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Laboratory Evaluation of Some Disinfectants Used in Poultry Farms Against Some Bacterial Isolates

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ABSTRACT

This study was designed to evaluate the effect of the four disinfectants; Quaternary Ammonium Compound QAC (Vrocid), Iodine compound (Iocid 30), Formalin (Aldekol des o3) and H_2O_2 (Aquaclean) used by commercial poultry integrator against four bacterial isolates; Escherichia coli (E.coli), Staphylococcus aeuraus, Proteus ssp and Pseudomonas ssp, and the effect of the heat in relation to time on recommended concentration. This experiment consist of tow-parts; in the first part these disinfectants were made in five concentrations one according to the manufacturers recommendation, three were higher and one was lower than manufacturer recommendation these concentrations were made in disk from filter paper. Five concentration of each disinfectant were put in a plate contained the bacterial culture. The disc which was saturated by the recommended concentration of each disinfectant was put in the middle of the plate contain bacterial isolates and the lower and higher concentration was rounded. The plates were incubated at 37^{o}C overnight and then observed, the diameter of clear inhibition zone surrounded each disc was measure by using a ruler. In the second part only the recommended concentration of each disinfectant was used, each disinfectant was divided in four groups and submerged in a water bath at 37, 45, 50, 60^{o}C each group contained 4 tubes. The tubes were collected 5, 10, 15, 30 minutes after the starts of the experiment, the disk was put inside the tube of each disinfectants then taken immediately and was put in the plate contain bacterial isolates which were departed in 4 department. Incubated overnight at 37 ^{o}C, then the plate were observed, the diameter of clear inhibition zone surrounded each disk was measure by using a ruler. The laboratory evaluation indicated that H_2O_2, QAC and Formaldehyde respectively was effective against E.coli, Staphylococcus aureus and Proteus ssp and pseudomonas ssp while the iodine don't show any effect. It was concluded that the disinfectant used in this study was very effective and recommended to use in poultry house.

Keywords : Disinfectants, *Escherichia coli, Staphylococcus aeuraus, Proteus, Pseudomonas, spp.*

INTRODUCTION

The poultry industry has been the most of dynamic and ever expanding sectors in world during last two decade. It has been source of high quality protein for human consumption, so that source must

be free from infectious agent. Poultry diseases are costly to poultry production and are difficult to control (Fussell, 1998). The major economic losses for the poultry industry in the form of mortality, production losses, contamination and cost of preventive medication are due to infectious diseases caused by viruses, bacteria, fungi and parasites. Bacterial diseases are one of these causes and the most important bacteria are: *Escherichia Coli* (E.coli), *Salmonella spp, Clostridium spp*, *Pasteurella spp, Staphylococcus spp* and *Campylobacter. Escherichia coli* is one of the main etiologic agents that cause inflammatory processes in chicks which often results in a downgraded of carcass (Barnes *et al.,* 2003). Outbreak of necrotic enteritis case increased morbidity and significant economic losses (villegas, 1998; van lmmerseesl *et al.,* 2004). The subclinical form of *Clostridium perfringens* associated with necrotic enteritis (NE) causes a reduction in performance and overall health of poultry (Kaldhusdal and Lovland, 2000).

Staphylococcus aureus infection increase morbidity and mortality from yolk sac infection and secondary infection affecting the bone, tendon sheaths and leg joint (Moya, 1986). *Salmonella* continues to be a predominant food born pathogen worldwide; with poultry and poultry considered as a common vehicle for this pathogen. *Campylobacter* enteritis in the United States is responsible for approximately 2 million human cases of enteritis each year (Tauxe, 1997). *Mycoplasma gallisepticum* (MG) is the most pathogenic and economically significant pathogen of poultry. Airsacculitis in chickens or turkeys resulting from MG infections, with or without complicating pathogens, causes increased condemnations at processing. The presence of a high population of pathogenic bacteria in broiler grow-out houses can contribute in declining the wellness of the flock and lead to a sensitive production loss (Payne *et al.,* 2002 and Payne *et al.,* 2005). The principal of disease prevention and control largely rely on biosecurity, disinfectants are important components of a biosecurity program. Classes of disinfectants used include Phenolics, Quaternary Ammonium Compounds (QAC), Halogens, Oxidizing agents, Chlorhexidine compounds and Alcohols (Smith and June, 1999; Dvorak, 2005). The objective of disinfection is to reduce microbial populations (Eckman, 1994), 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). Disinfectants may have a limited life span after their initial dilution and it is possible that heat, sunlight, time, organic matter (OM) and adulterants may reduce their efficacy (Sainsbury, 1982).
To my knowledge no study was conducted in the Sudan to evaluate the disinfectant used in poultry farms.This study was designed to evaluate the effect of the four disinfectants used by commercial poultry integrator against bacterial isolates and the effect of the heat in relation to time on recommended concentration.

