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IN-VITRO EVALUATION BY DISC-DIFFUSION AND PITS METHODS OF ANTIMICROBICIDAL EFFICACY OF DISINFECTANTS USED IN FOUR BROILER CHICKEN HATCHERIES IN BABIL CITY\IRAQ

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ABSTRACT

A controlled experimental study was carried out during the period from (March to June\2013), in (4) separate commercial broiler chicken hatcheries. A total of (132) sample, (33) for each one, (22) sample revealed no growth, were randomly collected by surface swabbing, before and after disinfected conditions of different parts within these hatcheries as: Hatcher, incubator, and rooms of (worker's, egg-sorting and chick-processing), by gets (3) samples from each site. A total of (110) isolates, (100= 74G+ve & 26G-ve) for bacteria and (10) fungi, (15) species, (9= 5G+ve & 4G-ve) for bacteria and 6 fungi, were identified in this study from these hatcheries, by microscopic examination of gram's stained smears to show growth characteristics, then biochemical reactions (Culture on differential media, enzymatic reactions, antibiotic sensitivity and IMVC tests). Hatchery sanitation evaluated using (12) different disinfectants dilutions most commonly used in hatcheries, against these randomly selected species, which applied in two (Disc-diffusion and Pit) newly attempted methods in Babil\Iraq. The results include**:** total percentage of microbism prevalent in hatchery environment were (83.3%) of which, bacterial isolates gives the higher percentage (91%) than fungi (9.1%), the majority of bacterial isolates are gram positive (67.3%), than gram negative (23.6%). According to bacterial species, *S.aureus* gives the higher rate (32.7%), followed by *B.subtilis* (14.5%), while the lower *K.pneumoniae* (1.8%). According to hatcheries, Asaa'd gives the higher rate (42%), followed by Babil (28.2%), while the lower Chiflawi (11.8%). Totally, according to hatchery sites egg-sorting room gives the higher rate (24.5%), followed by chick-processing room (21,8%), while the lowest rate in Hatcher (15.5%), and in hatchery sites According to hatchery conditions (before and after disinfected), after using disinfectants a significant drop in bacterial and fungal contamination rates observed. According to disinfection, before disinfected gives the higher rate (70.9%), than after disinfected (29.1%). According hatcheries conditions, before disinfected gives higher rate in Asaa'd (30%), followed by Babil (20%), while the lowest in Chiflawi (8.2%), while after disinfected gives higher rate (11.8%) in Asaa'd, followed by Babil (8.2%), while the lowest is shown in Chiflawi too (3.6%). The percentages of microbial isolates susceptibility to disinfectants used in hatcheries using a Disc-diffusion method, total sensitivity rate (92.2%), were higher (85.3%) for bacterial isolates, than (33.9%) fungi. According to disinfectants used, bacterial isolates gives the lowest rate for Al-cohol (53.3%), Sarttol (66.7%), and Hypochlorite (86.7%), while the (100%) for all the others, while in Pits method, total sensitivity rate (87.7%), were higher (55%) for bacterial isolates, than (32.8%) fungi. According to disinfectants used, bacterial isolates gives higher rate (100%) for each: Formaldehyde, H_2O_2, combined Remas + TH_4^+, Remas and Intercept, while the lowest in Hypochlorite (53.3%).

It was concluded that total percentage of microbism prevalent in hatchery environments were higher, bacterial isolates higher than fungi, the majority of bacterial isolates are G+ve than G-ve. According to bacterial species, *S.aureus*, *B.subtilis* most prevalent. According to hatcheries higher rates in Asaa'd, than others. (9) species of bacterial isolates were identified, while (6) unidentified species for fungi. According to hatchery

sites egg-sorting room higher than others. According to disinfection before higher than after disinfected. According disinfection conditions in hatcheries, before and after disinfected higher in Asaa'd than others. Also study has shown variations in the degree of commercial disinfectants efficacy in hatcheries, all these disinfectants were relatively active with broad spectrum of action, some isolates especially fungi show resistance to disinfectants efficacy when apply these methods.

Keywords: Evaluation, pit, disc-diffusion, method, antimicrobicidal, disinfectants, efficacy, broiler chicken, hatcheries.

INTRODUCTION

Hatchery hygiene is recognized as an important factor and common concern in healthy poultry production (Thermote, 2006). So, the development and maintenance of an effective hatchery sanitation program is essential for the successful operation of a poultry hatchery, Hatchery sanitation plays a crucial role in prevention and control of pathogens (Gehan *etal.*, 2004). A good sanitation program (management practices, treatment, disinfectants) that include complex and critical issues, can benefit the grower by optimizing contaminated hatcheries, thus, it is important to routinely evaluate its effectiveness in hatchery (Chima *etal.*, 2012). Hatchery eggs leaving breeder house and carry many bacteria on shell (Wells *etal.*, 2011; Cox *etal.*, 1994), and explosion of contaminated eggs cause air-borne infections (Agabou, 2009).

Risk analysis data designate hatcheries to be a major risk factor in proper health safe guarding in poultry industry, as it possible of incubating eggs contaminated with pathogens, during it there are conditions for occurrence and maintenance of microbism and development of incubator infections in hatcheries (Hrncar *etal.*, 2012; Zhelev *etal.*, 2012; Copur *etal.*, 2011). When cleaning and disinfection procedures are not performed properly, conditions present for transfer pathogens among the different batches of newly hatched chickens, this lead to significant epidemiological and economical risks (MSU, 2008; Jeffery, 2005).

