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RESEARCH ARTICLE

Evaluating the Bactericidal Activity of Various Disinfectants against *Pseudomonas aeruginosa* Contamination in Broiler Chicken Hatcheries

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ABSTRACT

Hatcheries are hubs for incoming eggs and progeny flock output, making them a crucial component of the poultry production chain. This study involved performing quantitative microbiological tests in a commercial hatchery with numerous compartments, including an egg handling room, cold storeroom, setter room, hatcher room, and chick production hall. There were 150 air samples and 180 surface swabs collected in the incubator before and after disinfection over ten visits, in addition to 250 yolk sac and organ samples taken from late-dead embryos. As a result, surface swabbing could detect higher contamination levels than open-plate methods in all hatchery visits, mainly in handling eggs, cold storage, and hatcher halls. This study examines the presence of *Pseudomonas aeruginosa* strains in hatchery environments and dead embryos. Biochemical and PCR testing were used to identify *P. aeruginosa* using 16SrDNA primers at 956 bp and the *toxA* gene at 396 bp. In hatchery environmental samples, the incidence rate was 10.7%, and in dead embryos, it was 10%; therefore, maintaining good hygiene, especially in hatcheries, is essential for *Pseudomonas* species control. Subsequently, in this study, a virulent strain of *P. aeruginosa* was subjected to an *in vitro* test with 10 disinfectants from six chemical groups. Iodine compounds with phosphoric acids, per-acetic acid, sodium di-chloroisocyanurate, and quaternary ammonium compounds (QACs) with glutaraldehyde compounds showed 100% microbial reduction even in the presence of organic matter with exposure times of 30 min. It was concluded that the most effective and cost-effective way to prevent and control infections is to use a disinfectant with sufficient concentration and exposure time in hatcheries.

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INTRODUCTION

A hatchery is an essential component of poultry production and provides an opportunity to segregate between generations of birds microbiologically; otherwise, control infectious diseases spread via the application of proper biosecurity measures (Abd El-Hack *et al.,* 2023; Yousef *et al.,* 2023). Unfortunately, rather than acting as a firebreak for infectious agents, hatcheries may enable the spread and multiplication of infections among generations of poultry (Wales and Davies, 2020).

Microorganisms can adversely affect hatchability and cause embryonic death in a poultry hatchery environment (Kumar *et al.,* 2012). A hatchery is vulnerable to pathogens that may be carried on or within eggs, on staff, on objects like trolleys and trays, or by air (Mcmullin, 2009). A commercial broiler hatchery was evaluated for hatchery hygiene using a surface swap and an open-plate method; despite the application of various sanitation measures, epidemiological investigations have revealed significant variances in microbial populations over time (Shehata *et al.,* 2019; Sallam *et al.,* 2023). Different bacteria were recovered from dead shell embryos that contaminate hatcheries (Bakheet and Torra, 2020); a particular hatchery-born pathogen is *P. aeruginosa*, known as gram-negative, non-spore-forming rods, motile, and fruity scent is produced because of the production of watery soluble green pigments (Elshafiee *et al.,* 2022; Salem *et al.,* 2024).

Chickens and embryos become infected with *P. aeruginosa*, causing respiratory complications, septicemia, and even death (Shahat *et al.,* 2019). *P. aeruginosa* virulence factors cause microbial invasion, multiplication, dissemination, tissue destruction, and septicemia, with high mortality rates in humans and animals (Poursina *et al.,* 2023). *P. aeruginosa* exotoxin A, a virulence factor that inhibits the biosynthesis of proteins at the polypeptide chain elongation factor 2, can cause severe damage to tissues and organs (Aljebory, 2018). According to Walker *et al.* (2002) and Marouf *et al.* (2023), *Pseudomonas* species are more germicide-resistant than other bacteria. In addition, the concentrations of disinfectants effective against *Salmonella* and *Staphylococcus* species are lower than those effective against *Pseudomonas* species (Gehan, 2009; Beier *et al.,* 2021a, b; Rabie *et al.,* 2023).

Further, appropriate hygienic measures like regular cleaning with wide-spectrum disinfectants are vital to eliminate pathogenic organisms from farms and hinder the re-infection of farm workers and animals (Davies and Wales, 2019). Thus, hatchery sanitation programs require safe and effective disinfectants that inhibit microorganism growth and maintain the hatchability of the eggs treated with them (McElreath, 2019). Additionally, convenient disinfectants that minimize sanitization time and are easy to use are needed (Gehan, 2009).

This study aimed to determine the sanitary situations in a poultry hatchery by bacteriological investigation of hatchery environmental samples during production (before and after disinfection) so they can identify the sources of bacterial contamination and implement appropriate preventive measures. In addition, an *in vitro* trial was conducted to evaluate the effectiveness of 10 disinfectants of different chemical groups at various contact times of 10, 30, and 60 minutes against a virulent strain of *Pseudomonas aeruginosa* isolated from poultry hatcheries were also investigated.

