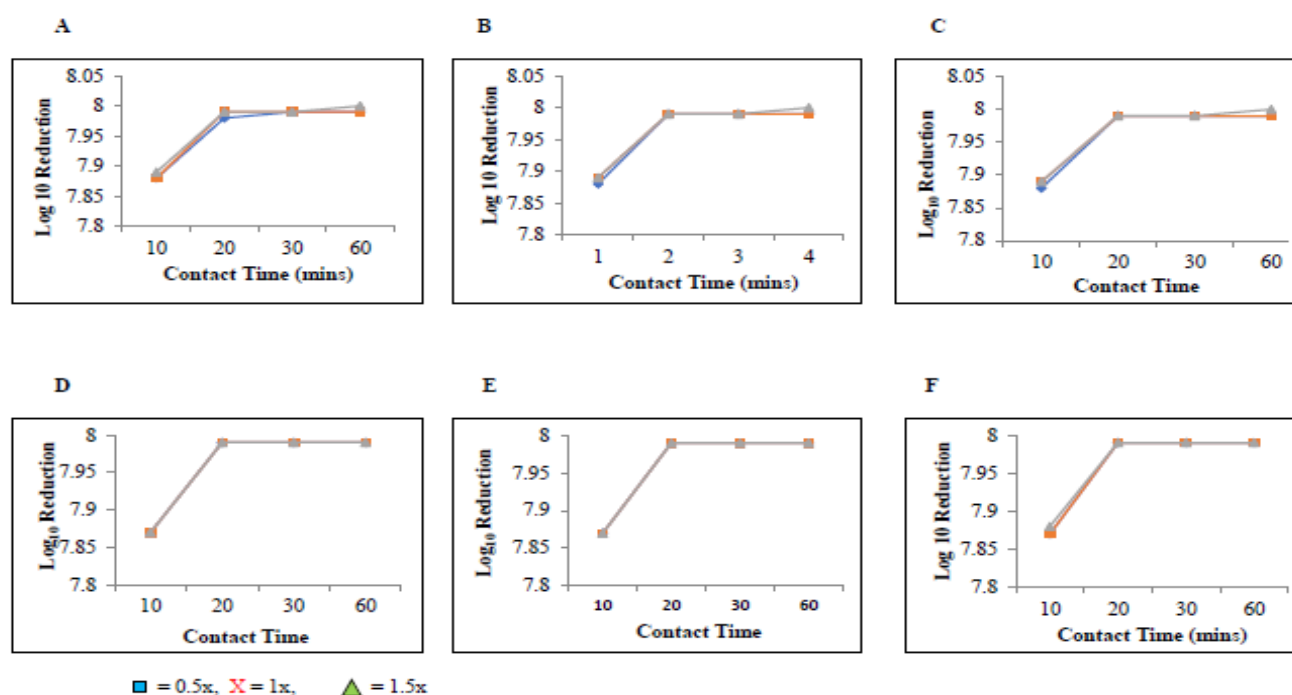


Fig .1: Effect of different concentrations of Iodasteryl on (A) *Pseudomonas putida* FaF12, (B) *P. mendocina* FaW48, (C) *P. stutzeri* FaM35, (D) *P. aeruginosa* FbW30, (E) *P. shigellioides* FbM36, (F) *P. fluorescens* FbC32