The environment of a poultry hatchery is very susceptible to contamination by microorganisms, which can adversely affect hatchability of the eggs and can results in embryonic and chick deaths (Metawea and El-Shibiny, 2013). And poor standards of hatchery hygiene may lead ultimately to an explosion of pathogenic organisms resulting in severe economic loss (Rashid *etal.*, 2011). and great agglomeration of poultry, chicks and eggs induce increased pathogenicity of some microbial agents, especially bacteria, which cause infection with high rate of morbidity and mortality (Wang, 2009).

Zoonotic potential of microbial agents present a special epidemiological problem in hatchery, as these agents pose a permanent risk for human health and hatcheries, mostly to people who work with poultry or to the consumers who eat contaminated poultry meat or eggs (Al-Jaff, 2005; Khan *etal.*, 2003). A high population of pathogenic bacteria in hatchery contributes to a decline in wellness, and the spread of pathogens to processing equipment can increase the chance of contaminating hatchery. The presence of microorganisms in the hatchery is directly related to deficiencies in hygiene, which can result in elevated first week chick mortality and depressed growth rate (Agabou, 2009).

The principles of disease prevention and control within hatcheries are based on: biosecurity, preventive vaccination and sanitation. Bio-security which regularly includes cleaning and disinfection, is one of the best methods to reduce the microbes (Mrigen, 2006), and although chemical disinfectants present an active alternative to antibiotics, but as the latter due to up and irregular use it gives raise chance and prevalence of resistant strains, and loss its activity

or they grow on, and sanitary program should include safe and easy procedures outlining the correct application of detergents and disinfectants, also proper use of application equipment and efficient monitoring (Gehan *etal.*, 2009; Moubarak, 2007).

To eradicate this infectious agents it is necessary to disinfect hatcheries, especially if health problems occur (Ilic *etal.*, 2009), and hatchery sanitation programs should include the use of one or more disinfectants to inhibit the growth of microorganisms and maintain a desirable level of hatchability (Metawea and El-Shibiny, 2013). A need exists for safe and effective disinfectants for use in hatchery, that convenient to use and can minimize the time required for satisfactory sanitation (Gehan, 2009; Jeffrey, 2005). All these facts emphasize the importance of disinfection as a part of general set of anti-epidemic measures in hatcheries. No single disinfectant is best for all purposes (Thermote, 2006). So, the disinfection process is complex and multifaceted, and influenced by number of factors and conditions as: disinfectant properties, type and resistance of microorganisms and the environmental conditions where disinfection done (Lyutskanov *etal.*, 2010; MSU, 2008). Application of effective disinfectants at manufacturer's recommended dilution levels is included in any hatchery sanitation program (Khan *etal.*, 2003; Samburg and Meroz, 1995), and must be careful in mixing it and any addition to it must approved by manufacturer as it could reduce efficacy of one or more in mixture (Kennedy *etal.*, 2006; McDonnell and Russell, 1999). Ability of disinfectant to function varies in presence of organic matter, temperature under 20ºC, pH extremes, Humidity, soap residues or problem in selecting a suitable one (Meroz and Samborg, 1995; Khars, 1995). The correct use of disinfectants is one of the means, which can be usefully applied against hatchery and poultry contamination and spread of invasive infections (Mrigen, 2006; Kennedy *etal.*, 2006). and the goal of any disinfectant is to prevent, reduce or destroy microbial populations on inanimate objects, surfaces or the premises (EPA, 2009; Payne *etal.*, 2005). Proper sanitation practices and the use of efficacious disinfectants in hatchery have no any effect on chick quality (Agabou, 2009), weight of hatched chick and egg, and hatchability (Hrncar *etal.*, 2012; Rashid *etal.*, 2011; Milakovic-Novak and Brukner, 1990).

So, the objects of the present study are to investigate and compare the antimicrobicidal efficacy (using two in-vitro susceptibility profile methods) of some available, most utilized commercial disinfectants of different chemical groups in a trial to evaluate and prove their effects in controlling the main isolated contaminant species of commercial hatcheries.

MATERIALS AND METHODS

The controlled experimental work was carried out during the period from (March to June\ 2013), in (4) separate commercial broiler chicken hatcheries (Babil, Al-A'mer, Chiflawi and Asaa'd), located in Babil City.

Microbial contamination information can only be gained by periodically surveying the microbial populations of many surfaces and objects which may harbor organisms in hatchery, and degree of contamination was first measured numerically by the microbiological examination of hatchery environment (equipments, rooms, and floor) by sampling technique, which is used extensively to monitor microbial levels.

Sample Processing and Handling

A total of (132) sample, (33) for each one, were randomly collected twice before and after disinfected conditions, were taken from surfaces of different parts within these hatcheries as: Hatcher, incubator, and rooms of (worker's, egg-sorting and chick-processing), gets (3)

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samples from each site, by rubbing a sterile cotton swab, then delivered to the Microbiology Laboratory, Community Health Dept., Technical Institute\Babil.