MATERIALS AND METHODS

Study area: The Institutional Animal Care and Use Committee Vet ethically approves this work. CU. IACUC with code "Vet CU 08072023693". The current study was adopted in a broiler hatchery in the El Beheira governorate. A total of 40 incubators and 21 hatchers (multistage) were available, and each incubator held 115200 chicken eggs (Cobb). Each hatcher can hold approximately 38400 eggs. The relative humidity and temperature were 25% and 36.5°C, respectively, for 18 days in the setting period, while the relative humidity and temperature of the hatcher were 30% and 36.0°C, respectively, for the rest of the three days.

Sampling location and time: Samples were taken on ten different morning dates as hatching chicks were processed after the hatchery had been cleaned and disinfected. During each visit, air samples and surface swabs were collected from egg handling rooms, cold storerooms, setter rooms, hatcher rooms, chick production halls, and organ and yolk sac swabs collected after hatching from the dead in-shell embryos. One hundred fifty air samples (inlet of airflow, Air conditioner, air ducts, and fans) were collected for bacterial load detection, and 180 swabs were collected from the surfaces of equipment and facilities (wall, floor, Trolly, Box). The hatchery was checked ten times from September 2020 to October 2021. The first, second, third, fifth, and seventh investigations were conducted before cleaning and disinfection in September 2020, December 2020, January 2021, April 2021, and June 2021, respectively. The fourth, sixth, eighth, ninth, and tenth examinations were performed immediately post cleaning and disinfection (after disinfection) of the hatchery post the elimination of chicks in March 2021, April 2021, July 2021, September 2021, and October 2021, respectively. Also, 200 swabs from yolk sacs of late dead in-shell embryos were taken in sterile plastic containers, preserved in ice boxes, and rapidly transferred to the laboratory for further examinations, and 50 samples were collected from hatched dead chicks (obtained from these visits) at the first two weeks of age.

Examination for the bacterial load of air, the surface of equipment & facilities in the studied hatchery: For air samples, the open-plate method was used; plate count agar (Difco, Detroit, MI) was used for aerobic bacteria. The prepared agar plates were subjected to be uncovered for 10 min at a meter height from the floor surface (Berrang *et al.,* 1995). Once the plates were submitted to the lab, they were kept for 24hrs. at 37ºC. Microbial loads were expressed as colony forming units (CFU) per 10 cm diameter plate, after which they were scored for bacterial multiplication following Table 1.

Surface samples were collected using microbiological swabs. Sterile cotton swabs were rubbed in sterile saline solution on a surface of 16cm^2 using sterile metal templates. The samples were transported in tubes containing 10 mL of sterile saline solution and refrigerated in the lab. A sterile saline solution was used to prepare ten-fold dilutions. Aerobic bacteria were quantified using aliquots of 0.1 mL from the primary dilution and decimal dilutions to plate count agar (Hi Media, India) (Lazarov *et al.,* 2018).

Bacteriological isolation and identification of *P. aeruginosa:* A total of 108 samples were collected from hatchery environment (50 aerial samples were tested using an open-plate method, and 58 samples from hatchery surfaces). Also, 200 swabs from late dead in shell embryos and 50 pooled liver, heart blood, and yolk sac samples from the investigated hatchery were collected for further bacteriological examination. Samples were taken

Table 1: Interpretation reference scored for bacterial growth inside poultry hatchery.

Colony Count	Score	Grade
0-5	0	None or very slight contamination (considered excellent)
6-15	1	Slight contamination (considered good)
16-30	2	Moderate contamination (borderline acceptable)
31-50	3	Significant contamination (poor)
>50	4	heavy contamination (unacceptable)
TNTC	5	very heavy contamination (unacceptable)

from the different hatchery rooms and labeled along the whole hatchery's processing pathway, from the egg handling room to the chick production hall.

Samples were quickly transported to the lab in ice boxes and kept in sterile plastic containers. Then the swab samples were put in nutrient broth and kept at 37ºC for 24 hrs., then sub-cultured onto selective agar (MacConkey and *Pseudomonas* agar base medium with C-N supplement) and incubated at 37ºC for 24 hrs. In addition, subculture onto a nutrient agar plate to observe the *P. aeruginosa* pigmentation (Elshafiee *et al.,* 2022). The supposed colonies were exposed to biochemical examinations such as catalase, oxidase, gelatin liquefaction, methyl red, arginine hydrolysis, indole, and urease (Cheesbrough, 2006).