MATERIAL AND METHODS

Bacterial isolates

A total of four bacterial isolates including: *Escherichia coli (E.coli), Staphylococcus aeurous, Proteus spp* and *Pseudomonas spp* isolated from poultry were obtained from the Veterinary Research Institute, Soba, department of poultry disease. Disinfectants

A total of four disinfectants used in poultry farm, Quaternary ammonium compound QAC (Vrocid), Iodine compound (iocid 30), Formaline (aldekol des o3) and H_2O_2 (Aquaclean) were used (Table1).

Table 1: Disinfectants used in the study

Disinfectants	Ingredients
Quaternary ammonium	-Quaternary ammonium compound

compound (Vrocid)	Alkyldimethylbenzylammoniumchloride 17% Didecyldimethylammoniumchloride 7.8% - Aldehydes: gluteraldhyde 10.7% -Alcohol: isopropanol 14.6% - Terpentine derivatives- pine oil 2%
Iodine compound (iocid30)	Iodine 2.8%
Formaline(aldekol des o3)	-Glutaral 24.8% - Fromaldehyde C12/C16 18.4% -Alkyldimethylammoniumchloride 2.5%
H_2O_2 (Aquaclean)	H_2O_2 and silver nitrate

Culture media

Solid and liquid media were used in the present investigation for preservation and identification of the isolates.

Nutrient Broth

The medium was prepared according to the manufacture instruction by adding 25gms of powder in 1000ml of distilled water and sterilized by autoclaving in 15 pounds for 15min at 121^{o}C.

Nutrient Agar (Oxid)

This was obtained as blood agar base, which contained heart infusion, tryptose, sodium chloride and agar. It was prepared according to the manufacture instruction by dissolving 40 gms of powder in one liter of distilled water, it was distributed in 250 ml amount in flask and sterilized by autoclaving in 15 pounds for 15 min at 121^{o}C.

Methods

Preparation of the disc

Filter papers were punched by punching tool to make disc of 7mm in diameter, these disc were saturated in each concentration of disinfectants.

Preparation of different concentration of disinfectant

The disinfectant were made in five concentration one according to the manufacturers recommendation, three were higher and one was lower than manufacturer recommendation. The dilution was made in distilled water (Table 2).

Table 2: Concentrations of the disinfectants

disinfectant	**Tested concentrations**		
	Lower concentration	**Recommended concentration (REC)**	**Higher concentrations**

QAC	0.15%	0.25 %	0.35%	0.45%	0.55%
H_2O_2	9%	10%	11%	12%	13%
Formalin	0.4%	0.5%	0.6%	0.7%	0.8%
Iodine	0.15%	0.25%	0.35%	0.45%	0.55%

Preparation of Bacterial isolates

Some colony of each isolates was looped in tube contain nutrient broth, shacked well and then incubated at 37°C overnight till used. Bacterial isolates from each tube were taken by swab and cultured in the nutrient agar plate.

Method

Five concentration of each disinfectant were put in a plate contained the bacterial culture. The disc which was saturated by the recommended concentration of each disinfectant was put in the middle of the plate contain bacterial isolates and the lower and higher concentration was rounded (Figure1). The plate incubated at 37^{o}C overnight and then observed, the diameter of clear inhibition zone surrounded each disk was measure by using a ruler (Chima *et al*., 2013).

Effect of heat and time on disinfectants efficacy

Only the recommended concentration of each disinfectant was used, each disinfectant was divided in four groups and submerged in a water bath at 37, 45, 50, 60^{o}C each group contained 4 tubes. The tubes were collected at 5, 10, 15, 30 minutes after the starts of the experiment. The disc was put inside the tube of each disinfectants then taken immediately and was put in the plate contain bacterial isolates which were departed in 4 department (Figure 2), then incubated overnight at 37 °C, the plate was examined, the diameter of clear inhibition zone surrounded each disk was measure by using a ruler (Stringfellow *et al.,* 2009).

RESULT AND DISCUSSION

Efficacy and clarity of the disinfectants

Iodine the results showed that the iodine was not acted against all bacterial isolates.