Samples were aseptically processed by routine microbiological laboratory methods for bacterial cultivation and identification were first immediately cultured aerobically by inoculate nutrient agar plates as enrichment media for cultivation of bacteria, by streaking of segmented sterile, 25ml of 24hr age previously prepared petri dish agars at 37ºC for 24hr, colonies then subcultured by streaking inoculate nutrient agar plates for purification, then incubated at 37ºC for 24hr.

Bacterial Isolation & Identification

Bacteriological analysis in isolation and identification of suspected colonies were achieved according to Leboff and Pierce (2011); Alexander and Strete (2001); MacFaddin (2000); Holt and Krieg (1994). These isolates were assayed for the following:

1. Gram's staining of smears prepared on glass slides were taken from each colony, then examined microscopically to distinguish bacterial cell characteristics as staining, shape, numbers and arrangement.
2. Biochemical reactions:
   A- Culture on differential media:
      a- Blood agar, for colony growth characteristics and type of hemolysis.
      b- MacConkey agar, to separate gram negative enteric and lactose fermenting bacteria from others.
      c- Mannitole salt agar (MSA), to differentiate isolated Staphylococci and *Bacillus* spp.
      d- Kligler's iron agar (KIA), to differentiate bacteria on slope and bottom colours, H_2S and gas production.
3. Enzymatic reactions as: Oxidase, Catalase, Coagulase and Urease.
4. Antibiotic sensitivity for Optochin and Bacitracin discs to differentiate Streptococci.
5. IMVC tests especially for gram negative isolates to differentiate bacteria on: Indole, Methyl red, Voges Proscaouer and Simmon's citrate reactions.

Fungal Isolation & Identification

Suspected isolates as fungi were inoculated on nutrient agar plates for additional time (2-3 days), then examined microscopically using gram stained smears to distinguish yeast cells as: Staining, numerous, large size than bacteria, spore-forming, with obvious rigid cell wall, also thread-like branches of molds hayphae by naked eye, then prepare inoculums for culture and tested by these two methods according to Carter and Wise (2004).

Tested Microorganisms

Microbial isolates from different sites in hatcheries, include (15) species (9= 5G+ve & 4G-ve) for bacteria and (6) unidentified fungi.

Bacteria: (G+ve): *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Streptococcus pyogenes* A, *Bacillus cereus*, *Bacillus subtilis*, (G-ve): *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Proteus mirabilis.*

Preparation of Bacterial Diluted Inoculums

Purified bacterial isolates stored in vials at 4ºC as stock cultures for work. Bacterial species were randomly selected for disinfectant sensitivity testing, the sterile cotton swab dipped inside the bacterial dilute prepared, then applied to the surface of agar plate to create a good surface coverage.

Preparation of Disinfectants Solution Dilutions

In this study we test antimicrobicidal efficacy of (12) different commercial disinfectants most commonly used in hatcheries against (15) microbial species isolates, as shown in (Table 1).

Table (1). Disinfectants, its dilutions (concentrations) used and properties.

Disinfectants Used	*Dilution used (Concentration)*	*Type/ Group & Active gradients*	*Usage & Application*	*Uses in Hatchery & Poultry*
Al-cohol	70%	Ethanol Al-cohol	Spray	Equipments& Devices, Tools, surfaces.
Sarttol	1:10 (v/v) 10%	Isopropyl Al-cohol	Spray	Equipments& Devices, Tools, surfaces.
Iodospec 2.8	1:500 (v/v), 10% I	Halogens-Iodophors-Oxidizing agent	Spray	Footbath & Sites, Devices-Incubator, Water.
Hypochlorite	2:100 (v/v) 2%	Detergents (Bleach)	Spray	Egg washing & dipping, Devices, Tools, surfaces.
Virkon-S	1:100 (w/v) 1%	Combined Peroxygens-Oxidizing agent	Spray, Fogging Add to water	Poultry houses, Devices, Porous surfaces, Water & Air.
TH_4^+	1:500 (v/v)	Combined	Spray, Add to water	Footbath & Sites, Poultry houses, Rooms.
Remas	1:200 (v/v) 0.5%	Combined	Spray	Footbath & Sites, Poultry houses, Rooms.
Remas + TH_4^+	1:200&1:500 (v/v)	Combined	Spray	Footbath & Sites, Poultry houses, Rooms.
Intercept	1:100 (v/v) 1%	Combined Gluteraldehyde 15% + QAC 15%	Spray, Floating	Poultry houses, premises, Rooms, Hatching equipments- Incubator, Tools. Floor
H_2O_2	3%	Oxidizing agent-Peroxygens	Spray, Fogging	Poultry houses, Rooms, Hatching equipments.
NaOH	2%	Alkalines	Spray	Poultry houses, Rooms, Hatching equipments.
Formaldehyde	40%	Aldehydes-Alkalines	Fumigation, Spray, Add to water	Poultry houses, Rooms, Devices-Incubator & Hatcher, Eggs, Tools, Surfaces.

All disinfectants were prepared at various dilutions of different concentrations at a common usage level, mostly in minimum levels using sterile distilled water as recommended by manufacturers on the label of each stock commercial bottle, some disinfectants used as received concentration without dilution. These solutions represent either single or combined treatments, then labeled for work with its name and concentration (Gehan *etal.*, 2009; EPA, 2009; HACCP, 2008), which turned in hatcheries as solutions for apply as spray, added to drinking water, fumigation or fogging (Kennedy *etal.*, 2006).

