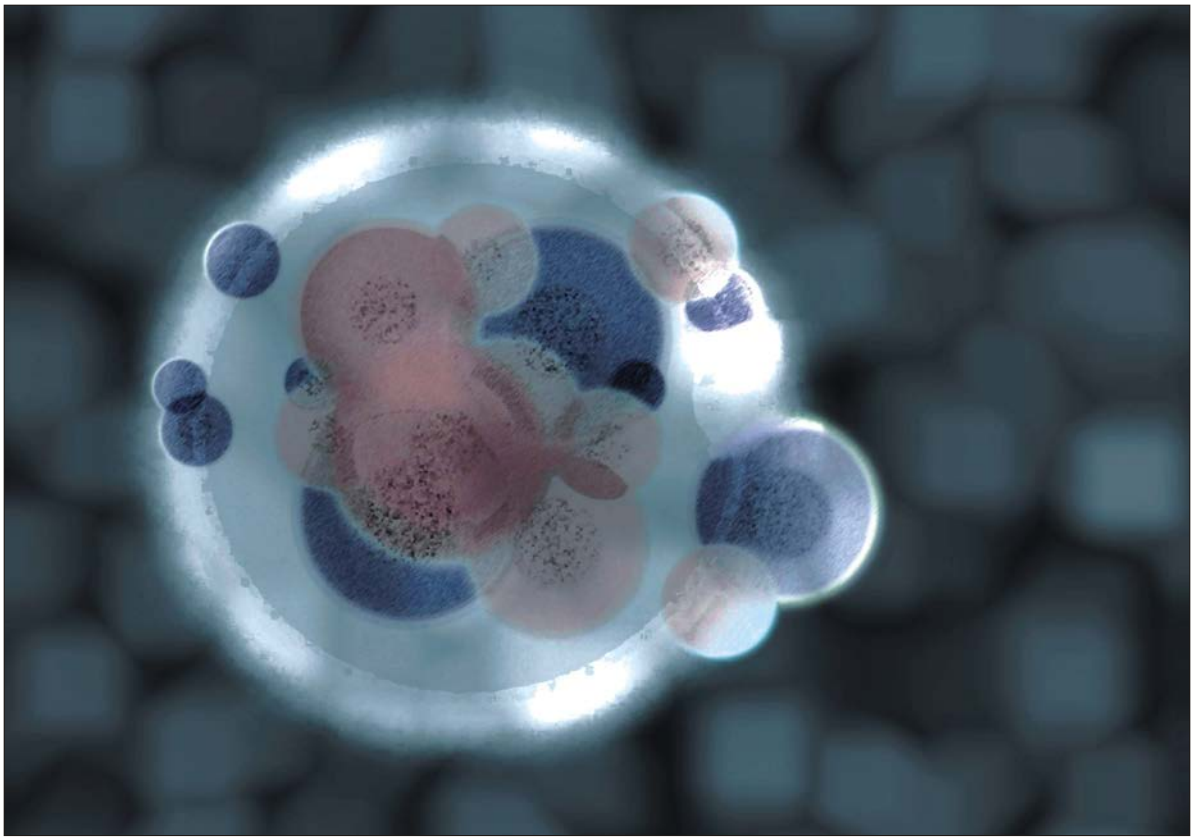

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Evaluation of the Efficiency of Disinfectants used Against Bacterial Isolates from Intensive Poultry Farming Environments in Imo State, Nigeria

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Abstract: Diseases and infections have always been a major concern to intensive poultry production industry. Pathogen contamination of farming environments can be prevented using proper health care products such as disinfectants. This study evaluated the efficiency of commonly used disinfectants against bacteria occurring in intensive poultry farming environments in Imo State, Nigeria. The efficacy of six commercial disinfectants namely; Izal®, Z-germicide®, Diskol®, Virkon®, Vox® and CID 20® in reducing the number of micro-organisms was assessed. Among the tested disinfectants, Virkon® (oxidizing agents) was the most efficient, reducing the micro-organisms by 95%. The *in vitro* test carried out to verify the effectiveness of disinfectants did not consider the adverse conditions found in the poultry farms. Therefore, the evaluation of the efficacy of on-farm reconstituted disinfectants over time was also carried out. The results indicated that efficacy of all the disinfectants was reduced during the afternoon. However, efficacy gradually increased during the evening for all the disinfectants but not as much as was observed in the morning. Temperature, it seems affects the activity of the disinfectants against the bacterial organisms.