Molecular identification of *P. aeruginosa:* The DNA was extracted using the QIA amp DNA Mini Kit (Qiagen, Germany) from all biochemically positive *P. aeruginosa* (n=36; 11 environmental samples and 25 dead embryos) maintained overnight in TSB broth. Using primers from Metabion, Germany, PCR was used to identify virulence-associated genes (*toxA*) and Pseudomonas species-specific genes (16S rDNA). The reaction volume (25 µL) contained 12.5 µl of 2x premix Emerald Amp GT PCR master mix (Takara), forward and reversed primer (20 p mol; 1 µl each), PCR grade water (4.5 µl) and template DNA (6 µl). The reaction circumstances were summarized in Table 2 and the PCR products were inserted in electrophoresis in 1.5% agarose gel (AB gene). A 100 bp DNA Ladder (Qiagen, USA) determines the fragment sizes. The gel picture was taken via a documentation system; then, the data was kept via computer software.

In vitro* evaluation of disinfectants against *P. aeruginosa: Using a modified version of the quantitative suspension test established by the British Standard Institute (BSI, 2019), the effect of disinfectants on *P. aeruginosa* strains was evaluated. The manufacturer's instructions state that the disinfectants were made in sterile distilled water (Table 3). *P. aeruginosa* was cultured in TSB broth overnight to produce bacterial suspension, which was then turbidity-adjusted to 0.5 MacFarland standards. In sterile test tubes, ten microliters of disinfectant were mixed with 0.1 mL of adjusted bacterial broth in each tube after being individually evaluated. Control tubes contain only diluted bacterial suspensions. At room temperature (25°C), each disinfectant was incubated with a bacterial suspension for various contact durations of 10, 30, and 60 min. The disinfectant was neutralized using 8 mL of neutralizing broth (Sigma) containing 0.5% sodium thiosulphate. Similar tests were conducted again using organic matter (2% yeast extract). Following a neutralizing period, triplicate subcultures from each set of tubes were placed on cetrimide agar plates, and the plates were left at 37°C overnight to assess the microbial proliferation. The equation log reduction = log10 (initial CFU/ml) = log10 (final CFU/ml) was used to compute the logarithmic reduction of the growth in each solution and the control.

Statistical analysis: The statistical analysis was performed utilizing SPSS software and one-way ANOVA (Park, 2009). All examined means (treatments) were compared using the LSD test, ensuring significance at a probability of *P*<0.05.

RESULTS

Microbiological examination of air and surface samples: Table 4 shows the results of aerobic bacterial air pollution. The mean count from the before disinfection investigation (first, second, third, fifth, and seventh) sampling times, which collected samples during egg incubation, was higher than that of hatchery sampling sites after cleaning and disinfection. The higher mean bacterial count isolation was from the air duct of setter hall 1, measuring; (410 CFU/Ø Petri-dish) whereas in the other sampling sites, hatchery hall 2, vaccine preparation room, and boxes washing room, it was minimal, TBC about (200 CFU/Ø petri-dish). The average bacterial score for every visit was calculated, and we observed that before disinfection, hatchers' visits ranged from slight to moderate contamination. In contrast, the other after-disinfection hatcher's visits show non-contamination (score 0-1), as shown in Fig. 1.

Table 5 shows the surface contamination caused by aerobic bacteria on the equipment and facilities. The most contaminated sites in post-disinfection hatcher's visits were the floor and eggshell in the egg handling room of more than (10^3 CFU /16 cm^2) and the eggshell and floor of the cold storeroom, followed by the floor of the setter and floor of the hatchery and box in the chick production hall. Regardless of the hatchery's cleanliness and sanitization, except the egg handling room, aerobic bacteria after disinfection hatcher's visits were moderate, measuring less than (100 CFU/16 cm^2) at all sampling times, floor of cold storeroom, floor of setter, and box in chick production hall measuring (1.26×10^3, 14.68×10^2, 14.68×10^2, and 0.810×10^3 CFU/16 cm^2) respectively. The remaining sampling sites were contaminated but to a relatively low level, measuring around 10 CFU/16 cm^2 for aerobic bacteria. The microbial contamination of the chick processing room in the two hatchery investigation sets was higher than in the other sampling sites. Surface swabbing detected a higher degree of microbial contamination than the open-plate method in the investigated hatchery along different sampling times, as shown in Fig. 2.

