



A Study on Bacterial Contamination of dead in shell Chicken Embryos and Culled One Day Chicks

M.M. Amer^{1*}, Kh. M. Elbayoumi², Zeinab M.S. Amin Girh², Hoda M. Mekky² and Nagwa S. Rabie²

¹Department of Poultry Diseases., Faculty of Veterinary Medicine, Cairo University, Egypt.

² Department of Poultry Diseases, Veterinary Research Division, National Research Centre, Egypt.

ABSTRACT

A total of 360 samples (160 dead in shell and 200 day old chicks) were collected from 10 commercial hatcheries were subjected to microbiological analyses for detection of bacterial contamination. A total bacterial species were isolated from dead in shell and one day old chicks in rate of 21.67% (78/360) including 23.12% from dead in shell and 20.5% from one day old chick. Isolation of 9 bacterial species including 2 gram positive Streptococcus and Staphylococcus and 7 gram negative including Salmonella spp., E.coli, Citrobacter spp., Proteus spp., Campylobacter spp., Pseudomonas spp. and Klebsiella spp.. The isolated bacterial spp. has been reported to be associated with infection of yolk sac and death of chicken embryos.

The gram positive isolates were 1 Streptococcus (S) and 17 Staphylococcus (Staph) 14 coagulase negative (CoNS) including 4 S. epidermidis, 1 S. haemolyticus, 6 S. xylosum and 3 S. sciuri). and 3 S. aureus coagulase positive (CoPS). The Gram negative isolates were 4 Salmonella Enteritidis (S. Enteritidis), 28 Escherichia coli (E. coli), 4 Citrobacter (C.frundii), 9 Proteus (P.vulgaris), 2 Campylobacter (C.jejuni) and 7 Pseudomonas (P.aeruginosa) and 4 Klebsiella (K.pneumonia). Four S. Enteritidis 1.11% (one isolate was obtained from dead in shell and other 3 isolates from chicks). The most isolated strains were E. coli in rate of 9.4% and 6.5 out of dead in shell and culled chicks with total rate of 7.78%. Streptococcus was isolated only from culled 1 day old chicks. Staph.aureus were isolated from both dead in shell and culled chick.

E.coli isolates showed sensitivity rate 52.1, 39.3, 32.1, 28.6, 60.1, 78.5, 64.3 to Cefotaxime, Enrofloxacin, Oxytetracycline, Oxacillin, Kanamycin, Calindamycin and Gentamycin; respectively. Isolates of S. enteritidis, P.vulgaris, C.frundii, K. pneumonia, C.jejuni, Staph.aureus, Streptococcus and S. sciuri are sensitive to Cefotaxime, Enrofloxacin, Kanamycin and Gentamycin with rate 50- 100%. P.aeruginosa was generally resistant to all tested antibacterial, while S. haemolyticus and S.xylosum are sensitive only to Oxytetracycline. Most of tested organisms are resistant to Oxytetracycline and Oxacillin. Trimethoprim+Sulphamethoxazole still effective on S. enteritidis, P.vulgaris, C.frundii, S. haemolyticus, S. sciuri and Streptococcus.

Therefore we recommended the application of restricted hatchery sanitation together with use of suitable disinfectant to minimize the risk of bacterial contamination and the possible related effect on hatchability and health of produced one day old chicks. Control usage of antibacterial agents to get good effect and avoid drug resistance.

Key Words: Dead in shell, Day old-chicks, Bacterial contamination, Hatchery, AntibioGram

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Corresponding author M.M. Amer

Address: Department of Poultry Diseases., Faculty of Veterinary Medicine, Cairo University, Egypt.

e-mail ✉ profdramer@yahoo.com

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INTRODUCTION

Hygiene is an important link, not only in terms of health and production performance but also in terms of food safety [1]. Hatchery can be an important source of spread of a variety of pathogenic microorganisms that can cause diseases problems in poultry farm[2],[3].

Hatchery waste: eggshell debris and fluff, infertile eggs, dead embryos, culled chicks, egg fluids, as well as wastewater from cleaning and disinfecting equipment and processing areas. Campylobacteriosis and Salmonellosis are two zoonotic infections that can be transmitted to human by contact with either the poultry itself or their eggs [4].