Key words: Disinfectants, poultry farms, bacterial isolates, susceptibility tests

INTRODUCTION

Intensification of poultry operations has always resulted in increased disease incidence in poultry due chiefly to accumulation of pathogens in the farming environment. These diseases and infections result from the presence of bacteria, fungi, parasitic and viral pathogens in the farming environment (Damerow, 1994). A high population of disease producing organisms in the poultry producing environment usually lead to poor flock wellbeing and decline in production (Payne *et al*., 2005). Such microbiological contamination and accumulation could be prevented and controlled using proper management, hygiene, biosecurity, strategic and point treatment practices. Healthcare products such as disinfectants remain indispensable tools for the destruction and break in the cycle of disease causing organisms in the poultry farming environment (MSU, 2008; Chima *et al*., 2011).

Disinfectants, which are chemicals used as germicides are targeted at preventing pathogenic microorganisms from contaminating livestock and their products. Regrettably, the application of these disinfectants sometimes does not seem to achieve the purpose for which they are used (Onah, 2004). However, according to Block (2001) and Chima *et al*. (2011) proper disinfectantion practice targets the reduction of the number of pathogens in the farming environment thereby reducing the frequency and intensity of disease occurrence in poultry. The mode of action of the disinfectant chemicals is usually to disrupt significant cellular structures or processes in order to destroy and eliminate the micro organisms (Allen *et al*., 2006).

Commercially available disinfectants that usually exhibit excellent results against microbes during standard laboratory tests may not show the expected efficacy against disease causing pathogens, especially under tropical small scale farming conditions (Corlett, 1998). There are many reasons for this which may include the fact that the carefully controlled conditions of the standard test methods are not usually the same in the farm environment and also the variations in the disinfection practices at different the poultry farms, especially at the small scale level in many developing countries.

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Okoli *et al.* (2004) had reported that many poultry farmers and farm attendants are not very literate. Therefore such operators may fail to reconstitute disinfectants according to the manufacture's instructions. Chima *et al.* (2011) has also reported that cost is a major factor for selection of disinfectants among small scale poultry farmers and animal health practitioners in Imo state and that financial consideration may cause them to chose a disinfectant and not on effective prescriptions that may attract higher costs. These mundane decisions by the small scale poultry farmers may exacerbate the already high disease occurrence in intensive poultry production in Nigeria (Okoli, 2004). While biosecurity measures, which include cleaning and disinfection in the poultry industry remain critical to the production process, the efficacy of the disinfectants used at small scale of poultry operation has been scantly reported (Herrera, 2004; Chima *et al.*, 2012). There is therefore an urgent need to evaluate the efficacy of disinfectants as well as the disinfection practices at the small scale poultry farming level. This is because earlier studies have shown serious cases of drugs and medication abuses in the industry (Okoli *et al.*, 2002). Similarly, a recent survey by Chima *et al.* (2011), highlighted the predominant use of domestic phenolic products by small scale poultry farmers in Imo State, Nigeria. The study also reported common poor disinfection practices in the area especially general lack of functional disinfectant footbath and where available, lack of adequate information on its use by workers or visitors as well as inappropriate reconstitution of disinfectants according to the manufacturers instructions.

In evaluating disinfection at the small scale farming level, it is important to consider the type of micro organisms present as well as the physical characteristics of the water in use at the farms. These factors may vary from farm to farm and may drive efficacious disinfection (Jeffrey, 2005). Furthermore, as recently stated by Chima *et al.* (2012), available disinfectants could be evaluated using locally isolated microorganisms instead of reference cultures, while water used daily in the farm should form the diluents for the disinfectant being tested

This study was designed to evaluate the efficiency of disinfectants commonly used by small scale poultry farms against bacteria isolated from the local farming environments in Imo State, Nigeria.

MATERIALS AND METHODS

The study area: The study was carried out in the three geo-political zones of Imo State, which is situated in South-eastern agro-ecological zone of Nigeria. The state is divided into 27 Local Government Area (LGA) for administrative purposes. Both the geo-political and agro-ecological characteristics of the state have been described by Okoli (2004).

The highest population of chicken in the area is made of local breeds reared by rural farming families under the extensive scavenging system (Okeudo, 2004), with the number of birds ranging between 50 and 100 birds and is usually family affairs. In most of the backyard poultry, hygienic and biosecurity measures are usually poor with all the family members being involved in the daily management activities. Usually there is no organized effort at vermin and traffic control.