Table 2: Primer sequences for detection of *Pseudomonas* and specific Exotoxin A gene (toxA) of *P. aeruginosa*

Target	gene	Oligonucleotide sequence 5'-3'	Annealing temp. (°C)	Fragment size (bp)	References
Pseudomonas spp.	16S rDNA	GACGGGTGAGTAATGCCTA CACTGGTGTTCCTTCCTATA	20 sec. at 54°C	618	Spilker *et al.* (2004)
P. aeruginosa	16S rDNA	GGGGGATCTTCGGACCTCA TCCTTAGAGTGCCCACCCG	20 secs at 54°C	956	
P. aeruginosa (Virulence gene)	*toxA*	GACAACGCCCTCAGCATCACCAGC CGCTGGCCCATTCGCTCCAGCGCT	55°C 45 sec.-	396	Matar *et al.* (2002)

Table 3: The disinfectants used for in vitro testing efficacy against virulent strains of *P. aeruginosa* isolated from the hatchery. were classified according to their active compounds and concentration recommendations

Active compound	Disinfectant	Producer country	Composition	Concentration
Aldehyde & quaternary ammonium compounds	A	Hungary	Glutraldehyde 150 gm.-QACs100gm- Draymarin Brilliant Blue 0.4gm- Nrinrazine 0.3gm-Azorobin 0.3gm	0.5%
	B	England	Glutraldehyde15% - QACs 10%	0.5%
Acidic compound	C	England	Phosphoric acid 10%- Sulfonic acid 30% - chlorinated phenols 40%	0.4%
	D	Egypt	Orthophosphoric acid 60% - Formic acid 10%	1%
Iodine	E	England	Iodine5% -Phosphoric acid 14%- Alcohol ethoxylate 24%	0.25%
	F	USA	1.75% titratable iodine	0.4%
Peracetic acid-hydrogen peroxide	G	Belgium	The stabilized mixture of Peracetic acid-hydrogen peroxide -organic acids- wetting agents -belong term stabilizer	2%
Ethoxylated Alcohol	H	Egypt	Sodium Hydroxide N-oxide amine Ethoxylated Alcohol	1.7%
Sodium	I	Ireland.	2.5 gm DiChloro Iso Cyanurates 62% in form of tablet	1/15 1
Dichloroisocyanurate15	J	England	Potassium Persulfate 50% + sodium dichloroisocyanurate NaDCC 2.5%	0.5%

Table 4: Mean±SE of bacterial contamination (CFU/ Ø Petri dish) of air samples using the open plate method in the studied poultry hatchery at different sampling times (before disinfection, and h) and sampling sites from different hatchery's process steps

	Sampling site	Before disinfection						After disinfection					
		1st	2nd	3rd	5th	7th	*Mean ±SE	4th	6th	8th	9th	10th	*Mean ±SE
1	Air conditioner of Egg handling hall	3	42	11	1	14	14.2±7.36	1	2	0	2	0	1±0.45
2	Fan of preheating room	4	31	0	9	5	9.8±5.49	3	7	3	2	2	3.4±0.93
3	Air duct of Inovo hall	2	16	2	40	0	12±7.56	3	1	4	25	1	6.8±4.59
4	Air duct of setter hall 1	TNTC	TNTC	30	3	21	410.8±240.58	6	5	0	0	3	2.8±1.24
5	Air duct of setter hall 2	4	9	0	0	4	3.4±1.66	15	18	2	0	0	7±3.92
6	Setter 30 right side	1	0	2	4	8	3±1.41	0	21	6	10	4	8.2±3.95
7	Air duct of hatcher hall 1	42	3	4	0	4	10.6±7.88	7	1	1	0	1	2±1.26
8	Air duct of hatcher hall 2	4	4	TNTC	2	1	202.2±199.45	6	6	3	1	1	3.4±1.12
9	Air duct of hatcher hall 3	1	0	0	23	4	5.6±4.41	0	2	9	50	1	12.4±9.53
10	Hatcher	9	20	7	3	43	16.4±7.22	3	6	12	6	1	5.6±1.86
11	Air duct of Chick production hall	12	19	50	40	11	26.4±7.88	30	20	14	3	6	14.6±4.87
12	Vaccine storeroom	12	10	13	9	3	9.4±1.75	9	4	3	16	8	8±2.30
13	Vaccine preparation room (1 Day old)	50	TNTC	14	4	10	215.6±196.26	0	9	4	10	18	8.2±3.04
14	Air conditioner of cold storeroom	0	0	0	0	2	0.4±0.4	0	0	1	1	1	0.6±0.24
15	Boxes washing room	TNTC	40	45	4	4	218.6±195.54	3	10	4	1	0	3.6±1.75
	**P* value	0.03											

Cfu = colony forming unit, TNTC= Too Numerous to Count >300 CFU, SE= standard error, * *P* value <0.05 is significant between the 2 means values

Table 5: Mean ±SE of bacterial contamination (CFU/16 cm2) of walls, floor, and other surfaces in the poultry hatchery was investigated at different sampling times (before disinfection and After disinfection) and sampling sites based on the different hatchery process stages