Eggs can be contaminated by coming in contact with contaminants like dust or droppings in the nest or on the litter floor [5] but in fact, most of Salmonellosis originates from a feeding gradient and can cause gastrointestinal illness in human. E. coli are found naturally in the gastrointestinal tract of all warm blooded

animals. Both yolk sac infection (YSI) and dead-in-shell occur in chicks a few days before hatching, which result in decreased hatchability and increased mortality. Members of the Enterobacteriaceae family, such as E. coli, Salmonella spp. and Klebsiella spp., along with other bacteria such as Staphylococcus spp., Pseudomonas and Clostridia spp., and also Aspergillus fumigatus are common causes of YSI and dead-in-shell [6]. [7] studied on bacteriology, pathology and antimicrobial resistance of YSI in broiler chicks. [8] isolated Klebsiella spp. in 15% of bacterial from dead-in-shell ostrich embryos of ostrich, Staphylococcus spp. (25%), E. coli (10%) and Proteus spp. (5%). Of 79 pooled samples containing 632 dead-in-shell chicken embryos, cultured from two hatcheries in Nigeria, 13 isolates were Klebsiella spp. [8], [9] detected Gram-negative bacteria among canaries with clinical disease 6 of 88 isolates belonged to Klebsiella spp. in Suleimani district and reported K. pneumonia as 12% of bacterial isolates from yolk sac samples.

The most well-known bacterial contaminant chicken eggs are E. coli and Salmonella [10]. S. enterica is worldwide in both the environment and in warm blooded animals. Salmonella usually exists as normal flora for chickens. Bacteria have been isolated from chicken eggs. These including Protus, A. hydrophilia, Campylobacter, staphylococcus and streptococcus have been isolated from chicken eggs [11]. During the period of 39 months (May, 2002 to August, 2005), 330 samples from yolk and visceral organs were taken from chicks suffered from omphalitis. Various bacteria isolated were Escherichia coli (47.93%), Proteus (5.87%), mixed infection (3.59%), Streptococci (2.89%), Klebsiella (1.79%), Salmonella (0.5%), Staphylococci (0.5%), Pseudomonas (0.5%), Pasteurella (0.5%) and Yarseinia (0.5%) [12].

Miss using of antimicrobials in poultry production leads to an increase in resistance of pathogenic and commensals [13] and [14]. The aim of this study was to evaluate the hygienic conditions of commercial chicken

hatchery by detection of bacterial contamination and bacterial species variety of microorganisms in incubator wastes (dead in shell embryo's and culled day old chicks) as well as sensitivity test of bacterial isolates using the standard disk diffusion method to determine the current situation of their susceptibility to available antibacterial agents.

MATERIAL AND METHODS

Samples:

A total of 360 samples (160 dead in shell and 200 day old chicks) collected from 10 commercial hatcheries. Sixteen dead in shell embryos and 20 one day old chicks showing leg deformity or omphalitis were collected at the end of the hatching from each of different hatcheries. The collected samples were kept separately in sterile container and transfers quickly to the laboratory for microbiological evaluation and analyses.

The Culture media:

Fluid media (nutrient broth and selenite-F-broth media) and solid agar media including MacConkey agar media for Enterobacteriaceae, Nutrient and Blood agar media for Gram- positive bacteria as well as Skirrow's, Butzler, and thioglycolate media for Campylobacter and Nutrient agar medium for P. aeruginosa. were prepared and used according to [15], [16] and [17].

Isolation of organisms:

From the sample collected egg with fully developed dead embryos, the unabsorbed yolk was used. Outer shell was washed thoroughly with a disinfectant (2% tincture iodine) and after dryness they were mopped with alcohol. by 70% alcohol and broken with sterile blade, with using a sterile Pasteur pipette, 0.1ml of the unabsorbed yolk was inoculated separately on bacterial media.

One day old chicks were separately opened and samples from liver and non-absorbed yolk sac were inoculated used bacterial media. Culture media plates were labeled and incubated at the recommended temperature, time and precaution then examined for bacterial growth according to [18] and [15].

Identification of Isolates:

The obtained isolates were identified and characterized on the basis of the results obtained from their colonial, morphological, cultural and biochemical properties [16],[17]. Biochemical characterization was done on the basis API identification kits (API System, France) were analyzed using Bergey's manual of systematic bacteriology [19]. The results of these investigations are shown in table (1).

Antibiogram:

In vitro sensitivity test for bacterial isolates was determined using the standard disk diffusion method [20] using Mueller Hinton agar (Oxoid) plates and antibiotic discs of 8 available antibacterial agents. the strains were evaluated as sensitive, intermediate sensitivity and resistant by measuring the inhibition zones diameters around the antibiotic discs [21]. The tested antimicrobial agents and their concentrations

(µg) were as follows: Cefatoxaime 30 µg/ml (CTX), Enrofloxacin 5 µg/ml (ENR), Oxytetracycline 30 µg/ml (T30) , Oxacillin 30 µg/ml (OX), Kanamycin 30 µg/ml (K), Calindamycin 2 µg/ml (DA), Trimethoprime+Sulphamethexole 2.25/23.75 µg/ml (SXT) and Gentamycin 10 µg/ml (CN). The obtained results are shown in table (2 and 3).

