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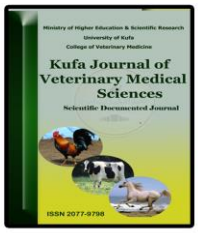

Isolation of Some Bacterial Contamination of Egg Shell and yolk in Najaf governante

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Abstract

A total of 60 eggs collected from different sources including small farms (15 eggs), large farms (15 eggs), road side vendors (15 eggs) and supermarkets (15 eggs) were cultured for the bacteria in Najaf governante, Bacteria on egg shells have been implicated as a source of bacterial contamination and some of them *Proteus spp* has been implicated in food poisoning, *E.coli* and *Salmonella spp* have been found in egg shell and yolk .The family *Enterobacteriaceae* were obtained with a higher proportion from shell membrane (54.4%) than from yolk samples (45.6%). There was no significant difference (P< 0.05) in the number of isolates between various sources indicating the contamination at farm level.

Key words: eggs, *E. coli*, *Salmonella*, contamination.

عزل بعض الملوثات البكتيرية من قشرة ومح بيض الدجاج في محافظة النجف الأشرف

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الخلاصة:

جمعت حوالي 60 بيضة دجاج من مصادر مختلفة شملت المزارع الصغيرة (15 بيضة), المزارع الكبيرة (15 بيضة), البائعة في جوانب الطريق(15 بيضة) ومحلات سوبرماركت(15 بيضة) في محافظة النجف الأشرف كدراسة التلوث البكتيري الذي يصيب قشرة البيض ومحها حيث اثبتت النتائج نسبة تلوث قشرة البيضة(54.4%) والتي كانتاكثر تلوثا في التسمم الغذائي الجرثومي مقارنة مع مح البيضة (45.6%) ببكتريا الجراثيم المعوية وخاصة الاشريشيا القولونية وكذلك تم التعرف على بكتريا السالمونيلا والمتقلبات ولايوجد فرق معنوي في نسبة العزل في المصادر المختلفة.

Introduction:

Eggs are laid by female birds which have been eaten by humans for thousands of years.[1] Chicken eggs consists of a protective eggshell, albumen (egg white), and vitellus (egg yolk) contained within various thin membranes. Popular choices for egg consumption are chicken, duck, quail, roe, and caviar, but the egg most often consumed by humans is the chicken egg by a wide margin [2].

Egg yolks and whole eggs store significant amounts of protein and choline, [3], [4] and are widely used in cookery. Due to their protein content, the United States Department of Agriculture (USDA) categorizes eggs as Meats within the Food Guide Pyramid. [2] Despite the nutritional value of eggs, there are some potential health issues arising from egg quality, storage, and individual allergies.

Bacteria on egg shells have been implicated as a source of bacterial contamination of broken-out eggs[5] Bacteria on shells may also under certain conditions, penetrate through the shells into the interior and cause spoilage[6] In the present the main goal of this study is to isolate and applied of antibiotic sensitivity against bacterial which contaminated yolk and egg shell in Najaf governante .

Material and Methods

1. Materials

1.1. Instruments and equipments

The instruments and equipments used in this work were listed in table (1) below:

Table (1): Instruments and equipments with their remarkers

No.	Instrument / equipment	Manufacturer / state
1	water bath	Gemmy industrial corp., Taiwan
2	Centrifuge	Elite – Medichem (India)

3	pH – meter	Hanna, Romania
4	Sensitive electric balance	Denver instrument, Germany
5	Distillator	AlabTech, Korea
6	Incubator	Binder, Germany
7	Electric oven	Binder, Germany
8	Autoclave	Monarch MSI, Germany
9	Hot plate with magnetic stirrer	Remi Equipments, India
10	Compound light microscope	Motic, Malaysia
11	Millipore filters	Merk, Germany
12	Laminar – flow cabinet	GallenKamp / England
13	Digital camera	Sony / Japan

1.2. Chemical and biological materials

The chemical and biological materials used in this work were listed in table (2) below:

Table (2): Chemical and biological materials

NO.	Name of Material	Manufacturers name
1	Ethanol	Tedia , USA
2	Urea solution	Oxoid, UK
3	Sodium chloride	BDH, England
4	Agar – Agar	BioLife, Italy
5	Pepton	Oxoid, UK
6	Trypton	Difco, USA
7	Yest extract	Difco, USA