Sampling site		Before disinfection						After disinfection					
Subunit/Surface samples		1th	2th	3th	5th	7th	Mean$\times10^{2}$ ±SE $\times10^{2}$	4th	6th	8th	9th	10th	Mean $\times10^{2}$ ±SE $\times10^{2}$
Egg handling room	Floor	20	20	3×10^{3}	–	5×10^{2}	13±7.4	100	0	__	__	0	33.3±33.3
	Wall	30	0	0	–	12	10.5±7.09	0	10	120	__	0	32.5±29.26
	Eggshell	3.2×10^{2}	4.2×10^{2}	40×10^{2}	–	6.4	13.5±8.87	__	30×10^{2}	1.2×10^{2}	__	0	10.33±9.83
Cold storeroom	Floor	33×10^{2}	30×10^{2}	40	300	9×10^{2}	14.7±6.44	2.9×10^{3}	1.1×10^{3}	0	0.2×10^{3}	10	8.4±5.69
	Wall	0	100	0	80	10	38 ±21.54	0	30	90	10	0	26± 16.91
	Eggshell	90	31×10^{2}	–	10.2×10^{2}	$\pm3.9\times10^{2}$	11.2± 6.54	20	6×10^{2}	10×10^{2}	10	0	3.26 ±2.03
Setter hall	Floor of setter hall	60	20×10^{2}	–	100	50	5.2±4.82	5×10^{2}	7×10^{2}	__	__	0	4 ±2.08
	Wall of Setter Hall	0	0	–	0	6×10^{2}	150 ±150	0	10	__	__	0	3.3 ±3.3
	Trolly	10	–	–	3.8×10^{2}	$\pm3.6\times10^{2}$	250±120.14	300	20×10^{2}	__	20	0	5.80±4.78
Hatcher halls	Floor of hatcher 5	80×10^{2}	55×10^{2}	80×10^{2}	6.8×10^{2}	150	5.9±120.69	20	0	4.2×10^{2}	20	0	92± 82.12
	Floor of hatcher 18	50	2×10^{3}	100	0.8×10^{3}	0.39×10^{3}	7±3.6		10	10×10^{2}	10	0	2.55±248.34
Chick production hall	Floor	6×10^{2}	210	8×10^{2}	4×10^{2}	4.5×10^{2}	4.9± 99.07	10	0	40	50	0	20 ±10.49
	Wall	10	10	0	100	0	24 ± 19.13	–	230	80	3.2×10^{2}	0	157.5±72.15
	Box	2.5×10^{3}	0.45×10^{3}	1.4×10^{3}	0.29×10^{3}	0.31×10^{3}	8±3.2	–	10	20×10^{2}	130	0	5.35 ±4.89
	Roll of Counting machine	7.5×10^{2}	–	10	9×10^{2}		5.5±275.10	0	–	–	0	0	0±0

Cfu= colony forming unit, SE= standard error, * P value <0.05 is significant between the 2 means values

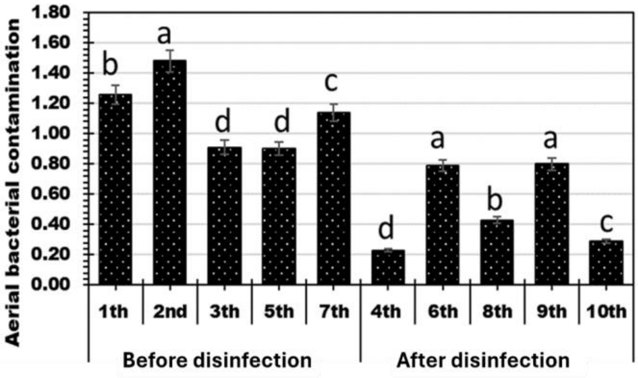

Fig. 1: Total mean score of aerial bacterial contamination of poultry hatchery in different sampling times.

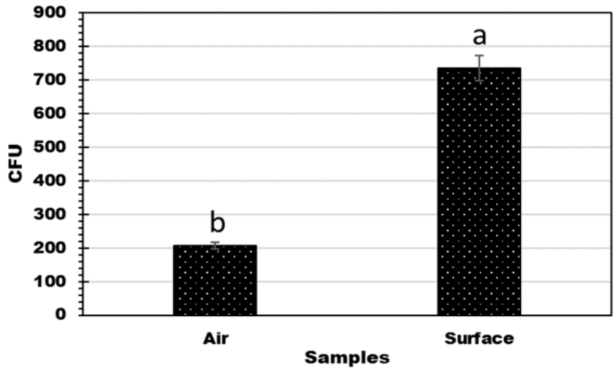

Fig. 2: Mean ± SE of bacterial contamination in poultry hatchery environment (from air samples and surfaces) at different sampling times.