In-Vitro Bacterial Sensitivity Testing Against Disinfectants

Performed in laboratory to evaluate disinfectant's efficacy in killing bacterial isolates (applied on fungi also) using two newly attempted methods:

Agar Disc-Diffusion (Filter Paper) Method

Bacterial isolates were subjected in this technique against (12) commercial hatchery disinfectants, which used according to the method of Kirby and Bauer as described by Brooks *etal.* (2013); Spicer (2008); Quinn and Markey (2003).

Sterile disinfectant discs of each dilution applied aseptically using forceps sterilized by alcohol and flame to transfer and press discs on the lowned surface of nutrient agar plates, then incubated reversely at 37°C for 24hr (Payne *etal.*, 2005), but the survival of fungi was examined after (2-3days) time of exposition to each disinfectant (Khan *etal.*, 2003).

The results were recorded by considering the clear zone of inhibition around different disinfectant discs (no growth- sensitive) or no clear zone (growth- resistant), using accurate ruler in millimeters (mm), then the growth was compared with the respective control (no disinfectant added).

Pits Method

By make pits into the lowned layer of nutrient agar plates using sterile cork cutter, then filled with a disinfectants solution by a sterile needles, then petri dishes incubated irreversely at 37°C for 24hr as described in Winn *etal.* (2006).

These two methods have the same principle in saturate the lawned agar with each disinfectant, used in this controlled study, to compare the effectiveness of disinfectants efficacy against some randomly selected bacterial and fungal isolates, as an alternative way and relatively new method attempted in Babil\Iraq instead of MIC dilutions, MBC, Phenol coefficient (PC) methods.

Control groups

Negative controls (untreated- performed using bacterial lawn only without a disinfectant, by add a distilled water instead of disinfectants as Placebo), were used for all plating procedures as treatment control, and to ensure that media had been properly sterilized and don't be contaminated.

Statistical Analysis

All samples has (3) replicates, and data were analyzed using percentage rates. Results up to 50% represent a statistical significance in bacterial and fungal prevalence, and its disinfectant-based treatment (Kim *et al.*, 2007).

RESULTS AND DISCUSSION

(132) samples were taken from (4) commercial broiler chicken hatcheries (Babil, Al-A'mer, Chiflawi and Asaa'd), (33) from each hatchery, from different sites (Incubator, Hatcher, and Worker's, egg-sorting and chick-processing rooms), (3) samples for each site. Numerous bacterial pathogens that contaminate hatcheries have been isolated from these various parts of poultry hatcheries. A total of (110) isolates, (100= 74G+ve & 26G-ve) for bacteria and (10) fungi, (15) species (9= 5G+ve & 4G-ve) for bacteria and (6) fungi, were identified in this study from hatcheries as in (table2). Total percentage of microbism of various microbial species prevalent in hatchery environment were (83.3%), of which bacterial isolates gives the

higher percentage (91%) than fungi (9.1%), the majority of bacterial isolates are a gram positive (67.3%), than gram negative (23.6%).

Table 2. Percentages of microbial isolates prevalence in hatcheries

Microbial Isolates	*Babil*		*Al-A'mer*		*Chiflawi*		*Asaa'd*		*Total*	
	No.	*%*	*No.*	*%*	*No.*	*%*	*No.*	*%*	*No.*	*%*
Staphylococcus Aureus	9	29	8	40	5	38.5	14	30.4	36	32.7
Staphylococcus Epidermidis	2	6.4	-	0	1	7.7	3	6.5	6	5.5
Streptococcus Pyogenes A	1	3.2	1	5	-	0	2	4.3	4	3.6
Bacillus Subtilis	5	16.1	3	15	2	15.4	6	13	16	14.5
Bacillus Cereus	2	6.4	2	10	3	23.1	5	11	12	11
Klebsiella Pneumoniae	1	3.2	-	0	-	0	1	2.2	2	1.8
Pseudomonas Aeruginosa	2	6.4	1	5	-	0	3	6.5	6	5.5
Proteus Mirabilis	2	6.4	1	5	-	0	3	6.5	6	5.5
Escherichia Coli	4	13	3	15	1	7.7	4	8.7	12	11
Total (Bacteria only)	28	25.5	19	17.2	12	11	41	37.2	100	91
	G+ve: 74 isolate/ 67.3%				G-ve: 26 isolate/ 23.6%					
Unidentified Fungi (Fungi only)	3	9.7	1	5	1	7.7	5	11	10	9.1
Total (Bacteria & Fungi)	31	28.2	20	18.2	13	11.8	46	42	83.3%	

According to bacterial species, *S.aureus* gives the higher rate (32.7%), followed by *B.subtilis* (14.5%), while the lower *K.pneumoniae* (1.8%). According to hatcheries, Asaa'd gives the higher rate (42%), followed by Babil (28.2%), while the lower Chiflawi (11.8%). Staphylococci and Streptococci spp. a G+ve normal gut flora, which help suppress other pathogens by their presence.

Hatchery sanitation evaluated using surface swabbing and microbiological examinations by microscopic examination of gram's stained smears to show growth characteristics, then biochemical reactions (Culture on differential media, enzymatic reactions, antibiotic sensitivity and IMVC tests) (McFaddin 2000; Holt and Krieg, 1994).