Poultry production in the study area could be divided into extensive, semi-intensive and intensive systems. Commercial intensive poultry production includes table eggs, broiler, parent stock, turkey, and started chicken and hatchery productions. These farming operations are distributed over urban, peri-urban and rural sites and have been described by Okoli (2004) to range from very small operations (50-100), to medium (101 to 1000 birds) and large scale (above 1000 birds).

Study outline and disinfectant selection: This involved a laboratory assessment of the efficacy of selected common disinfects using bacteria isolated from small scale farms in the study area and a further evaluation of the efficacy of on-farm re-constituents of these disinfectants across different periods of the day. Two farms each were randomly selected for the study from the three agricultural zones namely; Owerri, Orlu and Okigwe making a total of six farms to be studied. The six commonly used disinfectants were those identified in recent survey in the study area by Chima (2011). These included Diskol®, Izal®, CID 20®, Vox®, Z-Germicide® and Virkon®. Their characteristics as presented in the manufacturer's literatures have also been documented (Chima, 2011).

Test bacterial isolation: In each study farm, litter material was aseptically collected into sterile plastic bottles. The bottle was dipped into the litter at three different spots (near the entrance, feed trough and water trough) in the poultry house. Since there were more than one building in most of the farms, sample was collected from the buildings housing the oldest birds in the farm, in order to obtain a fair reflection of the endogenous bacteria population in the farms (Okoli, 2004). The samples were pooled and transported to the laboratory for analysis within 4 h of their collection.

About one gram of litter sample was added into 10 mL of sterile iodized water, thoroughly shaken and allowed to stand for 30 min. The resulting suspension was serially

diluted 10-folds in test tubes containing sterile iodized water as described by Okoli (2004). To 0.1 mL inoculum was pipetted out from the last test tube and used to inoculate the various media plates.

Nutrient agar (Himedia, India) and other media (MacConkey Agar Oxoid, England; Salmonella-shigella Agar, Antec Diagnostic Product, UK; Eosin Methylene Blue Agar, Bionark Laboratory, India; Thiosulphate Citrate Bile Salt Agar, Scharlau Chemie, S.A, Spain; Glutamate Starch Phenol-red-Agar, Oxoid, England and Mueller Hinton Agar, Lab. 39 Limited, UK) used were prepared according to manufacturer's specifications and used to isolate specific organisms.

The broth culture from each of the test tubes prepared were first streaked onto Nutrient agar plates and incubated at 37°C for 24 h to isolate viable bacterial cells. Discrete colonies of organisms on the nutrient agar plates were purified by sub-culturing pure isolates. Thereafter, appropriate media were used to characterize organisms on a given agar media using selective growth and colony characteristics (Cheesbrough, 2000).

Furthermore, biochemical tests such as Gram's reaction, motility test, citrate utilization test, catalase reaction, indole production, Oxidase reduction potential, nitrate reduction, urease test as well as pigmentation were performed. Carbohydrate utilization and fermentation tests were also carried out for glucose, sucrose, lactose, maltose, xylose, arabinose, mannitol, inositol and sorbitol. The results of these tests were carefully complied and identification of the isolates was accomplished by comparing the characteristics of the cultures with the cultural characteristics of known taxa using the schemes of Buchanan *et al.* (1974) and Cheesbrough (2000). Thus, the test organisms isolated were, *Salmonella* spp., *Shigella* spp., *E. coli*, *Klebsiella* spp., *Pseudomonas* spp., *Aeromonas* spp. and *Vibrio* spp. The organisms were stored in Nutrient agar slants at 4°C until needed.

Disinfectants sensitivity tests: The isolated organisms were screened for their susceptibilities to the test disinfectants using the disc diffusion method (Bauer *et al.*, 1966). Disinfectants impregnated discs used were locally produced at the ISEPA Laboratory, Owerri, Imo State.

Agar plate cultures of the slanted bacteria were prepared using nutrient broth (Oxoid, England). Thirteen gram of the powdered agar was weighed out and dissolved thoroughly in one litre of distilled water. Five milliliter of the solution was thereafter distributed into each of seven test tubes and sterilized at 120°C for 15 min at 15 psi. The sterile solutions were allowed to cool to room temperature. The characterized bacteria were inoculated onto them, with sterile wire loop and incubated for 6 h. The essence of this was to enhance growth of the stored bacteria. Thereafter, discs impregnated with known concentration of the six different test disinfectants were placed on the surface of the lawn of organisms with sterile forceps. The plates were then allowed to stand for a pre diffusion period of about 1 h before being incubated at 37°C over night.