1.3. Culture media

Culture media used in this work were listed in table (3) below:

Table (3): Culture media

NO.	Medium	Manufacturer (origin)
1	Mackonkey agar	Himedia, India
2	Salmonella – Shigella agar	Himedia, India
3	Birllent green agar	Himedia, India
4	Nutrient broth	Himedia, India
5	Triple Sugar Iron agar	Himedia, India
6	Urea base agar	Himedia, India
7	Brain heart infusion agar	Difco, USA
8	Brain heart infusion broth	Difco, USA
9	Muller – Hinton agar	Difco, USA

1.5. Antimicrobials

For the detection of antimicrobials discs bacterial isolates from egg shell and yolk , many types of antibiotic discs were chosen which are supplement Bioanalys Co. (Turkey) are listed in table (5) .

Table (4): Type of Antimicrobials and their concentration

NO.	Antimicrobials	Abbreviation	Concentration (mg /ml)
1	Ampicillin	AM	10
2	Amikacin	AK	30
3	Chloramphenicol	C	30

4	Erythromycin	E	15
5	Gentamicin	CN	10
6	Ciprofloxacin	CIP	10

2. Methods

2.1. Preparation of cultural media

2.1.1. Ready prepared media

Media used in this study (Tab. 3) were prepared according to the manufacturer's instructions on their containers and sterilized according to the suitable method.

2.1.2. Laboratory prepared media

2. Pepton water medium : It was prepared by dissolving 5 gm peptone and 5 gm sodium chloride in 1000 ml of D.W ., after that the pH was adjusted to 7.5 [7] .

3. Urea agar medium

This medium was prepared by dissolving 24 gm from urea agar base in 950 ml of D.W., and then the pH was adjusted to 6.8 and sterilized by autoclaving. 50 ml of 40 % urea solution which was sterilized previously by filtration through Millipore filters (0.45 µm) was added to the sterilized medium. The Urea agar medium was distributed in 5 ml amount in sterile tubes, and allowed to solidify in a slop form [7].

5. Maintenance medium

This medium is composed of nutrient agar as basal medium supplement with 15 % glycerol, after autoclaving, distributed in 5 ml amount in sterile tubes, and allowed to solidify in a slop form, then kept at 4 C˚ until used [8].

2.4. Samples collection

A total of 60 egg samples (60 small farm-house , large house , road vendor , supermarket) were collected from Najaf governorate . All egg samples were placed in sterile plastic bags, labeled and transported to the laboratory in portable coolers at 4 C˚, to be processed within 3 – 4 hours of collection. This study was conducted through a period extended from December 2013 to February 2014.

2.5. Isolation and identification of some bacterial contamination and (Egg rotting):

According to modified procedure by [9] The outer surface of the eggs was disinfected by wiping with surgical gauze soaked in 5% iodine solution and opened around the air sac area. After draining the

albumin, the yolk with intact vitelline membrane was transferred to a sterile beaker. The shell membranes were peeled off the shell and collected aseptically. Yolk contents and egg shell added into dish containing 20 ml of peptone water broth. The mixture was incubated at 37 C˚ for overnight. . Subsequently, a loopful of broth was streaked on surface of S.S, Mackonkey agar, Salmonella-shigella agar and Berillent green agar and then incubated at 37C˚ for 24 hrs.

The biochemical characters of non – lactose fermenting bacteria was determined using TSI agar and Urease test as in Figure (1).