Table 6: Incidence of identified *P. aeruginosa* isolated along the study period from the from the investigated hatcher environmental samples (air and surface; n=103)

Nature of sample		Before disinfection		After disinfection		
		No. of samples	No. of positive (%)	No. of samples	No. of positive (%)	Total
Air samples	Air conditioner of Egg handling hall	5	0(0)	5	0(0)	2/50 (4%)
	Hatcher air samples	5	0(0)	5	1(20)	
	Setter air samples	5	0(0)	5	0(0)	
	Air duct of Chick production hall	5	0(0)	5	0(0)	
	Box storeroom	5	0(0)	5	1(20)	
Surfaces Swabs	The floor of the egg handling room	4	0(0)	-	-	9/53 (16.98%)
	cold storeroom	-	-	3	1(33.3)	
	The floor of the setter hall	5	1(20)	5	0(0)	
	Exhaust setter	3	0(0)	3	1(33.3)	
	Floor of hatcher	5	2(40)	5	1(20)	
	floor of Chick production hall	5	2(40)	5	0(0)	
	Vacuum of Chick production hall	5	0(0)	5	1(20)	
		Total				11/103 (10.67%)

The chi-square statistic between environmental samples (air and water) is 4.54, and the *p*-value is .033, Significant at ($p<0.05$).

Table 7: The incidence of *P. aeruginosa* isolated from chicken in Elbehira governorate hatchery

Type of samples	Site of collection	No examined samples	No positive isolates	%
Dead In Shell Embryos (swabs from liver, heart blood, and yolk sacs)	Before disinfection hatchery investigations	100	7	7
	After disinfection hatchery investigations	100	8	8
Young chicks (1-2 weeks of chick incubation)	At farm level	50	10	20
Total	–	250	25	10

***P. aeruginosa* isolation from hatcheries and molecular detection:** The prevalence of *P. aeruginosa* in environmental samples was 10.7%, with eleven isolates found out of 103 total samples, as shown in Table 6. Morphologically, *P. aeruginosa* appears as pale colonies of non-lactose fermenter on MacConkey agar. The organism is characterized by its greenish diffusible pigment and fruity smell. Biochemically, it shows positive results for oxidase, catalase, urea, citrate utilization, and gelatin hydrolysis but negative for indole, methyl red, and Voges Proskauer.

The highest contamination of *P. aeruginosa* was found on surfaces in the hatchery (16.9%), particularly in the cold storeroom and exhaust setter. Air samples taken after cleaning and disinfection had lower contamination levels (4%). *P. aeruginosa* is not found in earlier processing rooms, such as the egg handling and setter rooms. Out of 250 samples taken from all hatchery visits (200 from dead in-shell embryos and 50 from freshly dead birds), only 25 samples were *P. aeruginosa* positive (10%); this information is presented in Table 7. The freshly dead birds showed postmortem lesions that appeared in shape with varied degrees of congestion, omphalitis, severe pneumonic lungs, air saculitis, kidney lesions with distended ureters with ureates, enteritis, and unabsorbed yolk sac (in some cases greenish discoloration of yolk sac has been noticed) (Fig. 3).

In vitro* efficacy of disinfectants on *P. aeruginosa: As indicated in Table 8, the outcomes demonstrated that all disinfectants were effective against the tested *P. aeruginosa* isolates. In the presence of organic matter, the disinfectants C and B showed 100% reduction after 30 min, disinfectant (F) showed 100% at 60 min, while disinfectant (H) failed to eliminate contamination post 60 min. While in the absence of organic matter, Except for disinfectant (B); show 100% reduction after 30 and 60 minutes, and disinfectant (H) which resulted in 98.6% reduction after 60 minutes, the rest disinfectants demonstrated superior effectiveness in removing the microbes (100% reduction after 10 min).

Table 8: The Efficacy of ten different disinfectants belong to six different chemical groups at various contact times of 10, 30, and 60 minutes against a virulent strain of *Pseudomonas aeruginosa* (titer of 1.5×10^8/ml) isolated from studied poultry hatchery

The used disinfectants		Concentration	Presence organic matter						Absence organic matter					
			10 min		30 min		60 min		10 min		30 min		60 min	
			Count	R %	Count	R %	Count	R %	Count	R %	Count	R %	Count	R %
1	A	0.5%	–	100	–	100	–	100	–	100	–	100	–	100
2	B	0.5%	3×10^6	98	–	100	–	100	5.3×10^5	99.6	–	100	–	100
3	C	0.4%	2×10^6	98.6	–	100	–	100	–	100	–	100	–	100
4	D	1%	–	100	–	100	–	100	–	100	–	100	–	100
5	E	0.25%	–	100	–	100	–	100	–	100	–	100	–	100
6	F	0.4%	9×10^6	94	3.5×10^6	97.6	–	100	–	100	–	100	–	100
7	G	2%	–	100	–	100	–	100	–	100	–	100	–	100
8	H	1.7%	3×10^7	80	22×10^6	85.33	45×10^5	97	21×10^6	86	12×10^6	92	2×10^6	98.66
9	I	1/151	–	100	–	100	–	100	–	100	–	100	–	100
10	J	0.5%	–	100	–	100	–	100	–	100	–	100	–	100