Susceptibility of the bacteria to the disinfectants was measured by the zone of inhibition or clearance. Susceptibility data were recorded quantitatively by measuring the size of the diameter with a meter rule. For the purpose of this study the zone of inhibition were interpreted as:

- No inhibition at all was regarded as resistant and was represented with 0
- Minimal inhibition was regarded as less effective and was represented with+ and assigned 1
- Intermediate inhibition was regarded as effective and represented with ++ and assigned 2
- Wide zone of inhibition was regarded as very effective and represented with +++ and assigned 3

On-farm disinfectants tests: This phase of the study involved the determination of on-farm reconstituted disinfectants efficacy across selected periods of the day. Six farms with a known history of disinfectant usage were randomly selected, two farms from each study zone of the state as earlier stated. Furthermore the six brands of commonly used disinfectants earlier tested were bought and each assigned to one of the six farms selected for this study (Table 1).

The fresh morning reconstituted samples served as control in each case. In each farm, the test disinfectant was reconstituted according to the manufacturer's instruction and placed in the footbath at the entrance of each study poultry house, which were stocked with birds at the time of the experiment. According to Morley (2005), footbath is a small bathtub for disinfecting the foot and is a common method used in the of control infectious diseases in poultry farms.

Collection of sample: Sample of the reconstituted disinfectants in the footbath was collected using sterile bottle to scoop the disinfectant at each time of sampling. Collection was done at 6.00 a.m. (immediately after reconstitution), 12 noon and 6.00 p.m. on the same day of sampling. After collection the sample were transported to the laboratory for analysis. The samples collected at 6.00 p.m. was stored in the refrigerator at 4°C pending the analyses the next day.

Table 1: Out lay of experimental design

Location of farm	Disinfectant	Time of collection		
		a.m.	noon	p.m.
Owerri Zone		6	12	6
Patok farm Naze Owerri North	Virkon®	6	12	6
Imo Livestock Dev. Project Vet. Compound	Diskol®	6	12	6
Orlu Zone				
Simuuna Farm Ltd Orlu LGA	Izal®	6	12	6
More Kosult Farm Awo Idemili	CID 20®	6	12	6
Okigwe Zone				
Dr. E. C Ndubuisi Farm Umulolo Okigwe	Vox®	6	12	6
Agrey Farm Ubahu Okigwe	Z-Germicide®	6	12	6

The fresh morning reconstituted sample served as control in each case. In each farm, the test disinfectant was reconstituted according to the manufacturer's instruction and placed in the footbath at the entrance of each study poultry house, which are stocked with birds at the time of the experiment. According to Morley (2005) footbath is a small bathtub for disinfecting the foot and is a common method used in the of control infectious diseases in poultry farms.

Sensitivity test: From the cultured broth, a sterile swab stick was used to make lawns of the test organisms on already prepared Mueller Hinton agar medium. Discs impregnated with known concentration of the six different brand of disinfectant samples collected 6.00 am, 12 noon and 6.00 p.m. from the farms according to the methods previously described were carefully placed on the agar media using sterile forceps. The plates were then allowed to stand for per-diffusion period of about 1 h before being incubated at 37°C over night.

Again, susceptibility or efficacy of the on farm disinfectants was measured by the zone of inhibition. The data were recorded quantitatively by measuring the size of the diameters of the inhibition zones and interpreted as previously outlined.

Data analyses: Completely Randomized Block Design (CRBD) was employed to evaluate the efficacy of on-farm reconstituted disinfectants over a period of time. Data collected for all parameters were subjected to Analysis of Variance (ANOVA) as outlined by Obi (1990). Where significant differences were established among means, they were separated using SAS statistical software (SAS Institute, 1999).

RESULTS AND DISCUSSION

Efficacy of disinfectants on isolated bacteria: The efficacy of the different disinfectants on the various organisms is presented in Table 2. The mean efficacy evaluation results for the six test disinfectants showed the least effective disinfectants were Izal® (51.1%), Z-germicide® (66.7%) and Diskol® (66.8%). The relatively lower values reported for these disinfectant products, were driven by their general poor efficacy against *E. coli*, *Klebsiella* specie and *Pseudomonas* species (Izal®), *E. coli* (Diskol®), *Klebsiella* species and *Aeromonas* specie (Z-germicide®).