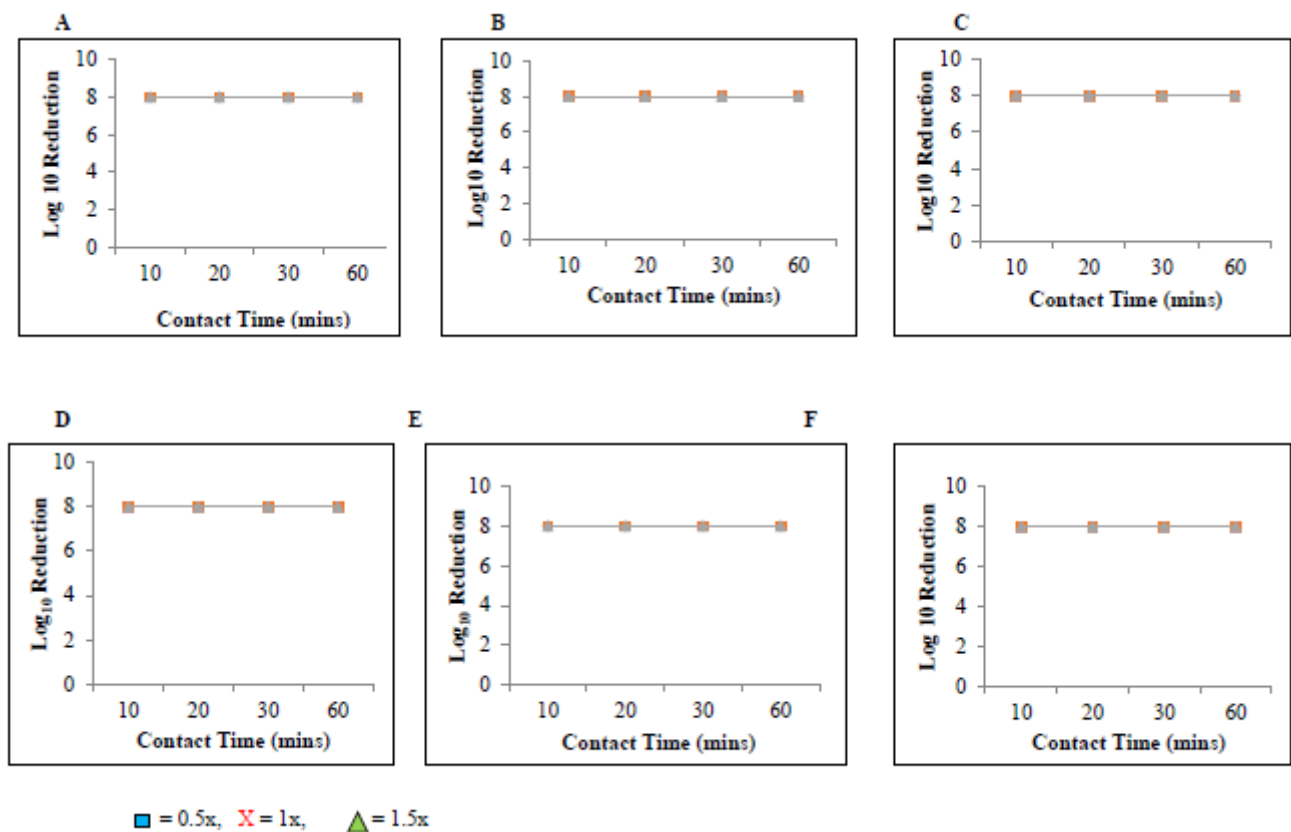


Fig.2: Effect of different concentrations of Diskol on (A) *Pseudomonas putida* FaF12, (B) *P. mendocina* FaW48, (C) *P. stutzeri* FaM35, (D) *P. aeruginosa* FbW30, (E) *P. shigelliodes* FbM36, (F) *P. fluorescens* FbC32