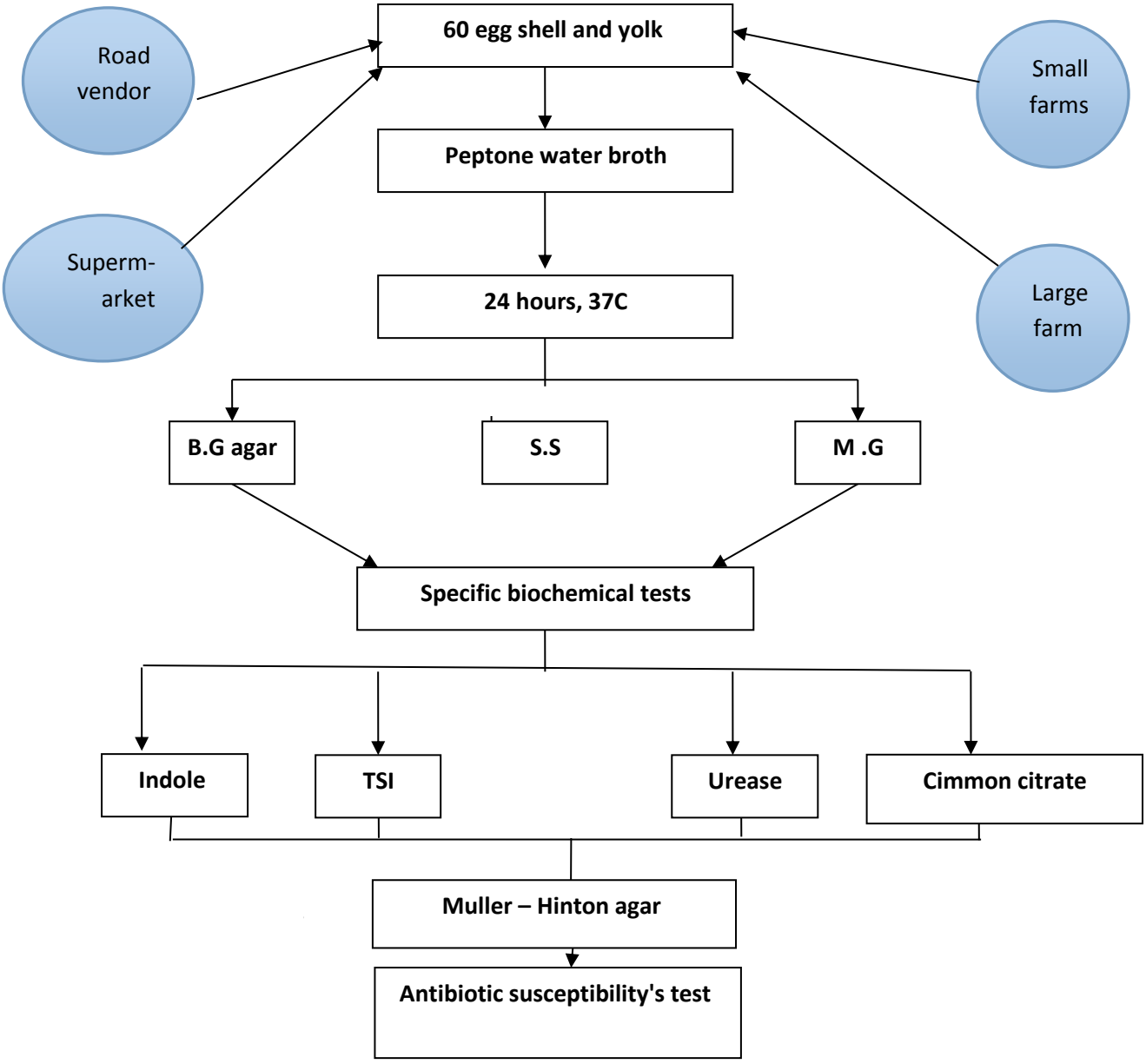


Figure (1): Isolation and identification of egg rotting bacterial contamination

2.6. Identification of bacterial profile

2.6.1. Cultural characteristic

The growing colonies on S.S, MG and B.G agar were examined by naked eye concerning the colour, shape and size of it.

2.6.2. Specific biochemical tests

1. Triple Sugar Iron (TSI) medium

A loopful inoculum was streaked over the surface of the slop of TSI medium and stabbed into the butt, incubated at 37 C˚ for 24 hrs. The results were interpreted in table (6) according to [8].

Table (6): Interpretation of bacterial growth on TSI medium

Area of reaction	Result	Fermentation
Butt	Yellow	Gloucose Fermentation
	Red or unchanged	No – gloucose Fermentation
	Black	Formation of hydrogen sulfide
	Bubbles or cracks	Gas formation
Slant surface	Yellow	Lactose and/or sucrose Fermentation
	Red or unchanged	No- lactose and sucrose Fermentation

2. Urease test

The agar slant surface was streaked and incubated at 37 C˚ for 24 hrs. and examined at intervals. If the reaction is positive, splitting of urea liberates ammonia which changes the color of

phenol red to rose pink, and later to deep cerise. The reaction is often apparent after 2- 4 hrs [7].