It is interesting that Izal® and Z-germicide®, which are phenolic products, are the most readily available in the animal health outfits and also constitute the most frequently used disinfectants in the farms (Chima *et al*., 2011). The implication of these results is that both the farmers and the animal health practitioners are not guided by sound scientific information in choosing or prescribing the disinfectants for use in the farms. Such professional misused based on poor information has been reported in the use of antibiotics by skilled veterinarians in Nigeria (Okoli *et al*., 2002).

Specifically Virkon®, Vox®, CID 20® and Izal® were significantly more effective against slamonella species ($p<0.05$). Z-germicide® and Diskol® differed from the other few disinfectants in efficacy; and as such were not as effective as the other four in eliminating salmonella species. This result is in line with the report by MSU (2008), which stated that when selecting disinfectants, consideration should be on the effectiveness against the organism that are of greatest concern. This is because not all disinfectants are effective on all types of species of organism. Also Izal® and Z-germicide® which are both phenolic products differ significantly in efficacy in eliminating salmonella species. This finding agrees with the reports by Pilotto *et al*. (2007), who observed some differences between compounds with the same active ingredients when assessing the effects of phenols on salmonella and concluded that these differences resulted from the dilution recommended by the manufacturers and from the different concentrations of the active ingredients.

Efficacy of the disinfectants against shegella species in the laboratory study as shown in Table 2 indicates that Virkon®, Izal®, Vox®, CID 20®, Diskol® showed superiority in action against Z-germicide®. This is in line with the report by Gamage (2003) that phenol products are mainly used in households and domestic disinfection.

In assessing the efficacy of the six disinfectants against *E. coli,* Virkon® was shown to be more effective

Table 2: The efficacy of the different disinfectants on the various organisms

	Test organisms								
Disinfectant	*Salmonella* spp.	*Shigella* spp.	*E. coli*	*Klebsiella* spp.	*Aeromonas* spp.	*Vibrio* spp.	*Pseadomonas* spp.	Mean	SEM
Izal®	67^b	100^a	33^c	33^c	67^b	67^b	33^c	51.1	9.61
Virkon®	100^a	100^a	100^a	100^a	67^b	100^a	100^a	95.2	4.71
Diskol®	67^a	100^a	0^b	67^a	67^a	67^a	100^a	66.8	12.60
Vox®	100^a	100^a	33^c	67^b	67^b	67^b	100^a	76.2	9.54
Z-germicide®	100^a	67^b	67^b	33^c	33^c	67^b	100^a	66.7	10.34
CID 20®	100^a	100^a	67^b	67^b	100^a	67^b	100^a	85.8	6.67

Means on the row with different superscripts are significantly different

than the others while Diskol® did not have any bactericidal efficacy against the organism. Virkon® being significantly more effective than the other disinfectants is in line with the study done by El-Naggar *et al.* (2001), which reported that Virkon® is one of the most potent disinfectants used against *E. coli* infection in poultry farms. The very high resistance of *E. coli* against most common disinfectants in the study areas is of public health and economic significances.

The result of the efficacy of the disinfectants against Klebsiella species showed that again Virkon® is the most effective and significantly differed from the other disinfectants ($p<0.05$). This result is in line with the reports of Gasparini *et al.* (1995) in which the activities of Virkon® (dipotassium peroxodesulphate) was compared with phenolic and gluteraldehyde products and was found to be more effective.

CID 20® was found to be significantly more effective in eliminating aeromonas species than the other disinfectants ($p<0.05$). Efficacy against aeromonas species could be because of CID 20® having a mixture of three active ingredients from three chemical groups (quaterinary ammonium compound, gluteraldehyde and alcohol). This result agrees with the reports of Pilotto *et al.* (2007) that the use of two or more effective ingredient in commercial disinfectants preparations is expected to increase the antibacterial effects of the product.

The result of the efficacy of the disinfectants against Vibro species indicated that Virkon® was again significantly more effective than the other disinfectants. This is in line with the reports of DuPont (2010), which quoted Virkon® as having a unique formulation that can not be compared with any other disinfectant. In comparing the efficacy of the different disinfectant against pseudomonas species, Virkon®, Vox®, Diskol®, CID 20® and Z-germicide® were found to be more effective than Izal®. This could be because of Izal® not being recommended by the manufacturers for use in poultry farm but mostly for use in homes.

Therefore, the efficacy of the disinfectants from the laboratory study indicates that Virkon® (oxidizing agent) showed significant superiority in action that was obvious. This was followed by CID 20® (QAC, aldehyde and alcohol), Vox® (Halogen) Izal® (phenol), Z-germicide® (Phenol) and Diskol® (aldehyde). This study indicates that disinfectants do not always guarantee elimination of problem causing bacteria as shown from the inability of Diskol® to eliminate *E. coli* in the laboratory trails. When selecting disinfectants, care should be taken to consider the effectiveness on the organisms that are of great concern, as not all disinfectants are effective on all types of bacteria organisms.