Quaternary ammonium compound (QAC) results showed that the QAC was effective against *E.coli*, *Staphylococcus aureus, proteus spp* and *pseudomonas spp* (Table 3).

Hydrogen peroxide (H_2O_2) the results showed that the H_2O_2 was effective against *E.coli* and *proteus spp*(Table 4). Also H_2O_2 was effective against *pseudomonas spp* and *Staphylococcus aureus*.

Formaldehyde Formaldehyde was found effective against *E.coli*, *Staphylococcus aureus*, *proteus ssp* and *pseudomonas spp* (Table 5).

Effectiveness

E.coli the results revealed that the H_2O_2, QAC and Formaldehyde respectively showed effectiveness against *E.coli*, while Iodine has never been act on *E.coli.*

Staphylococcus aureus the results showed that the H_2O_2, QAC and Formaldehyde respectively showed effectiveness against *Staphylococcus aureus*, while Iodine has never been act on *Staphylococcus aureus*.

Proteus spp the results showed that the H_2O_2, QAC and Formaldehyde respectively showed effectiveness against *Proteus spp*, while Iodine has never been act on *Proteus spp*.

Pseudomonas spp the results showed that the QAC showed higher effectiveness against *pseudomonas spp* and H_2O_2 then formaldehyde respectively, while Iodine has never been act on *pseudomonas spp.*

Effective concentration against bacterial isolates

The effective concentration against *E.coli* observed by H_2O_2 was 13% which was higher than recommended concentration, QAC was 0.35%, 0.45% and 0.55% which were higher than recommended concentration, and Formaldehyde was 0.8% which was higher than recommended concentration, while Iodine do not showed any action on *E.coli*. The effective concentration against *staphylococcus aureus* observed by H_2O_2 was 11% which was higher than recommended concentration, QAC was 0.55% which was higher than recommended concentration and Formaldehyde was 0.5% which was recommended concentration, while Iodine do not showed any action on *E.coli*. The effective concentration against *Proteus spp* observed by H_2O_2 was 10% which was recommended concentration, QAC was 0.15% which was lower than recommended concentration, 0.25% which was recommended concentration and 0.45% which was higher than recommended concentration and Formaldehyde was 0.4% which was lower than recommended concentration, 0.5% which was recommended concentration, 0.6%, 0.7% and 0.8% which were higher than recommended concentration, while Iodine do not showed any action on *Proteus spp*. The effective concentration against *Pseudomonas spp* observed by H_2O_2 was 10% which was recommended concentration, 13% which was higher than recommended concentration, QAC was 0.55% which was higher than recommended concentration and Formaldehyde was 0.6% which was higher than recommended concentration, while Iodine do not showed any action on *Pseudomonas spp* (Table 6).

Effect of heat and time in the recommended concentration of the disinfectant

Effect of heat and time in the recommended concentration of the H_2O_2 against bacterial isolates

On 37^{o}C the zones around the disk were clear on all bacterial isolates expect in *Proteus spp* and it is constant in *Pseudomonas spp* and *Proteus spp* but there is a little variation in *E.coli* and *Staph* zones. The variation is minimizing with increase of time in *Staph* but it same after 15 and 30 min. In *E.coli* the zone increases at 10 min, then minimize after that.

On 40^{o}C the zones around the disk clear on all bacterial isolates expect on *Proteus spp* and there is a little variation in the zones around the disk in all plats of bacterial isolates. On 45^{o}C the zones around the disk clear on all bacterial isolates expect on *Pseudomonas spp* and there was a little variation in the zones around the disk in all bacterial isolates. On 60^{o}C the zones around the disk clear on all bacterial isolates expect on *Proteus spp* and there is a little variation in the zones around the disk in all plats of bacterial isolates (Table 7).

Effect of heat and time in the recommended concentration of the Formaldehyde against bacterial isolates

On 37^{o}C the zones around the disk were clear on all bacterial isolates and there was a little variation in the zones around the disk in all plats of bacterial isolate. On 40^{o}C the zones around the disk clear on all bacterial isolates and there was a little variation in the zone around the disk in *E.coli* and *Staph*. On 45^{o}C the zones around the disk clear on all bacterial isolates and there was a little variation in the zones around the disk in *E.coli* and *Staph*. On 60^{o}C the zones around the disk clear

on all bacterial isolates and it was constant in all bacterial isolates (Table 8).