RESULTS AND DISCUSSION

Hen's eggs can be contaminated or infected horizontally (Through the shell) or vertically (transovarially) that makes them a potential source of pathogen participating in the etiology of diseases in poultry or food borne diseases in human [10] , [22]. Omphalitis or YSI is a common cause of death in chicks during the first week of life and most common with artificially hatched chicks. It is a bacterial infection of the yolk sac. Various bacteria may be involved in yolk sack infection including *E.coli*, *Staphylococci*, *Proteus*, *Clostridia*, *fecalis* and *Pseudomonas* [10] , [12]. Most chicks with a yolk sac infection die within 24 hours of hatching, peaking at 5 to 7 days.

A total of 9 bacterial genera of gram positive (2 out of 9) and gram negative were isolated from all the examined samples with different percentage (Table 1). Regarding isolates it was related to comes in accordance of [23]. It was found that mostly isolated bacterial contaminant is *E.coli* in both dead in shell and one day old chicks which was 9.4% and 6.5% respectively when compared with other contaminating microorganism this may be due to its virulence factors including [24] ;[25].

The most isolated strains were *E.coli* in total rate of 7.78%. Organism motility have an important role in avian pathogenic *E.coli* virulence including egg penetration.[26] Seven gram negative (Table 1) including *Salmonella* spp., *E.coli*, *Citrobacter* spp., *Proteus* spp., *Campylobacter* spp., *Pseudomonas* spp and *Klebsiella* spp. had been isolated from examend samples. Same Gram-negative bacteria such as *Citrobacter* spp., *Klebsiella* spp., *Proteus* spp., *Campylobacter* spp, and *Pseudomonas* spp, and *Salmonella* spp. have also been found in eggs with intact or damaged shells with low proportion which seem to be in agreement with those reported by [22] and [27] who found that *Escherichia* was present on most eggs examined but in small numbers; while, *Pseudomonas*, *Proteus*, and *Serratia* were occasionally recovered. Moreover, [28] correlated the presence of *E. coli*, *Proteus*, *Pseudomonas* and *Aerobacter* with different percentage in tested eggs. [29] isolated *Citrobacter* , *Escherichia*, *Klebsiella* and *Salmonella* from the shells of eggs examined. The isolated bacterial species and isolates were reported by many authors [10] , [30] , [12], [8],[9]. Regarding identified bacterial isolates including the gram positive isolates were 1 *Streptococcus* and 17 *Staph.* out of them 14 coagulase negative (CoNS) including 4 *S. epidermidis* , 1 *S. haemolyticus*, 6 *S. xylosus* and 3 *S. scuiri*. and 3 *S. auras* coagulase positive (CoPS) [31] and [32].

Table(1):Bacterial isolates obtained from examined samples.

Bacterial spp.	Bacterial isolates	dead in shell (160)		1 day old chicks (200)		Total 360	
		No	%	No	%	No	%
Salmonella	<i>S. Enteritidis</i>	3	1.9	1	0.5	4	1.11
E.coli	<i>E.coli</i>	15	9.4	13	6.5	28	7.78
Protus	<i>P.vulgaris</i>	5	3.1	4	2.0	9	2.50
Citrobacter	<i>C.frundii</i>	1	0.6	3	1.5	4	1.11
<i>Klebsiella</i>	<i>K. pneumonia</i>	2	1.2	2	1.0	4	1.11
<i>Pseudomonas</i>	<i>P. aeruginosa</i>	2	1.2	5	2.5	7	1.67
<i>Campylobacter</i>	<i>C.jejuni</i>	1	0.6	1	0.5	2	0.56
Staphylococcus .	<i>Staph. aureus</i>	2	1.2	1	0.5	3	0.83
	<i>S. epidermidis</i>	2	1.2	2	1.0	4	1.11
	<i>s. xylosus</i>	2	1.2	4	2.0	6	1.67
	<i>S. haemolyticus</i>	1	0.6	-	-	1	0.28
	<i>S. scuiri</i>	1	0.6	2	1.0	3	0.83
Streptococcus	<i>Streptococcus</i>	-	-	1	0.5	1	0.28
un typed	un typed	-	-	2	1.0	2	0.56
	Total number bacterial isolates	37	23.12	41	20.5	78	21.67

The Gram negative isolates were 4 Salmonella Enteritidis (*S. Enteritidis*), 2 Escherchia *E.coli*, 4 *C.frundi*, 9 *P.vulgaris*, 2 *C.jejuni* and 7 *P.aeruginosa* and 4 *K.pneumonia*. [33] and [34].