Investigations have revealed a large microbial populations in these hatcheries despite the application of various sanitation measures, and the quantitative microbiological studies established that different surfaces in hatcheries (floor, walls, devices) were contaminated to a different extent, this coincides the results of Metawea and El-Shibiny (2013); Lyutskanov *etal.* (2010); Mamman *et al* (2008); Al-Jaff, (2005); Khan *et al* (2003), as air flow, employee activity, soiled eggshells and contaminated water supply are responsible for the dissemination of these contaminants within hatchery environment, in addition to mistakes during process of sorting, removing unfertile eggs, which tend to explode during incubation, thus

contaminating the surfaces, also lack of thorough cleaning before disinfection (Lyutskanov *etal.*, 2010; Soliman *etal.*, 1999 a&b).

Table (3) shows the results of morphological characteristics and diagnostic biochemical reactions of bacterial isolates from hatcheries. In morphological characteristics it classified on Gram's stain to (G+ve& G-ve). Also in shape (cocci and bacilli), arrangement (single, pairs-chains and grape-like clusters), motile or non, capsule present or absent. According to growth characteristics of colonies on differential culture media, on Blood agar to shows growth and type of hemolysis, MacConkey agar a selective medium to isolate gram negative bacteria only that classified as lactose fermenters or non, especially enterobacteriaceae family, Mannitole salt agar (MSA) a selective medium also to isolate gram positive staphylococci and *Bacillus* spp. only that grow on and classified as mannitole fermenters or non.

Also Kligler's iron agar (KIA) slants a differential medium especially for gram negative bacteria, which contain (4) reactions (slope, bottom, H_2S & gas). Also (4) enzymatic reactions used (Oxidase, Catalase, Coagulase & Urease) for both gram negative and positive. In addition to IMVC test which contain (4) reactions (Indole, MR, VP & Citrate).

Table (4) shows the percentages of microbial prevalence in hatchery sites according to hatchery conditions before and after apply disinfectants. After using disinfectants a significant drop in bacterial and fungal contamination rates observed. Totally, according to hatchery sites egg-sorting room gives the higher rate (24.5%), followed by chick-processing room (21.8%), while the lowest rate in Hatcher (15.5%). According to disinfection, before disinfected gives the higher rate (70.9%), than after disinfected (29.1%). According disinfection conditions in hatcheries, before disinfected gives higher rate in Asaa'd (30%), followed by Babil (20%), while the lowest in Chiflawi (8.2%), while after disinfected gives higher rate (11.8%) in Asaa'd, followed by Babil (8.2%), while the lowest is shown in Chiflawi too (3.6%).

Microbial contamination of the egg-sorting and chick-processing rooms was higher than others, this findings coincides with Metawea and El-Shibiny (2013); Lyutskanov *etal.* (2010); Gehan (2009), as bacteria in horizontal surfaces become air-borne from employee activity and drawn in walls, floor, equipments, egg and chick baskets, chick fluff and feces, also broken eggs and diseased or dead chicks in these rooms, also chicks dried off organisms on fluff and dust spread through the rooms where they again settled and this cycle could be repeated with each hatch. So, surfaces should be smooth, impervious and dried for good sanitary results. Also, removal of old litters, followed by cleaning (organic matter, dust, soil) (can reduce 80-90% of microbism) and disinfecting of facilities, can help reduce pathogen loads, break disease cycles and prevent transmission via food chain.

According hatcheries, we noticed that sanitary conditions of Chiflawi is better than in others, this should result in lower degree of contamination of hatching eggs and consequently enhance the hatchability and improve the chick quality (Khan *etal.*, 2003). Incubator also higher as eggshell may be contaminated and penetrate it into contents (Zhelev *etal.*, 2012; Al-Jaff, 2005; Spikle *etal.*, no date), also drawn into hatchers where they multiplied rapidly during hatching although cleaning and disinfecting processes.

Table 5 shows the percentages of microbial isolates susceptibility to disinfectants used in hatcheries using a Disc-diffusion method, according to Kirby and Bauer method presented by Brooks *et al.* (2013) and Winn *etal.* (2006). The inhibition zone diameters determined and the results recorded on the following scale: 0-6 resistant (R), 7-12 intermediate (I) and 13-more sensitive (S). Total sensitivity rate (92.2%), were higher (58.3%) for bacterial isolates, than (33.9%) fungi. According to disinfectants used, these isolates gives the lower rate for Al-

cohol (53.3%), Sarttol (66.7%), and Hypochlorite (86.7%), while the (100%) for all the others. Most of microbial isolates were sensitive for disinfectants, but according to bacterial species resistance shown in , *B.cereus* for Al-cohol, *E.coli* for Al-cohol and Sarttol, while in fungi it shown in F2-F6 for Al-cohol, F3-F6 for Sarttol, F5&F6 for Hypochlorite.