2.7. Antimicrobial susceptibility testing

The antimicrobial susceptibility testing was done by the agar discs diffusion method as that described by Bioanalyse® sensitivity discs Co. as follows:

1. Preparation of bacterial inoculum
   - At least 3-5 well isolated colonies were suspended in 4-5 ml Brain heart infusion.
   - The broth culture was incubated at 37 C° for 8 hrs.
   - The turbidity of the actively growing broth culture was adjusted with sterile broth to obtain turbidity optically comparable to the 0.5 McFarland standards.
2. Streaking of test plates
   - A sterile cotton swab was dipped into the adjusted suspension, then rotated several times firmly on the inside wall of the tube above the fluid level to remove excess inoculum from the swab.
   - The entire surface of a Mueller – Hinton agar plate was streaked with the dipping swab. The streaking was repeated two more times and the plate was rotated approximately 60° each time to ensure an even distribution of inoculum. As a final step the rim of the agar was swabbed.
   - The Petri dishes allowed drying for 15 – 20 minute at room temperature before disc application.
3. Application of discs
   - The antimicrobial discs were dispensed onto the surface of the inoculated agar plate. Each disc was pressed gently to ensure complete contact with the agar surface by helping flamed and cooled forceps.
   - The plates were inverted and placed in an incubator at 37 C° for overnight.
   - After overnight incubation clear inhibition zones must be calculated by using transparent ruler. The diameter of the inhibition zone was calculated from the underside of the dish.
   - The results were compared with the minimum inhibition

diameter of the Bioanalys Co. (Turkey).

2.8. Statistical analysis

In order to determine the statistical significance among different variables, SPSS program (Statistical Program for Social Sciences) version 11 was used. Chi – square was applied to test the obtained results.

Results and Discussion

4.1. Prevalence of Egg rotting isolates according to diagnostic tests

The results of the conventional methods carried out on egg isolation showed that all tests were able to detect *E. coli, Salmonella spp, Proteus Spp* after 24 hrs of incubation of the pre enrichment broth. The colonies of *E. coli* on Maconkey agar were small , circular , pink color lactose fermentation colonies (Fig 2)[10].The colonies of *Salmonella spp* on S.S agar were circular , smooth , convex and pale in color with black center on S.S agar (Fig 3) this methods was used by procedure 7 and was perfectly matched with a researcher[11] who used the S-S agar medium method to isolated Salmonelosis from egg hatching .