The isolated bacterial spp. has been reported to be associated with infection of yolk sac and death of chicken embryos. The most common of these are Staphylococcus, Streptococcus, Klebsiella, E. coli, Enterobacter, Citrobacter, Proteus, Salmonella and Pseudomonas spp. [35], [36], [37], [38], [39] and [40]. Dead-in-shell embryos and culled chicks are common in chicken hatcheries with high bacterial contamination and it is important to dispose them hygienically to prevent source of spread to the poultry. Hatchery can be an important source of spread of a variety of pathogenic microorganisms that can cause diseases problems in poultry farm [2], [3].

Results of table (3) revealed that bacterial isolate under Egyptian field in 2016 have variable antibiotic sensitivity profile, as *S. enteritidis* was 100% sensitive to Cefotaxime, Enrofloxacin and Gentamycin, while *E.coli* has variable sensitivity varies from 14.3% to Trimethoprim+Sulphamethoxole to 64.3% sensitivity to Calindamycin this was matched with [41] and [42] who report variable sensitivity to different antibacterial medications for both *E.coli* and *Salmonella* spp..

P.vulgaris found to be 100% sensitive to Cefotaxime and lowest in sensitivity (33.3%) to Calindamycin, *C. Frundii* was 100% sensitive to Calindamycin and lowest sensitivity to Oxacillin. *K.pneumonia* was 100% sensitive to all used antibiotic except calindamycin which was 25% sensitivity, *P.aeruginosa* be resistant to both Oxytetracycline and Oxacillin and with variable sensitivity varied from 28.6% to Cefotaxime, Enrofloxacin and kanamycin reach 85.7% to Gentamycin. *C.jejuni* found to be 100% sensitive to Cefotaxime, Enrofloxacin and kanamycin while found to be resistant to Oxytetracycline, Oxacillin and Trimethoprim+Sulphamethoxole. *Staph.aureus* found to be 100% sensitive to Cefotaxime and Enrofloxacin while *S. epidermidis* found to be 100% sensitive only to Calindamycin, *S. xylosus* found to be 100% sensitive only to Oxytetracycline while *S. haemolyticus* found to be 100% sensitive to both Oxytetracycline and Trimethoprim+Sulphamethoxole, *S. scuiri* found to be 100% sensitive to all tested antibiotics except Oxytetracycline, Oxacillin and Cefotaxime and finally *Streptococcus* found to be 100% sensitive to all tested antibiotics except Oxytetracycline, Oxacillin and Kanamycin which were resistant. Emerging of resistant bacterial strains to antibacterial agents maybe due to several conditions such as hazzard used of antibiotics in field, lack of new commercial antibiotic development in market by pharmaceutical companies [43].

Table (2): Results of antibiogram of bacterial isolated from dead in shell and culled chicks.

Isolate	No	Antibacterial																							
		CTX			ENR			T30			OX			K			DA			CN			SXT		
		S	I	R	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R
<i>S. enteritidis</i>	4	4			4			1		3	2	1	1	3	2		2	1	1	4			3		1
<i>E. coli</i>	28	16	7	5	11	8	9	9	10	11	8	14	6	17	4	5	22	4	2	18	5	5	4	13	11
<i>P. vulgaris</i>	9	9			7	1	1	6	1	3	4	2	3	8		1	3	2	4	7			6	2	1
<i>C. frundii</i>	4	2	1	1	3	1		2		2	1	2	1	3	1		4			3		1	2	1	1
<i>K. pneumonia</i>	4	4			4			2	1	1	1	1	3	4			2		2	4				3	1
<i>P. aeruginosa</i>	7	2	1	4	2		5		7			7	2		5	5	1	1	6		1		2	5	
<i>C. jejuni</i>	2	2			2				2			2	2				1		1	1		1			2
<i>Staph. aureus</i>	3	3			3			2		2		2	1	1				2	1	1					2
<i>S. epidermidis</i>	4			3		1	2			3		4	1		3	4					4				4
<i>S. xylosus</i>	6		3	3	2		4		1	5		6	4		2		4	2	2	1	3			2	4
<i>S. haemolyticus</i>	1			1			1	1				1			1			1						1	
<i>S. scuiri</i>	3	2		1	3			1		2	1		2	3			3			3				3	
<i>Streptococcus</i>	1	1			1				1			1			1			1						1	

S: Sensitive

CTX; Cefatoxaime 30 µg/ml.