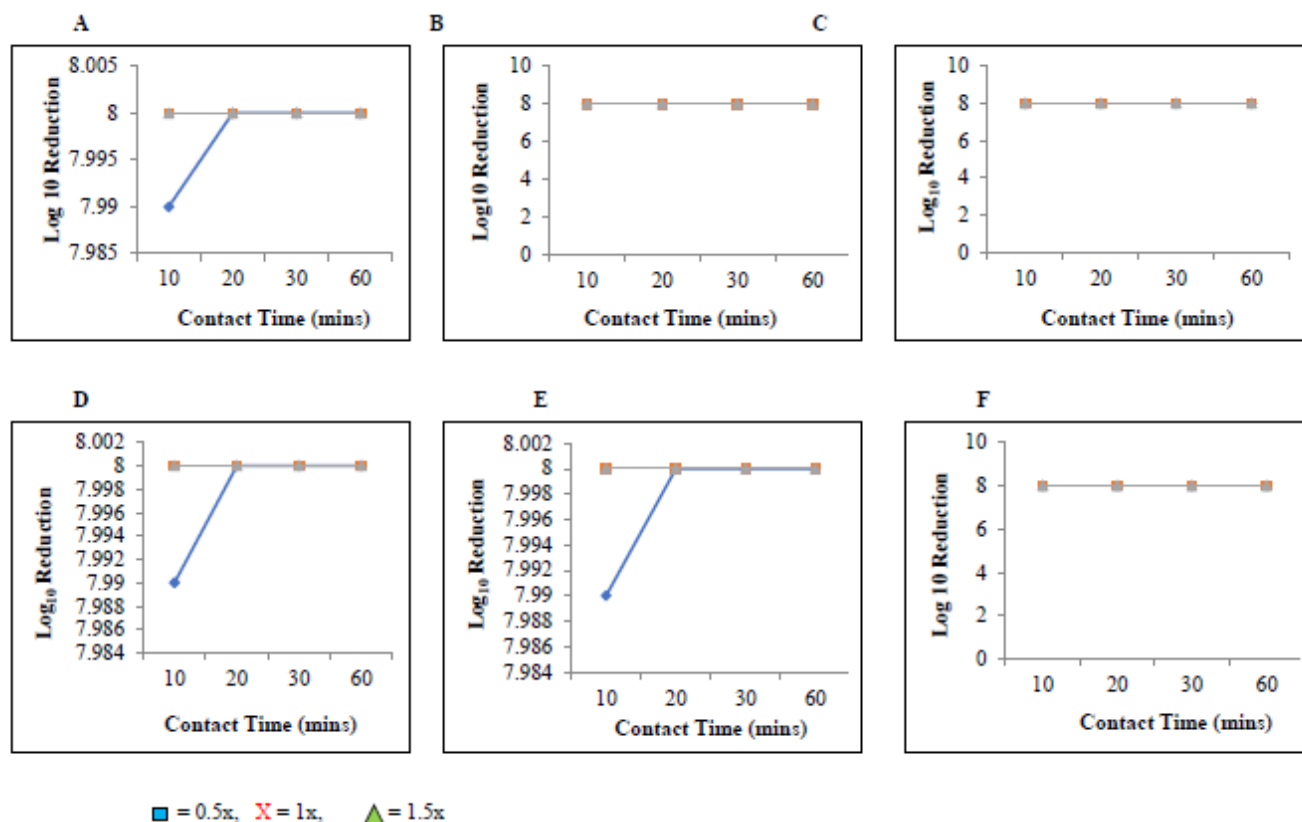


Fig.3: Effect of different concentrations of Morigad on (A) *Pseudomonas putida* FaF12, (B) *P. mendocina* FaW48, (C) *P. stutzeri* FaM35, (D) *P. aeruginosa* FbW30, (E) *P. shigelliodes* FbM36, (F) *P. fluorescens* FbC32