Effect of heat and time in the recommended concentration of the QAC against bacterial isolates

On 37^{o}C the zones around the disk clear on all bacterial isolates and there is a little variation in the zones around the disk in *Proteus spp* and *Staph.* On 40^{o}C the zones around the disk clear on all bacterial isolates and there was a little variation in the zones around the disk in *Proteus spp* and *Staph*, there was observed variation in the zone around the disk in *Pseudomonas ssp*. On 45^{o}C the zones around the disk clear on all bacterial isolates and there was a little variation in the zone around the disk in *Pseudomnas spp* and *Staph*. On 60^{o}C the zones around the disk clear on all bacterial isolates and it was constant in all bacterial isolates (Table 9).

Effect of heat and time in the recommended concentration of the Iodine against bacterial isolates

There no effect by Iodine in all bacterial isolates in all degrees of heat.

Table 3: Effect of different concentrations of QAC against different bacterial isolates

QAC Concentrations	Bacterial isolates			
	E.coli	Staph	Proteus	Pseudomonas
0.15%	9	1.8	1.6	1.5
0.25%(REC)	1	1.9	1.6	1.6
0.35%	1	1.7	1.5	1.7
0.45%	1	1.8	1.6	1.7
0.55%	1	1.8	1.5	1.8

REC: recommended concentration.

Numbers in table revealed to zone around the disk/ mm

Table 4: Effect of different concentrations of H_2O_2 against different bacterial isolates

H_2O_2 concentrations	Bacterial isolates			
	E.coli	Staph	Proteus	Pseudomonas
9%	1.6	1.9	1.7	1.3
10%(REC)	1.5	2.2	2.6	1.5
11%	1.7	2.3	1.8	1.4
12%	1.8	1.6	1.9	1.4
13%	1.9	1.8	1.8	1.5

REC: recommended concentration.

Numbers in table revealed to zone around the disk/ mm.

Table 5: Effect of different concentrations of Formaldehyde against different bacterial isolates

Formaldehyde concentrations	Bacterial isolates			
	E.coli	Staph	Proteus	Pseudomonas
0.4%	9	1.8	1.3	1.1
0.5 %(REC.)	1	1.9	1.3	1.2
0.6%	1	1.8	1.3	1.4
0.7%	1	1.8	1.3	1.3
0.8%	1.1	1.8	1.3	1.3

REC: recommended concentration.
Figures in table revealed to zone around the disk/ mm.

Table 6: Effective concentration against bacterial isolates

Dis. / Isolates	Concentrations %																			
	H_2O_2					QAC					Formaldehyde					Iodine				
	9	10 (REC)	11	12	13	0.15	0.25(REC)	0.35	0.45	0.55	0.4	0.5 (REC)	0.6	0.7	0.8	0.15	0.25(REC)	0.35	0.45	0.55
E.coli	-	-	-	-	+	-	-	+	+	+	-	-	-	-	+	-	-	-	-	-
Staph	-	-	+	-	-	-	-	-	-	+	-	+	-	-	-	-	-	-	-	-
Proteus	-	+	-	-	-	+	+	-	+	-	+	+	+	+	+	-	-	-	-	-
Pseudo	-	+	-	-	+	-	-	-	-	+	-	-	+	-	-	-	-	-	-	-

Dis.: disinfectants.

Pseudo: pseudomonas.

DISCUSSION

Diseases and infections have always been a major concern to intensive poultry production industry. Pathogenic organisms can be introduced into a poultry housing facility through a variety of ways, for this reason, biological risk management (BRM) protocols are necessary to prevent, contain and eliminate the spread of disease (Dvorak, 2008). The correct usage of disinfectants is an important component of a successful biosecurity program (Stringfellow *et al.,* 2009). Disinfection protocols, when implemented correctly, can be a cost-effective means of reducing pathogenic organisms and are an important step in any biological risk management program, disinfectants should be used after cleaning and removal of organic matter (blood, fecal, litter, fat, hatchery fluff), organic matter provides a physical barrier that protects microorganisms from contact with the disinfectants (Dvorak, 2005). Commercially available disinfectants are not all classified as broad spectrum agent;