Table (6) shows the percentages of microbial isolates susceptibility to disinfectants used in hatcheries using a Pits method. Total sensitivity rate (87.7%), were higher (55%) for bacterial isolates, than (32.8%) fungi. According to disinfectants used, these isolates gives higher rate (100%) for each: Formaldehyde, H_2O_2, combined Remas + TH_4^+, Remas and Intercept, while the lower in Hypochlorite (53.3%), due to action on DNA, were combined increase its efficacy as in Youseif *etal.* (2001). Susceptibility to Virkon-S as in Bolder (2009). Susceptibility to H_2O_2 as in Sander and Wilson (1999). Susceptibility to TH_4^+ as results of Gehan (2009); Soliman *etal.* (2009a&b), they proved that TH_4^+ is the most powerful disinfectant because its synergistic action of QAC and glutaraldehyde, QAC acts on cytoplasmic membrane resulting in leakage, also similar to results of Kassaify *etal.* (2007); Kaskova *etal.* (2007). High sensitivity of all organisms to 2% NaOH solution as results of Lyutiskanov *etal.* (2010), this due to the hydrolytic effects and its ability to dissolve organic residues, which allows a good in-depth penetration.

Most of microbial isolates were sensitive for disinfectants, but resistance (on number of disinfectants) shown in bacterial species, for (1) in *S.epidermidis*, *B.subtilis*, *E.coli*, for (2) *S.aureus*, *S.pyogenes* A & *P.mirabilis*, while in fungi for 1(F1), 2(F3&F5) and 5(F), mostly for Al-cohol, Sarttol and Hypochlorite.

Some gives intermediate resistance to disinfectants, which may need to use in higher concentration or long contact time, as death rates of organisms affected by length of exposure time to disinfectant (Payne *etal.*, 2005; Gasparini *etal.*, 1992). Disinfectants should be used subsequent to the cleaning and removal of organic matter on surfaces subjected to sanitation, and results show that bacterial isolates gives resistant to Al-cohol and Sarttol, and sensitive to the others. While fungi resist Al-cohol, Sarttol and hypochlorite only. These results illustrated that most of disinfectants used were effective against bacterial and fungal isolates at the used concentrations. Formaldehyde was outstanding as a disinfectant with best action on bacteria and fungi, this similar to results of Ilic *etal.* (2009).

As results of Chima *et al.* (2012), Al-cohol and Sarttol gives low efficacy, as it acts in dilute phospholipids at cell membranes resulting in leakage, have limited activity in presence of organic matter, with limited residual activity due to evaporation. Iodophors low efficacy as iodine require prolonged contact time and frequently applied, low active in presence of organic matter, poor residual activity and acts on amino groups in cell proteins.

S.aureus are highly susceptible to biocides (Rodgers *etal.*, 2001), and resistance of *B.subtilis* is not so important as it is non pathogenic organism in hatcheries (Khan *etal.*, 2003). *P.aeruginosa* gives intermediate resistance, which is noticed to be less sensitive to a variety of disinfectants because acquired resistance as a result of mutation, acquisition of plasmids or transposons, and able to degrade QAC.

Although in-vitro bacterial resistance to disinfectants was very low in hatcheries, yet it is anticipated that a high number of bacteria would show a resistance, but prolonged use of some disinfectants may have selected resistance to it, also individual bacteria of the same genus and species may have variations in sensitivity to disinfectants (Gehan *et al.*, 2009). So, disinfectants usually used in minimal concentrations, and altered to prevent the prevalence of microbial resistant strains, and Conner and Eckman (1993) noticed that rotating disinfectants guards against resistant strains even where biofilms formed in hatchery. Mamman *et al.*

(2008) noticed that G-ve bacteria were more resistant than G+ve due to their complex cell wall, have an outer layer impermeable for anticytoplasmic compounds. As results of Rashid *et al.* (2011); Kassaify *et al.* (2007) during the study period and findings of workers and breeders, none of any chemical commercial disinfectants used in these hatcheries shown to be affected on egg and chick weight and hatchability in treated eggs, this need further study.

The blends (combined or mixture) gives higher sensitivity rates in this study, similar to results of Kassaify *etal.* (2007), this suggest the use of blends of compatible compounds for disinfection to target a wider range of organisms, taking in consideration the compatibility of ingredients used and the nature of target microbial species.

Finally, all these disinfectants active in this study with broad spectrum in **Disc-diffusion method** against bacteria except Al-cohol, low for Sarttol, in Fungi Al-cohol and Sarttol, low for Hypochlorite, while in **Pits method** against bacteria except Sarttol and Hypochlorite, low for Al-cohol, Iodospec and NaOH, in fungi Al-cohol, Sarttol and Hypochlorite, low for Iodospec, Virkon-S, TH_4^+ and NaOH.

Antibacterial or bacteriocidal and high disinfectants efficacy and sensitivity of bacteria in this study coincides the results of Kadria *etal.* (2009); Reybrouck (2004); Ruano *etal.* (2001). Also, antifungal or fungicidal disinfectants efficacy and sensitivity of fungi that proved in this study, were similar to results of Nawaczawski *etal.* (2013); Kramomtong *etal.* (2010); Soliman *et al.* (2009b); Reybrouck (2004); Williams and Brake (2000).

Fungal isolates (depends on number) in these two experiments gives the higher resistance to disinfectants used than bacterial isolates, as bacteria found in vegetative stage be fragile and destroyed by most disinfectants, while fungi contain a rigid cell wall and spores that could survive and not affected by most disinfectants used, and Maillard (2002) noticed that the differences in sensitivities of bacteria and fungi and efficacies of disinfectants against it, due to effects of different target sites in these microorganisms.