OX: Oxacillin 30 µg/ml.

CN; Gentamycin 10 µg/ml (CN).

I: Intermediate

ENR: Enrofloxacin 5 µg/ml.

K: Kanamycin 30 µg/ml.

SXT: Trimethoprim+Sulphamethoxole 2.25/23.75 µg/ml.

R: Resistant

T30: Oxytetracycline 30 µg/ml.

DA: Calindamycin 2 µg/ml.



Table (3): Sensitivity pattern of bacterial isolates to antibacterial agents.

Isolate	No	Antibacterial															
		CTX		ENR		T30		OX		K		DA		CN		STX	
		S	%	S	%	S	%	S	%	S	%	S	%	S	%	S	%
<i>S. enteritidis</i>	4	4	100	4	100	1	25	2	50	3	75	2	50	4	100	3	75
<i>E. coli</i>	28	16	52.1	11	39.3	9	32.1	8	28.6	17	60.1	22	78.5	18	64.3	4	14.3
<i>P. vulgaris</i>	9	9	100	7	77.8	6	66.7	4	44.4	8	88.9	3	33.3	7	77.8	6	66.7
<i>C. frundii</i>	4	2	50	3	75	2	50	1	25	3	75	4	100	3	75	2	50
<i>K. pneumonia</i>	4	4	100	4	100	2	50	1	25	4	100	2	25	4	100	0	00
<i>P. aeruginosa</i>	7	2	28.6	2	28.6	0	00	0	00	2	28.6	5	71.4	6	85.7	0	00
<i>C. jejuni</i>	2	2	100	2	100	0	00	0	00	2	100	1	50	1	50	0	00
<i>Staph. aureus</i>	3	3	100	3	100	2	66.7	0	00	1	33.3	0	00	1	33.3	0	00
<i>S. epidermidis</i>	4	0	00	0	00	0	00	0	00	1	25	4	100	0	00	0	00
<i>S. xyloso</i>	6	0	00	2	33.3	0	00	0	00	4	66.7	0	00	2	33.3	0	00
<i>S. haemolyticus</i>	1	0	00	0	00	1	100	0	00	0	00	0	00	0	00	1	100
<i>S. scuri</i>	3	2	66.7	3	100	1	33.3	1	33.3	3	100	3	100	3	100	3	100
<i>Streptococcus</i>	1	1	100	1	100	0	00	0	00	0	00	1	100	1	100	1	100

S: Number of Sensitive isolates.

CTX; Cefatoxaime 30 µg/ml. ENR; Enrofloxacin 5 µg/ml.

T30: Oxytetracycline 30 µg/ml.

OX: Oxacillin 30 µg/ml.

K: Kanamycin 30 µg/ml.

DA: Calindamycin 2 µg/ml.

CN; Gentamycin 10 µg/ml (CN).

SXT: Trimethoprim+Sulphamethexole 2.25/23.75 µg/ml.

General speaking, *E. coli* isolates showed of sensitivity rate 52.1, 39.3, 32.1, 28.6, 60.1, 78.5, 64.3 to Cefatoxaime, Enrofloxacin, Oxytetracycline, Oxacillin, Kanamycin, Calindamycin and Gentamycin; respectively. Isolates of *S. enteritidis*, *P. vulgaris*, *C. frundii*, *K. pneumonia*, *C. jejuni*, *Staph. aureus*, *Streptococcus* and *S. scuri* are sensitive to Cefatoxaime, Enrofloxacin, Kanamycin and Gentamycin with rate 50- 100%. *P. aeruginosa* was generally resistant to all tested antibacterial, while *S. haemolyticus* and *S. xyloso* are sensitive only to Oxytetracycline. Most of tested organisms are resistant to Oxytetracycline and

Oxacillin. Trimethoprim+Sulphamethexole still effective on *S. enteritidis*, *P. vulgaris*, *C. frundii*, *S. haemolyticus*, *S. scuri* and *Streptococcus*. Our results indicate the usage of antibacterial agents must be good controlled to get good effect and avoid drug resistance

Therefore we recommended the application of restricted hatchery sanitation together with using suitable disinfectant to minimize the risk of bacterial contamination and the possible related effect on hatchability and health of produced one day old chicks. Usage of antibacterial agents must be used under control and according to sensitivity test.

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