multiple factors should be considered when disinfectant is chosen, such as organic matter on the surface to be treated, presence of organic matter in the diluents, quality of water, corrosiveness or toxicity of the product, application method, temperature, porosity of the surface being treated, length of the contact time, infectious organism targeted, susceptibility of the infectious organism and correct dilution (Prince *et al.,* 1991; Quinn and Markey, 2001; Drorak, 2005; Payne *et al.,* 2005). Prevention of disease is typically easier and more cost-effective than addressing an outbreak situation. Therefore, development and implementation of a step-by-step disinfection protocol for the control and prevention of infectious disease has become essential for farms and clinics, no single disinfectant is adequate for all situations, disinfection protocols used on a daily basis will differ from those needed to control an infectious disease outbreak, however, both have one Component in common; thorough cleaning and washing prior to the application of any disinfectant are essential (Dvorak, 2008). Disinfecting agents are substances used to control, prevent or destroy harmful microorganisms (bacteria, viruses, or fungi) on inanimate objects and surfaces. Disinfection is the process of eliminating infectious organisms by using chemical or physical agents. In addition to the necessary knowledge, successful disinfection procedures, guidelines or regulations require a clear, succinct plan of action for each specific disinfectant application. The efficacy of any selected disinfectant also depends on the target organisms, their requirements for multiplication, and their resistance to environmental conditions and chemicals. The effective concentration against *Proteus ssp* observed by H_2O_2 was 10%, QAC was 0.15%, 0.25% and 0.45% and Formaldehyde was 0.4%, 0.5%, 0.6%, 0.7% and 0.8%, while Iodine do not showed any action on *E.coli*. The effective concentration against *Pseudomonas ssp* observed by H_2O_2 was 10%, 13%, QAC was 0.55% and Formaldehyde was 0.6%, while Iodine also do not showed any action on *E.coli*. When H_2O_2 used on 37^{o}C, 40^{o}C and 60^{o}C the result showed that the zones around the disk clear on all bacterial isolates expect on *Proteus ssp*, the zone is constant in *Pseudomonas ssp* and *Proteus ssp* but there is a little variation in *E.coli* and *Staph* on 37^{o}C, the variation is minimizing with increase of time in *Staph* but it same after 15 and 30 mint. In *E.coli* the zone increases at 10 mint, then minimize after that but there is a little variation in the zones around the disk in all plat of bacterial isolates on 40^{o}C and 60^{o}C, when we used H_2O_2 on 45^{o}C the result showed that the zones around the disk were clear on all bacterial isolates expect on *Pseudomonas ssp*, and there was a little variation in the zones around the disk in all bacterial isolates. The result was revealed that QAC on 37^{o}C, 40^{o}C, 45^{o}C and 60^{o}C the zones around the disk clear on all bacterial isolates and there was a little variation in the zones around the disk in all bacterial isolate but it is constant in 60^{o}C, in 40^{o}C and 45^{o}C there was a little variation in the zone around the disk in *E.coli* and *Staph* which was agree with Stringfellow *et al.,* (2009) who found that all disinfectants remained effective against *staphylococcus aureus* regardless of temperature.

The zones around the disc when Formaldehyde was used on 37^{o}C, 40^{o}C, 45^{o}C and 60^{o}C was clear on all bacterial isolates, it was constant on 60^{o}C and there is a little variation in the zones around the disk in *Proteus* and *Staph* 37^{o}C and 40^{o}C and there was some variation was observed in the zone around the disk in *Pseudomonas* in 40^{o}C, also there was a little variation in the zone around the disk in *Pseudomnas ssp* and *Staph* in 45^{o}C.

The study revealed H_2O_2 the most effective disinfectant which disagrees with Chima *et al*., (2013) which they found gluteraldehyde the most effective disinfectant.

The resistance of microorganism to the disinfectant decrease when the contact time was long which agree with Gehan *et al*., (2009)

Chima *et al*., (2013) indicated that efficacy of disinfectants was reduced during the afternoon when testing efficacy of six commercial disinfectants namely: Izal. Z-germicide, Diskol, Virkol, Vox and

CID20. However, efficacy gradually increased during the evening, this was disgreed with my study results which revealed efficacy of disinfectants increase with increase of temperature. Gehan *et al*., (2009) were testing five commercially available disinfectants against 7 selected bacterial, fungal and viral isolates, with and without organic matter in different time. They found that the microorganism resistant to disinfectant in presence of organic matter.

CONCLUSION

-The laboratory evaluation indicated that H_2O_2, QAC and Formaldehyde respectively was effective against *E.coli, Staphylococcus aureus and Proteus,* while the iodine don't show any effect.

-The efficacy of disinfectant was increase with increase of temperature in the storage.

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