CONCLUSIONS

Total percentage of microbism prevalent in hatchery environments were higher, bacterial isolates higher than fungi, the majority of bacterial isolates are G+ve than G-ve. According to bacterial species, *S.aureus*, *B.subtilis* most prevalent. According to hatcheries higher rates were in Asaa'd, than others. (9) species of bacterial isolates were identified, while (6) unidentified species for fungi. According to hatchery sites egg-sorting room higher than others.

According to disinfection before were higher than after disinfected. According disinfection conditions in hatcheries, before and after disinfected higher in Asaa'd than others. Also study has shown variations in the degree of commercial disinfectants efficacy in hatcheries, all these disinfectants were relatively active with broad spectrum of action, some isolates especially fungi shows resistance to disinfectants efficacy when apply these newly attempted methods.

So, bacterial etiology presents important factors in poultry hatcheries, and cleaning and disinfecting of facilities can help reduce pathogen loads. Study indicates disinfectants efficacy and safety recommended to maintain control monitoring programs to reduce occurrence of these organisms in hatcheries.

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Table 3. Results of morphological characteristics, and diagnostic biochemical reactions of bacterial isolates from hatcheries

	Bacterial isolates	Cellular morphology						Growth characteristics of colonies on differential media							Biochemical reactions								
											Kligler's iron agar (KIA)				Fermentative/ Oxidative enzymes				IMVC			
		Gram's stain	Shape	Arrangement	Motility	Spore	Capsule	Blood agar & Hemolysis	MacConkey agar	Mannitole salt agar (MSA)	Slope	Bottom	H_2S	Gas	Oxidase	Catalase	Coagulase	Urease	Indole	Methyl Red	Voges Proskauer	Simmon's citrate
G +ve	*Staphylococcus aureus*	+	C	Cl	-	-	+	β	NG	G,F					+	+	+	+			+	
	Staphylococcus epidermidis	+	C	Cl	-	-	-	γ	NG	G					-	+	-	+			+	
	Streptococcus pyogenes A *	+	C	Ch	-	-	-	β , S	NG	NG						-					-	
	Bacillus subtilis	+	B	Ch	+	+	-		NG	G,F						V		V	-		+	+
	Bacillus cereus	+	B	Ch	+	+	-	β	NG	G						+		V	-		+	+
G -ve	*Klebsiella pneumoniae*	-	B	S	-	-	+		G,LF	NG	Y	Y	-	+	-	+		+	-	-	+	+
	Pseudomonas aeruginosa **	-	B	S	+	-	-		G	NG	R	R	-	-	+	+		V	-	-	-	+
	Proteus mirabilis	-	B	S	+	-	-	α,*	G	NG	R	Y	+	+	-	+		+	-	+	-	+
	Escherichia coli	-	B	S	+	-	-		GLF	NG	Y	Y	-	+	-	+		-	+	+	-	-

C: Cocci, B: Bacilli, Cl: Grape-like clusters, Ch: Chains, S: Swarming, α: Partial, β: Complete, γ: no lysis, V: Variable, H_2S: Hydrogen sulfide (Blackening), G: Growth, NG: No growth

F: Fermenter, LF: Lactose fermenter, +: Present, -: Absent, R: Red pink (alkaline reaction), Y: Yellow (acid rea.), * (Optochin R, Bacitracin S), ** (G at 42°C, NG at 4°C)

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Table 4. Percentages of microbial prevalence in hatchery sites according to hatchery conditions, before and after disinfected (R: Room)

Hatchery sites	*Babil*				*Al-A'mer*				*Chiflawi*				*Asaa'd*				*Total*					
	Before		*After*		*Before*		*After*		*Before*		*After*		*Before*		*After*		*Before*		*After*			
	No.	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**
Egg-sorting R.	5	16.1	2	6.4	4	20	2	10	3	23.1	1	7.7	7	15.2	3	6.5	19	24.3	8	25	27	24.5
Chick-processing R.	4	13	2	6.4	2	10	1	5	1	7.7	1	7.7	10	21.7	3	6.5	17	21.8	7	21.9	24	21.8
Workers R.	7	22.6	2	6.4	2	10	1	5	1	7.7	1	7.7	5	11	3	6.5	15	19.2	7	21.9	22	20
Incubator	3	9.7	2	6.4	3	15	1	5	3	23.1	-	0	6	13	2	4.3	15	19.2	5	15.6	20	18.2
Hatcher	3	9.7	1	3.2	3	15	1	5	1	7.7	1	7.7	5	11	2	4.3	12	15.4	5	15.6	17	15.5
Total	22	20	9	8.2	14	12.7	6	5.4	9	8.2	4	3.6	33	30	13	11.8	78	70.9	32	29.1		
	31	28.2			20	18.2			13	11.8			46	42			83.3%					

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Table 5. Percentages of microbial isolates susceptibility to disinfectants used in hatcheries, using Disc-diffusion method