Figure (2): Colonies of *E.coli* on Mackonkey agar

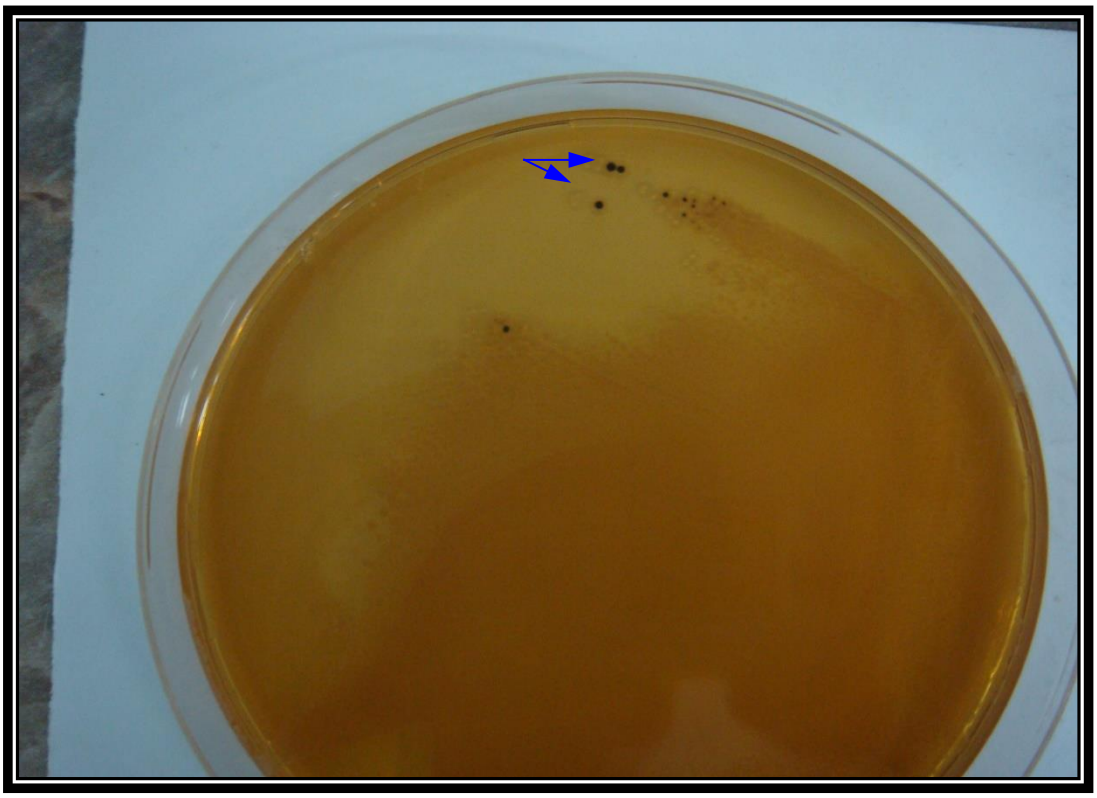


Figure (3): Colonies of *Salmonella spp* on S.S agar

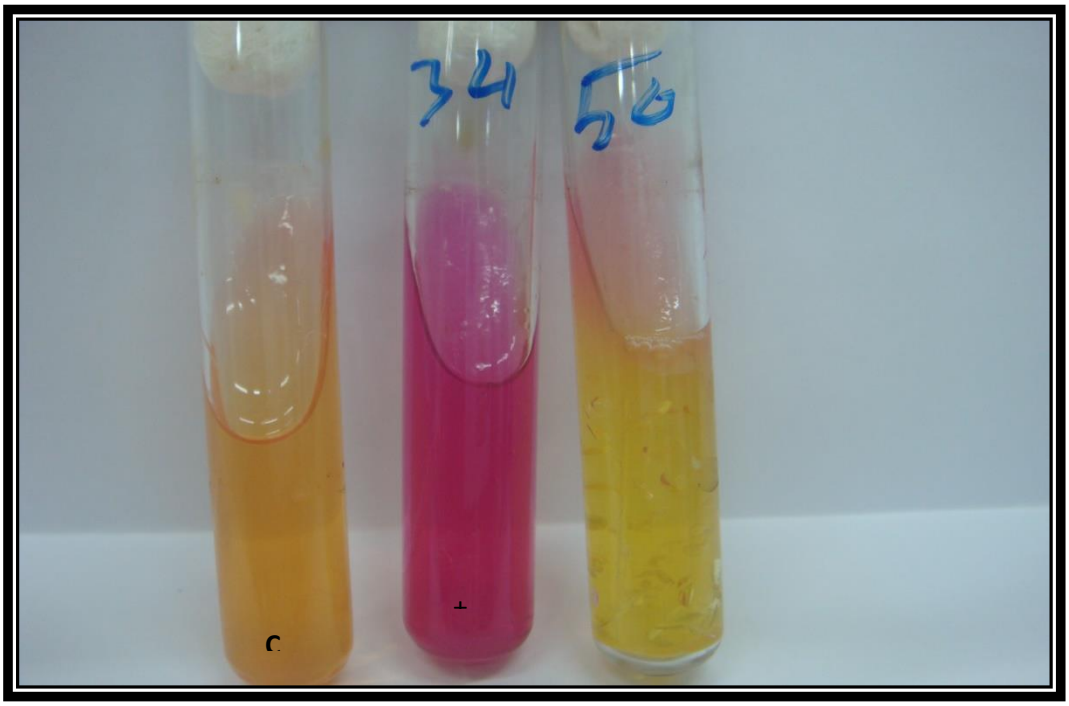
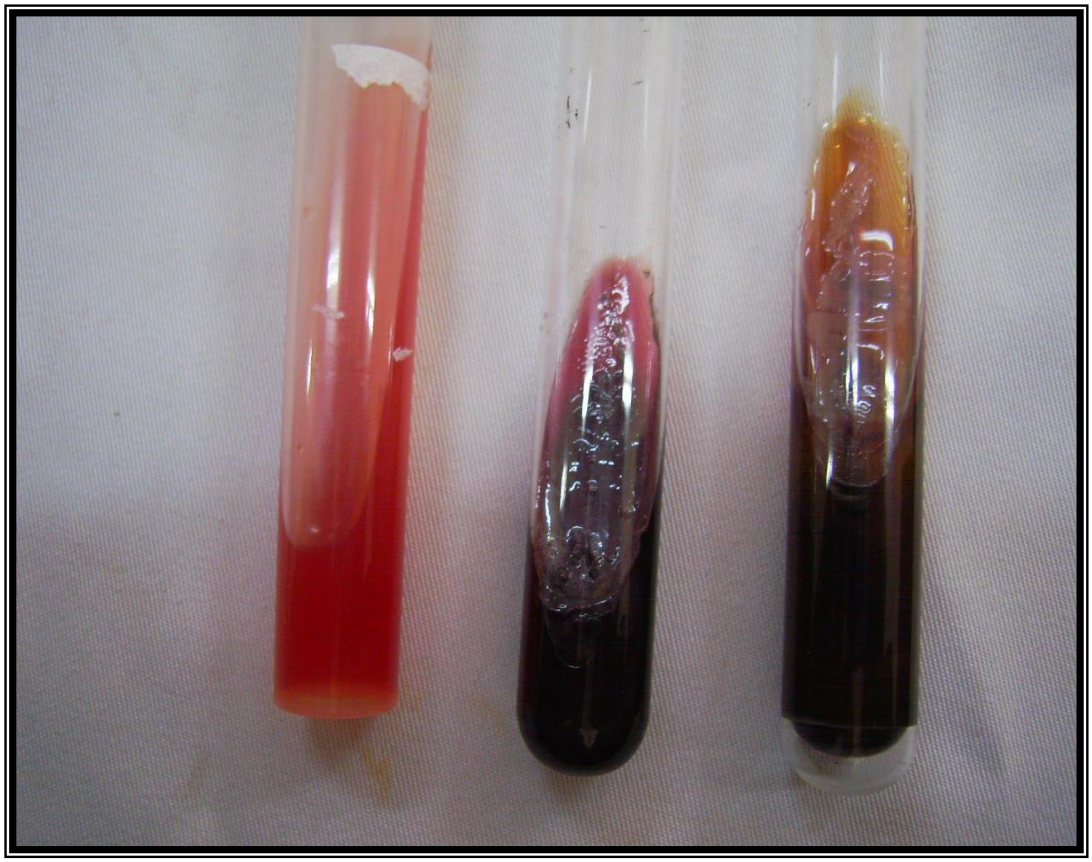