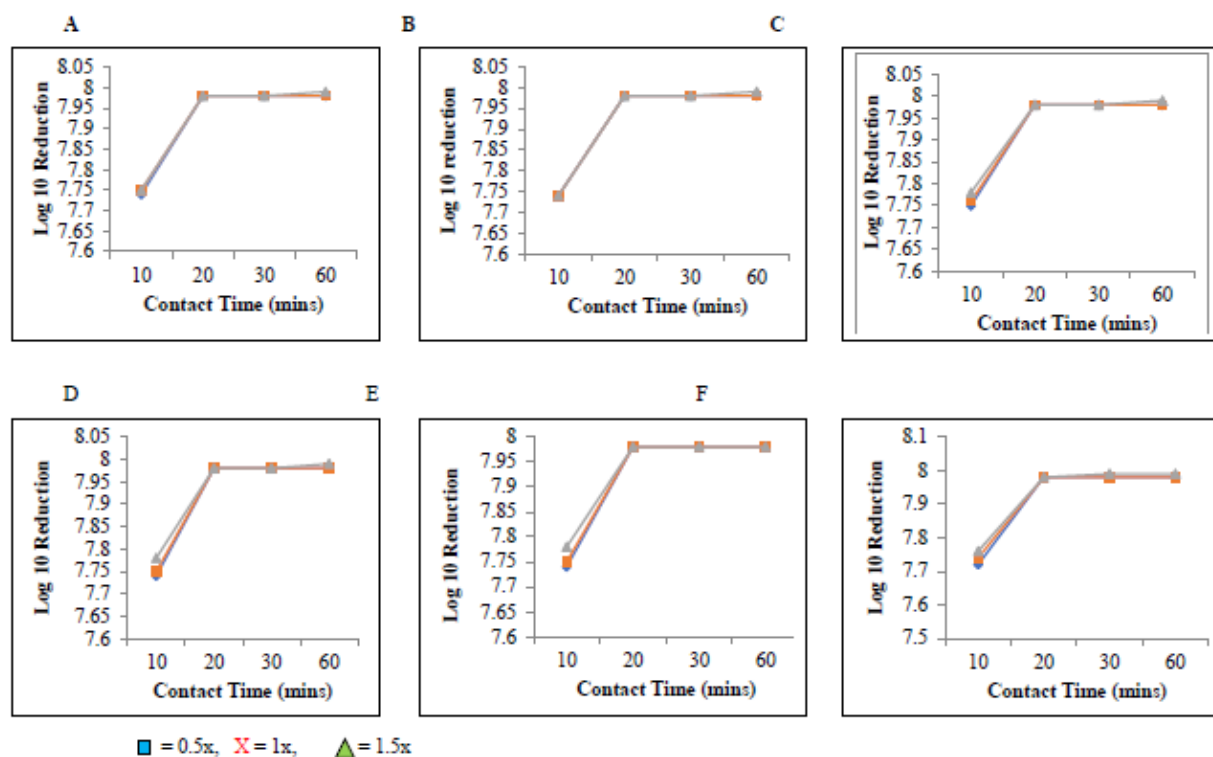


Fig.4: Effect of different concentrations of Iodasteryl on (A) *Staphylococcus lentus* SN1, (B) *S. lentus* SN2, (C) *S. sciuri* SN3, (D) *S. sciuri* SN4, (E) *S. aureus* SM1, (F) *S. aureus* SM2

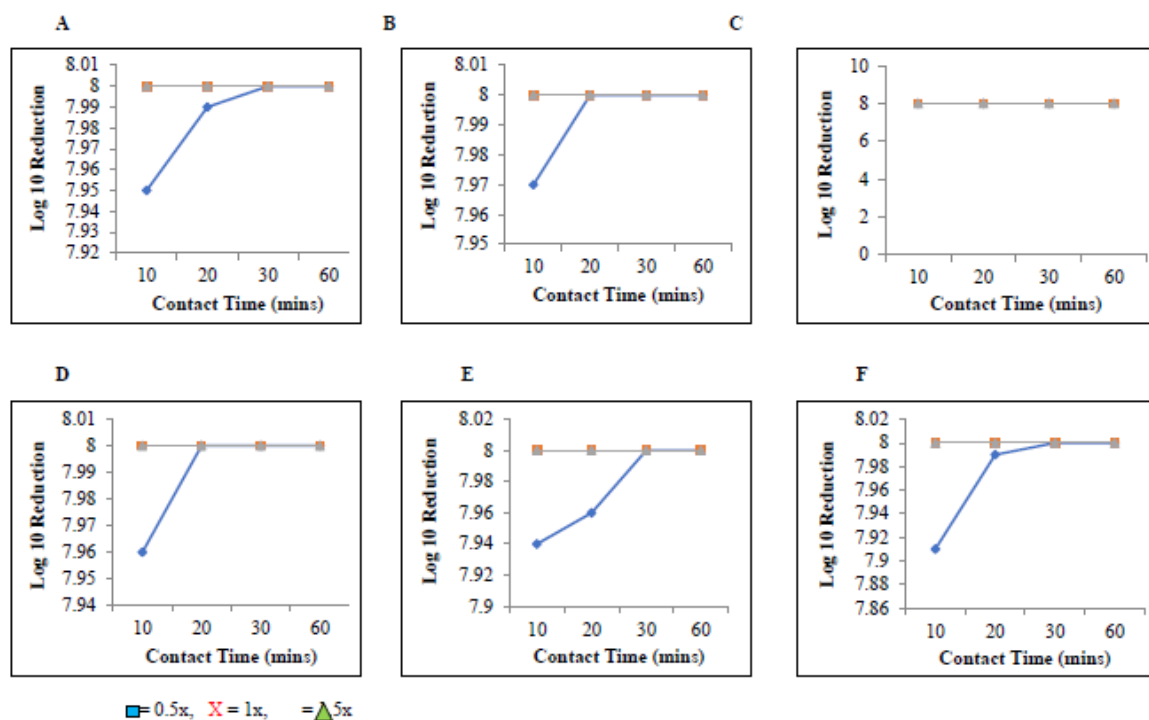


Fig.5: Effect of different concentrations of Diskol on (A) *Staphylococcus lentus* SN1, (B) *S. lentus* SN2, (C) *S. sciuri* SN3, (D) *S. sciuri* SN4, (E) *S. aureus* SM1, (F) *S. aureus* SM2