Izal®, which is seen from the survey study as the most widely stocked and used is not, indicated for use in poultry farms by the manufacturers. Inspite of its wide patronage and use, the efficacy is significantly lower than the other disinfectants. The same is also seen of Z-germicide®. This disparity in efficacy of the disinfectants could be attributed to the active ingredient used in producing the products. Varying effects of synergisms of the active ingredient with the inert ingredient might be responsible for the various levels of disinfection and different disinfectants, but no definitive conclusion can be made and these effects were not tested.

Efficacy of on-farm reconstituted disinfectants over time: Table 3 to 8 highlighted the results of the evaluation of on-farm reconstituted disinfectants over three period of the day (6 am, 12 noon and 6 pm). All the disinfectants were generally more efficacious during the morning (6 am) than during the afternoon or evenings. The general trend was for disinfectant efficacy to decrease appreciably during the afternoon and to increase again during the evenings but usually not as high as the morning values.

It is possible that as the temperature of the reconstituted solution increased with day temperature the efficacy of the disinfectants decreased. Most of the footbaths are actually built outside under the direct heat of the sun. There is the need to investigate the true cause of these changes.

Table 3 showed that CID 20® was completely ineffective against salmonella and *E. coli* during the afternoon and its efficacy against most organisms remained low in the evening after it had decreased during the afternoon heat. Table 4 showed that Diskol® was not efficacious against *E. coli* across the three periods

Table 3: Efficacy of CID 20® against isolated bacteria over time

CID 20®	*Salmonella* spp.	*Shigella* spp.	*E. coli* spp.	*Klebsiella* spp.	*Aeromonas* spp.	*Vibrio* spp.	*Pseudomonas* spp.	Mean
6 a.m.	100^{a}	100^{a}	67^{a}	67	100^{a}	67	100	85
12 noon	0^{c}	33^{b}	0^{b}	33	33^{b}	33	67	28
6 p.m.	33^{b}	33^{b}	67^{a}	67	33^{b}	33	67	47
SEM	19.43	18.26	16.41	14.98	18.26	12.51	20.03	

Means on the row with different superscripts are significantly different

Table 4: Efficacy of Diskol® against isolated bacteria

Diskol®	*Salmonella* spp.	*Shigella* spp.	*E. coli* spp.	*Klebsiella* spp.	*Aeromonas* spp.	*Vibrio* spp.	*Pseudomonas* spp.	Mean
6 a.m.	67	100^{a}	0	67	33	67	67	57.29
12 noon	33	33^{b}	0	67	33	33	67	38.00
6 p.m.	33	67^{a}	0	33	33	33	67	38.00
SEM	12.51	19.47	0.00	14.98	8.08	12.51	16.41	

Means on the row with different superscripts are significantly different

Table 5: Efficacy of Z-germicide against isolated bacteria over time

Z-germicide®	*Salmonella* spp.	*Shigella* spp.	*E. coli* spp.	*Klebsiella* spp.	*Aeromonas* spp.	*Vibrio* spp.	*Pseudo-monas* spp.	Mean
6 a.m.	100^{a}	67	67	33	33	67	100^{a}	66.71
12 noon	33^{c}	33	33	33	33	33	67^{b}	37
6 p.m.	67^{b}	33	33	33	33	67	67^{b}	47.57
SEM	19.47	12.51	12.51	8.08	8.08	14.98	20.03	

Means on the row with different superscripts are significantly different

Table 6: Efficacy of Izal against isolated bacteria over time

Izal®	*Salmonella* spp.	*Shigella* spp.	*E. coli* spp.	*Klebsiella* spp.	*Aeromonas* spp.	*Vibrio* spp.	*Pseudo-monas* spp.	Mean
6 a.m.	67	100^{a}	33^{a}	33	67	67	33	57.14
12 noon	33	0^{b}	0^{b}	33	33	67	33	28.43
6 p.m.	67	67^{a}	67^{a}	33	33	33	67	57.43
SEM	14.98	21.11	13.38	8.08	12.51	14.98	12.51	