Figure (4): Urease test



C: control, +: positive, - : incomplete positive
Figure (4): TSI test

Depending on the number of *Salmonella* isolates detected by culture methods, the differences were found in the frequency of *Salmonella* prevalence in egg shell and yolk .In egg shell *Salmonella* prevalence was more frequently observed in large farm (5 %) for both egg shell and egg yolk. On the other hand there are high frequency of *E.coli* occurrence in large farm (13.33%) followed by small farm (11.66%) and road vendor(10%) and less occurrence in supermarket (3.3%) egg shell, was also observed in egg yolk diagnosis, they are high frequency of *Proteus spp* as (10 %) followed by (6.66%) and (3.33%) in road vendor and small farm respectively, but less frequency of *proteus spp* prevalence was in supermarket as (1.66 %) in egg shell.(Table 4 – 1) .

Table (5): Percentage of different positive test of bacterial profile causes egg rotting in different places (Najaf government):

Egg samples (60)				
	Small farm	Large farm	Road vendor	Supermarket

Type of bacterial isolate	Shell 15	Yolk 15	Shell 15	Yolk 15	Shell 15	Yolk 15	Shell 15	Yolk 15
E.coli	7	1	8	3	6	2	2	0
Salmonella spp	0	0	3	3	1	0	0	0
Proteus spp	2	3	6	2	4	3	1	0
X^2(calculated) = 12.798, Degrees of freedom= 12, Yates' chi-square= 5.291 Non-significant								

5. *In vitro* antimicrobial susceptibility testing

By using disc diffusion method 57 isolates (27 isolates for *E.coli*, 21 isolates for *proteus spp* and 7 isolates for *Salmonella spp* for each species) of bacterial isolates were tested for their antimicrobial susceptibility toward 6 antimicrobials.

All tested isolates from eggshell and egg yolk showed high susceptibility (100 %) toward ciprofloxacin , and Chloromphenicol , and high resistance (100 %) against Ampecillin .On the other hand , these isolates revealed varying percentage of susceptibility and resistance toward other antibiotics (Fig 5 , Table 5) .