R %: reduction percent

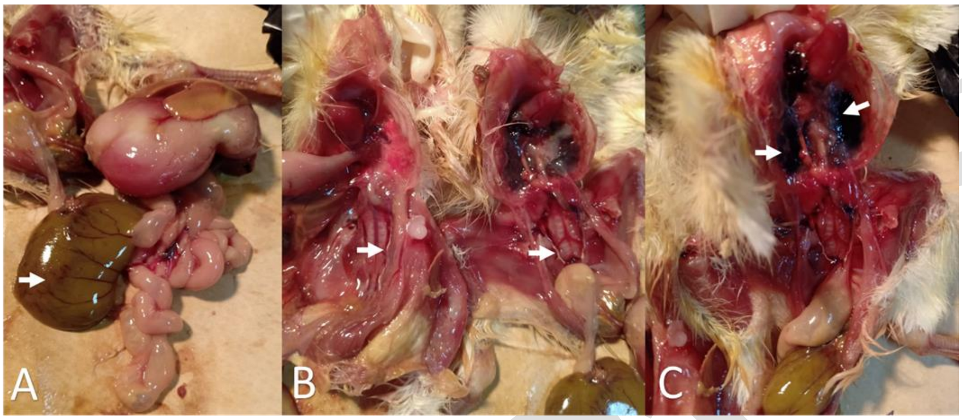

Fig. 3: The postmortem examination of freshly dead chicks (obtained from the investigated hatcheries) showed A: arrow refers to unabsorbed yolk sac with greenish discoloration with distended intestine with a mild degree of enteritis; B: arrows refer to pathological changes in the kidney with distended ureters with ureates; C: arrows refer to severe degree of lung congestion and pneumonia

DISCUSSION

Different pathogens transmitted due to hatcheries contamination, they resulted in financial losses in poultry industry (Abd El-Hack *et al.*, 2022a, b; El-Saadony *et al.*, 2022; Khalifa *et al.*, 2023). Several authors have suggested different ways to maintain biosecurity and hygiene in hatcheries (Bennett, 2017).

According to the results, surface swabbing identified a higher level of microbial contamination in the examined hatchery than the open-plate method. Compared to other frequently used rooms, the air duct of the setter hall had the highest level of aerobic bacterial contamination (410 CFU), followed by the air duct of the hatcher hall (202 CFU). Additionally, there are high counts of >200 CFU in the vaccine preparation and box washing rooms. The active hatchers had high concentrations of coliform, fungi, and aerobic bacteria in the air, measuring over 300 CFU /63.6 cm^2 (Kim and Kim, 2010).

The egg sorting room had a moderate level of contamination. However, this was not the case in other spaces, like the setter room, candling-transfer room, and chick counting room, where contamination was minimal and measured less than 10 CFU /63.6 cm^2 for aerobic bacteria. According to Fig. 1, the second hatchery's investigation time had the highest mean score for bacterial contamination (1.48), while the fourth hatchery's investigation time had the lowest (0.23) also, the chick processing room had microbial contamination levels over 100 CFU /16 cm^2, the highest of all the areas sampled. According to the current study, surfaces had higher bacterial count than samples collected from air. The results concur with McElreath's, (2019) discovery that although the open-plate method is quick, cheap, and easy to use, it can only identify living microorganisms. In contrast, Kim and Kim (2010) discovered that the levels of bacterial contamination on equipment and facilities were similar to those in the air. However, there were higher levels on surfaces in certain areas of the hatchery. *P. aeruginosa* is a serious hatchery-borne disease that can infect and colonize fertilized and embryonated eggs, causing in-shell death to embryos and chicks after hatching (Dinev *et al.*, 2013).

Among the most toxic virulent factors of pathogenic *P. aeruginosa* is exotoxin A, which inhibits eukaryotic protein synthesis and promotes tissue necrosis (Eman *et al.*, 2017). *P. aeruginosa* was isolated from environmental samples at a rate of 10.67%, with only two out of 50 air samples and 9 out of 53 surface samples being positive. Similarly, to Gehan, (2009), in current study it was found that some bacterial strains could not be isolated through the open-plate method but could be detected by surface swabs. The incidence of *P. aeruginosa* surface isolation differs significantly between samples from air and surface swabs.

The isolation of *P. aeruginosa* virulence strains from the environment is associated with the isolation from dead embryos, as environmental pollution causes a significant issue in chicken hatcheries as it affects recently hatched chickens, resulting in an elevated rate of embryo death (Dinev *et al.*, 2013). The incidence of *P. aeruginosa isolates* was 7.5% from the yolk sac of dead in-shell embryos and 20% from slow chicken. The current results are like those of Bakheet *et al.* (2017) and Shahat *et al.* (2019), who isolated *P. aeruginosa* from chicks with 18.6% and 20% incidence rates, respectively. Also, Hassan, (2013) recovered *P. aeruginosa* from freshly dead broiler chickens and one-day-old chicks in incidences of 25.3 and 10%, respectively.