Microbial isolates	Al-cohol	Sarttol	Iodospec 2.8	Hypochlorite	Virkon-S	$TH4^+$	Intercept	Remas	Remas + TH_4^+	H_2O_2	NaOH	Formaldehyde	Total			
													S		R	
	70%	1:10 (v/v) 10%	1:500 (v/v)	2:100 (v/v) 2%	1:100 (v/v) 1%	1:500 (v/v)	1:100 (v/v) 1%	1:200 (v/v) 0.5%	1:200 1:500 (v/v)	3%	2%	40%	No.	%	No.	%
Staphylococcus aureus	I	S	I	S	S	S	S	S	S	S	S	S	12	100	-	0
Staphylococcus epidermidis	I	S	I	I	S	S	S	S	S	S	I	S	12	100	-	0
Streptococcus pyogenes A	I	I	S	I	S	S	S	S	S	I	I	S	12	100	-	0
Bacillus subtilis	I	I	I	I	S	S	S	S	S	I	I	S	12	100	-	0
Bacillus cereus	R	I	I	S	S	S	I	S	S	S	S	S	11	93.3	1	6.7
Klebsiella pneumoniae	I	I	I	I	S	I	S	S	S	S	S	S	12	100	-	0
Pseudomonas aeruginosa	I	S	S	I	S	S	S	S	S	S	I	S	12	100	-	0
Proteus mirabilis	I	I	I	S	S	S	S	S	S	S	S	S	12	100	-	0
Escherichia coli	R	R	I	S	S	S	S	S	S	S	S	S	10	86.7	2	13.3
Total (S/R)- Bacteria	7/2	8/1	9/-	9/-	9/-	9/-	9/-	9/-	9/-	9/-	9/-	9/-	105	58.3	3	1.7
F1	I	I	S	I	S	S	S	S	S	S	S	S	12	100	-	0
F2	R	I	I	I	I	S	I	S	I	S	I	S	11	93.3	1	6.7
F3	R	R	I	I	S	S	S	S	S	S	I	S	10	86.7	2	13.3
F4	R	R	S	S	S	S	S	S	S	S	S	S	10	86.7	2	13.3
F5	R	R	S	R	S	S	S	S	S	S	I	S	9	80	3	20
F6	R	R	I	R	S	S	S	S	S	S	S	S	9	80	3	20
Total (S/R)- Fungi	1/5	2/4	6/-	4/2	6/-	6/-	6/-	6/-	6/-	6/-	6/-	6/-	61	33.9	11	6.11

Total		No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
	S	8	53.3	10	66.7	15	100	13	86.7	15	100	15	100	15	100	15	100	15	100	15	100	15	100	15	100	15	100	166	92.2
	R	7	46.7	5	33.3	-	0	2	13.3	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-	0	14	7.8

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Table 6. Percentages of microbial isolates susceptibility to disinfectants used in hatcheries, using Pits method

Microbial isolates	Al-cohol	Sarttol	Iodospec 2.8	Hypochlorite	Virkon-S	$TH4^+$	Intercept	Remas	Remas + $TH4^+$	H_2O_2	NaOH	Formaldehyde	Total			
													S		R	
	70%	1:10 (v/v) 10%	1:500 (v/v)	2:100 (v/v) 2%	1:100 (v/v) 1%	1:500 (v/v)	1:100 (v/v) 1%	1:200 (v/v) 0.5%	1:200 1:500 (v/v)	3%	2%	40%	No.	%	No.	%
Staphylococcus aureus	I	S	R	R	S	S	S	S	S	S	S	S	10	86.7	2	13.3
Staphylococcus epidermidis	I	R	S	I	S	S	S	S	S	S	S	S	11	93.3	1	6.7
Streptococcus pyogenes A	R	S	S	R	S	S	S	S	S	S	S	S	10	86.7	2	13.3
Bacillus subtilis	S	R	S	S	S	S	S	S	S	S	S	S	11	93.3	1	6.7
Bacillus cereus	I	I	I	S	S	S	I	S	S	S	S	S	12	100	-	0
Klebsiella pneumoniae	I	I	I	I	S	S	S	S	S	S	I	S	12	100	-	0
Pseudomonas aeruginosa	S	I	S	I	I	S	S	S	S	S	S	S	12	100	-	0
Proteus mirabilis	S	I	S	R	S	S	S	S	S	S	R	S	10	86.7	2	13.3
Escherichia coli	I	S	I	R	S	S	S	S	S	S	S	S	11	93.3	1	6.7
Total (S/R)- Bacteria	8/1	7/2	9/-	5/4	9/-	9/-	9/-	9/-	9/-	9/-	9/-	9/-	99	55	9	5
F1	R	S	S	S	S	S	S	S	S	S	I	S	11	93.3	1	6.7
F2	R	R	I	R	S	S	S	S	S	S	S	S	9	80	3	20
F3	R	R	S	I	S	S	S	S	S	S	S	S	10	86.7	2	13.3
F4	I	R	S	R	R	R	S	S	S	I	R	S	7	66.7	5	33.3
F5	I	S	R	R	S	S	S	S	S	S	S	S	10	86.7	2	13.3
F6	I	S	S	I	S	S	S	S	S	S	S	S	12	100	-	0
Total (S/R)- Fungi	3/3	3/3	5/1	3/3	6/-	6/-	6/-	6/-	6/-	6/-	5/1	6/-	59	32.8	13	7.2

Total		No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
	S	11	73.3	10	66.7	13	86.7	8	53.3	14	93.3	14	93.3	15	100	15	100	15	100	15	100	13	86.7	15	100	158	87.7
	R	4	26.7	5	33.3	2	13.3	7	46.7	1	6.7	1	6.7	-	0	-	0	-	0	-	0	2	13.3	-	0	22	12.2