We have proved practically that the Ciprofloxacin and chloromphenicol used to enteric bacteria due to the strength of those antibiotics depending on DNA gyrase degradation for Ciprofloxacin and protein inhibition for chloromphenicol[12].

In the present study, the most frequently isolated are *Salmonella* and *E. coli*, which return to family *Enterobacteriaceae* and the result resemble to [13] was obtained similar results when they analysed the frequency with which bacteria occurred in eggs depending on their source.

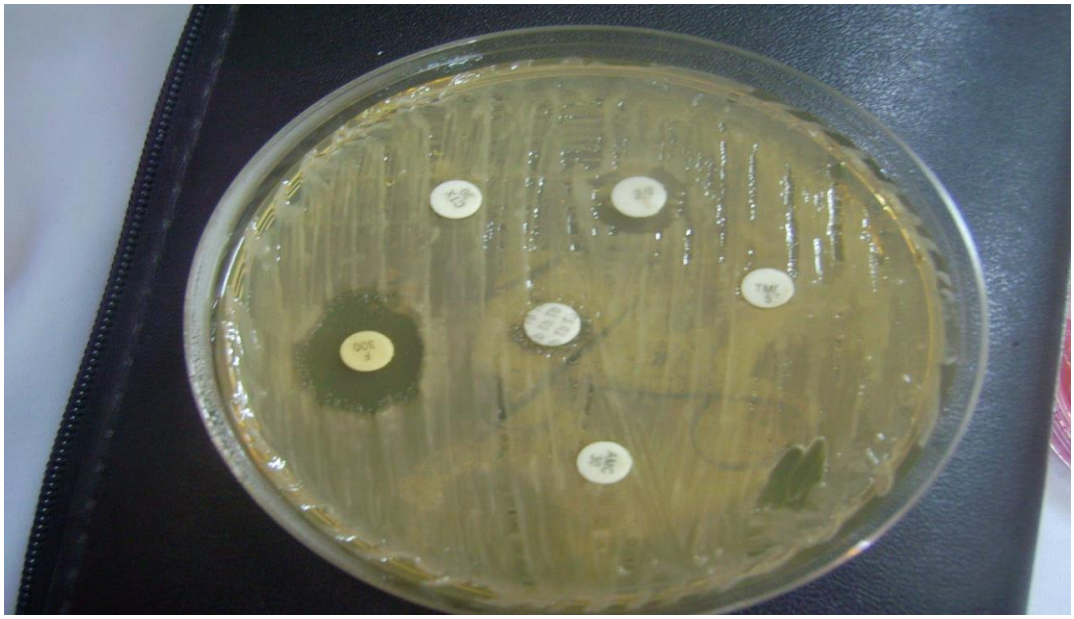


Figure (4-5) Antibiotic susceptibility of some bacterial isolates causing egg rotting

Table (4-2) : percentage of resistance of some bacterial isolates causing egg rotting against different antimicrobials .

N	Antimicrobial	*E.coli* 29 isolate		*Salmonella spp* 7 isolate		*Proteus spp* 21 isolate	
		NO. of Resistance Isolates	(%)	NO. of Resistance Isolates	(%)	NO. Resistance Isolates	%
1	Ampicillin	29	100%	7	100%	21	100%
2	Amikacin	19	56.5%	6	85.7%	8	38%
3	Chloramphenicol	0	0%	0	0%	0%	0%
4	Erythromycin	4	13.7%	2	28.5%	7	33.3%
5	Gentamicin	2	6.8%	5	71.4%	11	52.3%
	Ciprofloxacin	0	0%	0	0%	0%	0%

X^2(calculated) = 13 degree of freedom=6

P value= 0.031 significant (P>0.05)

Table (4 – 6) : Antimicrobials resistance bacterial profile causes egg rotting

Conclusion: The present study found that Gram-negative bacteria were isolated less often from yolks, Among the most frequently isolated *Enterobacteriaceae* bacteria on eggshell surfaces were E. coli, Proteus spp and Salmonella spp .The conditions applied, i.e. the temperature and duration of storage, were not found to significantly influence the prevalence of particular species of Gram-negative bacteria in the eggs. However, based on the analysis of Salmonella contamination of eggs depending on where they were purchased, it can be concluded that the system of housing of the hens affects the risk of infection with these pathogens.

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