Elsayed *et al.* (2016) isolated *P. aeruginosa* with a percentage of 22.9% and a high isolation rate from dead-in-shell embryos yolk sac (52%). Still, the liver samples 2- 40 days old, diseased, and freshly dead were (12%). These findings were validated by Kebede, (2010), who demonstrated that experimentally infection with *P. aeruginosa* during the hatching period from the environment or by infiltrating the eggshell of the embryo was the major cause of the high mortality rate in unhatched chicken and early chicks, which led to death.

In this study, the observed postmortem lesions in the freshly dead birds were omphalitis septicemic, including congestion of subcutaneous tissues and muscles with increased size and congestion of the parenchymatous organs and in some freshly dead birds, unabsorbed yolk sac, enteritis, air saculitis, and pneumonia were recorded. These findings concur with Walker *et al.* (2002) recorded in freshly dead SPF chicks due to *P. aeruginosa* experimental infection. Hatcheries are vulnerable to infectious agents that may enter on or within eggs, on staff, on objects like trolleys and trays, or as airborne contaminants (Marouf *et al.*, 2023; Saad *et al.*, 2023; Elsayed *et al.*, 2014).

The current research showed that compounds containing glutaraldehyde, such as disinfectant A, efficiently eliminated *P. aeruginosa* even with organic matter. According to Jiang *et al.* (2018), QACs eliminated 1% of the bacteria after 30 & 60 minutes, and disinfectant B demonstrated effectiveness after 30 minutes. The results supported earlier studies' results (Gehan, 2009). When no organic matter is present, the phenolic compound disinfectant C effectively eliminates germs within 10 minutes. It takes 30 minutes to be completely effective if organic matter is present. These findings align with those made by Mclaren *et al.* (2011), who found that phenolic disinfectants tend to be consistent in efficacy.

Even in organic matter, *P. aeruginosa* was quickly eradicated by disinfectant (D). This result is in line with the efficiency of organic acids as bactericidal agents, which are preferable to antibiotics because they do not result in the emergence of bacterial strains resistant to the drugs (Novickij *et al.*, 2019). Gram-negative bacteria, including *Pseudomonas* species, have long been known to be resistant to low pH. According to the current study, disinfectant (E) is more efficient than disinfectant (F) because it achieved a 100% reduction after only 10 minutes. Hydrogen peroxide and PAA are combined to create a disinfectant (G), effective against microbes even when organic matter is present. This concurs with the suggestion of Rodgers *et al.* (2001) to use H_2O_2 as a disinfectant in hatcheries. However, PAA was not recommended as the preferred sanitizing agent for chicken processing equipment by Rossoni and Gaylarde (2000).

A disinfectant (H) with a 0.5% concentration is specifically used to clean eggshells. However, in the current study, even after 60 minutes of contact time, both with and without organic matter, this concentration could not wholly eradicate *P. aeruginosa*. Only 98% and 97% of *P. aeruginosa* were reduced, respectively. This study discovered that with and without organic matter, sodium di-chloroisocyanurate compounds effectively eliminated microbes. The findings support earlier research that demonstrated sodium hypochlorite successfully lowered *P. aeruginosa* counts (Krause *et al.*, 2019). This contrasts with a prior study by Gharieb *et al.* (2022), which discovered that 3% sodium hypochlorite could only reduce the microbe count by 89% even after 60 minutes of contact with organic matter.

Overall, the current findings supported those of Moustafa *et al.* (2009), who claimed that QAC, glutaraldehyde, and per-acetic acid had demonstrated their efficacy in preventing the contamination of poultry hatcheries and could be used as effective formaldehyde substitutes. In a lab setting, Glutaraldehyde 1% and hydrogen peroxide 3% were effective in a short time on specifically *P. aeruginosa*. in the absence and presence of organic matter compared to other disinfectants, according to Gharieb *et al.* (2022).

Conclusion: Based on virulent genes and isolation from the hatchery environment, *P. aeruginosa* was a critical pathogen causing the deaths of newly hatched chicks during the first two weeks of their rearing age. Therefore, maintaining good hygiene, especially in hatcheries, is essential for the control of *Pseudomonas* spp. Iodine compound with phosphoric acids (disinfectant, E), per-acetic acid (disinfectant, G), sodium dichloroisocyanurate (disinfectant I; disinfectant J), and QAC with glutaraldehyde compound (disinfectant, A) all show *in vitro* 100% microbial reduction even in the presence of organic matter with exposure times of 30 min. Further studies are recommended to evaluate these disinfectants in hatcheries in the field.

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