Means on the row with different superscripts are significantly different

Table 7: Efficacy of Virkon against isolated bacteria over time

Virkon®	*Salmonella* spp.	*Shigella* spp.	*E. coli* spp.	*Klebsiella* spp.	*Aeromonas* spp.	*Vibrio* spp.	*Pseudomonas* spp.	Mean
6 a.m.	100^{a}	100^{a}	100^{a}	100^{a}	67	100^{a}	100^{a}	95.29
12 noon	33^{bc}	33^{b}	0^{b}	33^{b}	33	33^{b}	33^{b}	28.29
6 p.m.	67^{ab}	33^{b}	67^{a}	33^{b}	33	33^{b}	67^{a}	45.57
SEM	19.47	18.26	21.11	18.26	12.51	18.26	19.47	

Means on the row with different superscripts are significantly different

studied. The product was also lowly effective against *Aeromonas* spp., across the periods. Against *Salmonella* spp., *Vibro* spp. and *Aeromonas* spp., efficacy remained low up till evening after afternoon decreases.

Tables 5 and 6 showed that the efficacy trends for Z-germicide® and Izal® were similar and reduced greatly owing the afternoons. However, shegella and *E. coli* were completely resistant to the action of Izal® during the afternoons.

Although, Virkon® has been shown to be highly effective against *E. coli* under laboratory tests (Table 2), under field exposure; it lost its efficacy against the organism during the afternoon. This is probably because of afternoon heating of the reconstituted solution since it returned to 67.0% during cool evenings (Table 7). It was however completely effective on all the organisms in the mornings, with a general reaction on all the organisms during the afternoon. In the evening, the efficacy was slightly increased from what was observed in the morning.

The efficacy trend of Vox® was particularly similar to that of Virkon® especially with respect to its morning effects against the test microorganisms. It consistently returned very poor efficacy results (0-33.33%) against *E. coli* across the three period studied. It would seem that the organisms have developed appreciable resistance against the products. Evaluating the efficacy of on-farm reconstituted disinfectant over time indicates that disinfectant type and temperature at the time of application can impart on bacteria inhibition. However, it is important to remember that for the field trials; all surface debris and loose organic materials were removed from the footbath prior to disinfection.

The efficacy of all the disinfectants was reduced during the afternoon. The efficacy gradually increased during the evening for all the disinfectants but not as much as was observed in the morning. Temperature it seems affects the activity of the disinfectants against the bacterial organism as seen in this study. This result is in line with report by Gamage (2003) that increase in

Table 8: Efficacy of Vox® against isolated bacteria over time

Vox®	*Salmonella* spp.	*Shigella* spp.	*E. coli* spp.	*Klebsiella* spp.	*Aeromonas* spp.	*Vibrio* spp.	*Pseudomonas* spp.	Mean
6 a.m.	100^a	100^a	33^a	67	67	67	100^a	76.29
12 noon	33^b	33^b	0^b	33	33	33	33^b	28.29
6 p.m.	67^a	67^a	33^a	33	33	67	67^a	52.43
SEM	19.47	19.47	8.08	12.51	12.51	14.98	19.47	

Means on the row with different superscripts are significantly different

temperature cause disinfectants to degrade and weaken its germicidal activity. Corlett (1998) also stated that it is important to consider temperature in disinfection because chemical binding and reaction are strongly affected by temperature.

CONCLUSION

The laboratory efficacy evaluation indicated that Izal®, closely followed by Z-germicide® is the least effective. Despite this least effectiveness, they are the most widely used in poultry farms in the state Conversely, Virkon®, which has a superior activity over the other disinfectants, is the least used. The efficacy of on-farm reconstituted disinfectant over time showed that temperature at mid-day affects the efficacy of the disinfectants on the organisms. The result of this study therefore, indicates that disinfectant type and temperature of the day influences bacterial inhibition.

REFERENCES

Allen, M.J., G.F. White and A.P. Morby, 2006. The response of *Escherichia coli* to exposure to the biocide polyhexamethylene biguanide. Microbiology, 152: 989-1000.

Bauer, A.W., W.M. Kirby, J.C. Sherris and M. Turck, 1966. Antibiotic susceptibility testing by a standardized single disk method. Am. J. Clin. Pathol., 45: 493-496.

Block, S.S., 2001. Disinfection, Sterilization and Preservation. 5th Edn., Lippincott, Williams and William, Philadephia, PA., ISBN-13: 9780683307405 Pages: 1481,.

Buchanan, R.E., N.E. Gibbons, S.T. Cowan, T.G. Holt and J. Liston *et al*., 1974. Bergey's Manual of Determinative Bacteriology. 8th Edn., Williams and Wilkins, Baltimore, ISBN: 0683011170.