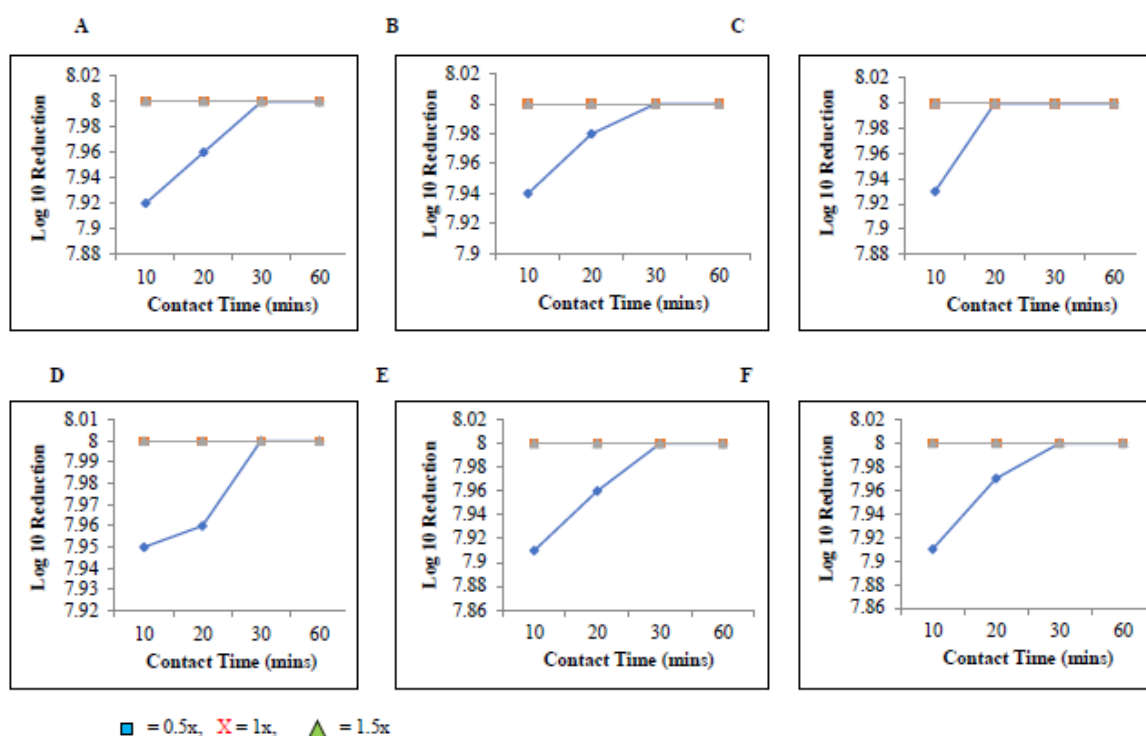


Fig.6: Effect of different concentrations of Morigad on (A) *Staphylococcus lentus* SN1, (B) *S. lentus* SN2, (C) *S. sciuri* SN3, (D) *S. sciuri* SN4, (E) *S. aureus* SM1, (F) *S. aureus* SM2

4. CONCLUSION

Diskol and Morigad had comparatively better performance against the test bacteria than Iodasteryl but Diskol was the most effective. The results indicated the high inhibitory capacity of Diskol and Morigad and therefore justify their use by poultry farmers for disinfecting poultry houses.

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AUTHORS' CONTRIBUTIONS

Grillo, Adebayo designed the study, performed the study protocol with the statistical analysis, arranged the literature searches and wrote the first draft of the manuscript. Olasupo, Nurudeen managed the analysis of the study and proofread the manuscript.

Both authors read and approved the final manuscript.

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