Cheesbrough, M., 2004. District Laboratory Practice in Tropical Countries Part 2. Cambridge Low Price Edn., Cambridge University Press, London.

Chima, I.U., 2011. Evaluation of disinfectants and disinfection practices of poultry farmers in Imo state Nigeria. Department of Animal Science and Technology, Federal University of Technology Owerri, Nigeria.

Chima, I.U., I.C. Unamba-Opara, C. Ugwu, A.C. Udebuani and C.G. Okoli *et al*., 2012. Biosecurity and disinfection controls of poultry microbial pathogen infections in Nigeria. J. World?s Poult. Res., 2: 5-17.

Chima, I.U., M.C. Uchegbu, I.C. Okoli and C.G. Okoli, 2011. Survey of chemical disinfectants used by poultry farmers in Imo State, Nigeria. Online J. Anim. Feed Res., 1: 223-230.

Corlett, D., 1998. HACCP Users Manual. Aspen Publishers, Inc., Gaithersburg MD.

Damerow, G., 1994. The Chicken Health Handbook. Storey, UK., ISBN-13: 9780882666112, Pages: 344.

DuPont, 2010. DuPont animal health solutions. Sudbury, SUFF CO10 2XD. Chilton Industrial Estate, USA. http://www.antecint.co.uk

El-Naggar, M.Y.M., M.A. Akeila, H.A. Turk, A.A. El-Ebady and M.Z. Sahaly, 2001. Evaluation of *in vitro* antibacterial activity of some disinfectants on *E. coli* serotypes. J. Gen. Applied Microbiol., 47: 63-73.

Gamage, B., 2003. Selection and use of disinfectants. BC Centre for disease Control.

Gasparini, R., T. Pozzi, R. Magnelli, D. Fatighenti and E. Giotti *et al*., 1995. Evaluation of *in vitro* efficacy of the disinfectant Virkon. Eur. J. Epidemiol., 11: 193-197.

Herrera, A.G., 2004. Nontraditional method of evaluating disinfectants: With isolated microorganisms from the food factory. Methods Mol. Biol., 268: 289-291.

Jeffrey, J.S., 2005. Sanitation-Disinfection basics for poultry flock. http://www.vetmed.ucdavis.edu/vetext/INF-PO_Sanitation.html

MSU, 2008. Sanitation: Cleaning and disinfection. http://www.MSUcares.com/poultry/diseases/sanitation.html

Morley, P.S., 2005. Evaluation of the efficacy of disinfectant footbath as used in veterinary hospitals. J. Am. Vet. Med. Assoc., 226: 2053-2058.

Obi, I.U., 1990. Statistical Methods of Detecting differences between Treatment Means. 2nd Edn., Snaap Press, Enugu Nigeria.

Okeudo, N.J., 2004. Empirical Studies of the Living Condition of Domestic Animals in Nigeria: Results from Nigeria. In: Studies of Sustainable Agriculture and Animal Science in Sub-Saharan Africa, Amalu, U.C. and F. Gottwald (Eds.). Peter Lang Publ., Germany, ISBN-13: 978-3631531389.

Okoli, I.C., 2004. Studies on anti-microbial resistance among *E. coli* isolates from feeds and poultry production units. Ph.D. Thesis, Federal University of Technology, Owerri, Nigeria.

Okoli, I.C., C.N. Anyaegbunam, E.B. Etuk, M.C. Uchegbu and A.B.I. Udedibie, 2004. Socio-economic characteristics of poultry business entrepreneurs in Imo state, Nigeria. J. Agric. Soc. Res., 4: 100-111.

Okoli, I.C., N.J. Okeudo, C.I. Nwosuh, C.G. Okoli and V.I. Ibekwe, 2002. Drug management of anti-microbial resistance in avian bacterial pathogen infections in Nigeria. Int. J. Environ. Health Human Dev., 3: 239-248.

Onah, D.N., 2004. Biosecurity in modern livestock production. Proceedings of the National Workshop on Continuing Education for Veterinary and Livestock Workers, June 21-25, 2004, Nsukka, Nigeria.

Payne, S.B., E.C. Kroger and S.E. Watkins, 2005. Evaluation of disinfectant efficiency when applied to the floor of poultry grow out facilities. J. Appl. Poult. Res., 14: 322-329.

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. Ciencia Avicola, 9: 127-131.

SAS Institute, 1999. SAS/STAT User?s Guide: Statistics. Version 8.00, SAS Inst. Inc., Cary, NC.