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STUDY OF BACTERIAL CONTAMINATION OF DEFECTED EGGSHELLS AND EGG CONTENTS IN BAGHDAD CITY

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Abstract

The main aim of the current research is to focus the light on some bacterial contamination on cracked eggshell and egg content plus studying the sensitivity of these bacterial isolates to antibiotics. For this purpose, a total of 50 eggs were collected from the markets in Baghdad city (Iraq) and examined for bacterial isolation from cracked eggshells and from the egg contents. The bacterial isolates were cultured and purified then transferred to a specific media to study its sensitivity against antibiotics. The results revealed that bacteria isolated from both cracked eggshells (46%) and egg contents (44%). The bacteria isolated include *E. coli, Staphylococcus, Enterococcus faecalis, Enterobacter* and *Pseudomonas.* The results of antibiotic sensitivity test showed that all bacteria are resistant to Bacitracin. It can be concluded that the consumers should get ride of cracked eggshells and never used for human consumption.

Key words: Bacterial contamination, eggshells, egg contents.

Introduction

Zohair and Amer, (2015) studied the contamination of eggshell and egg contents in cracked and uncracked eggs in Yemen. They found that the eggs were contaminated with *E. coli, Klebsiella spp., Proteus* spp., *Campylobacter* spp. *and Pseudomonas* spp., *Staphylococcus aureus* and *Streptococcus* at percentages less than 10% each.

In Jordan, bacteria were isolated from table eggs which include *Staphylococcus*, *Streptococcus* spp., *Pseudomonas* spp., *Proteus* spp., *Klebsiella* spp., *Escherichia coli*, *Bacillus* spp., *Listeria monocytogenes* and *Salmonella* (Al Momani *et al.*, 2018).

Staphylococcus, *Salmonella* and *Escherichia coli* were isolated from the contaminated table eggs (Chaemsanit *et al*., 2015). However, in Egypt, an attempt was made to isolate *E.coli*, yeasts and moulds from 170 table eggs. They did not detect *Salmonella* and *Pseudomonas* (EL-KHOLY *et al*., 2014).

Another study revealed isolation of bacteria from table eggs which involves *E. coli*, *Providencia rettgeri*, *Providencia alkalifaciens*, *Citrobacter freundii*, *Salmonella* spp. and *Enterobacter aerogenes* (Fardous and Shamsuzzaman, 2015).

In Iraq, a study was done in Al-Najaf Province to evaluate the effect of food poisoning bacteria in the eggshell which focused on 3 main bacteria *Proteus*, *E.coli* and *Salmonella* spp. (Hamzah, 2014). In Baghdad (Iraq) there were two studies on table eggs to study the penetrative effect of bacteria into the eggshell as well as eggs content (Abdul-Al-Mounam *et al*., 2012) and (Mounam *et al*., 2019).

Also (Al-Shadeedi, 2018) in Baghdad, Iraq studied the total bacterial count from the eggs containers and found high contamination of bacteria.

Material and Methods

A total of (50) random brown egg samples (5 eggs for each sample) were collected from different markets in Baghdad city. The samples were collected in sterile plastic container and transported aseptically to the laboratory of the Unit of Zoonotic Disease in the College of Veterinary Medicine, University of Baghdad. Eggs were cleaned and transferred into a clean bag divided into two parts homogenized shells and mixed internal egg

Table 1: Frequency distribution of isolated microorganism from examined cracked eggshells (50 eggs).

Isolated organism	No. of isolates	%
E. coli	9	18%
Staphylococcus aureus	9	18%
Enterococcus faecalis	3	6%
Enterobacter	2	4%
Total	23	46%

Table 2: Frequency distribution of isolated microorganism from examined eggs' contents (50 eggs).

Isolated organism	No. of isolates	%
E. coli	3	6%
Staphylococcus aureus	7	14%
Enterococcus faecalis	4	8%
Enterobacter	1	2%
Pseudomonas spp.	4	14%
Total	19	44%

contents homogenized by using a stomacher for (3-5) minutes in sterile plastic bags. A (1)ml or (1)gm of samples was inoculated to (9) ml of nutrient broth and incubated at 37°C for (24-48) hrs, the culture from each tube was inoculated on TSB agar for (*Vibrio* and *Enterococcus*) on MacCkonky agar and SS agar (for *E.coli*, *Salmonella*, *Enterobacter*, *Klebsiella* and *Proteus*), Mannitol salt agar and Staph1 10 (for *Staphylococcus aureus*), Nutrient agar, Pseudomonas agar (for *Pseudomonas*), Muller Hinton agar for antibiotic sensitivity test, tetrathionate broth and brain heart infusion broth was used also. All these media were prepared according to the instructions of manufacturers company.

Table 3: Mean zone of inhibition of antibiotics (mm).

Antibiotic	*E. coli*	*Staphylococcus aureus*	*Entero-bacter*	*Entero-coccus*
Ceftriaxon (CRO)30	R	7	20	10
Trimetheprime (TMP)5	R	14	23	30
Gentamycin (CN)30	12	22	19	10
Doxycyclin	15	28	11	22
Azithromycine (AZM)15	10	25	22	20
Carbencillin (Py)100	R	21	15	15
Tetracycline (TC)30	13	25	18	20
Amoxicillin clavulanic acid (Amc 30)	R	15	6	15
Bactircin (B)10mg	R	R	R	12

Isolates	Resistance	Sensitive
Staphylococcus aureus	B	CRO, TMP, DXO, CN, AZM, PY, TC, MC
E. coli	CRO, TMP, AMC, PY, B	CN, DOX, AZM, TC
Enterobacter	B	CRO, TMP, DXO, CN, AZM, PY, TC, AMC
Enterococcus	-	CRO, TMP, DXO, CN, AZM, PY, TC, AMC, B

Isolation and identification: single colony from bacterial growth was taken and according to their characteristic properties were examined microscopically before being isolated in pure culture on agar slop for further identification. The suspected cultured colonies were identified biochemically (TSI, Lactose fermentation, Urease test, Indole, Motility H_2S, Citrate utilization test) were done on isolated bacteria to confirm diagnosis according to (Quine *et al.,* 2004).

Antimicrobial Susceptibility Test

The Kirby-Bauer method is based on the diffusion of antibiotics impregnated in previously dried paper disks, deposited on the surface of Muller-Hinton agar. Then 4 to 5 colonies were transferred into an appropriate broth (Tryptone Soy broth and placed in a 37°C incubator (in general 2 to 5 hours) until an opacity is obtained which is equivalent to the standard opacity of a barium sulfate suspension (density of 0.5 on the MacFarland scale). A sterile swab was added to the inoculum and adjusted to the opacity standard and drain excess broth by pressing the swab on the walls of the tube. The swab was inoculated on Muller-Hinton agar. The swab was passed 2 or 3 times over the entire surface in order to obtain a homogeneous inoculum. The plates were allowed to dry for 10 minutes before depositing the disks. The antibiotic disks were applied using slight pressure to insure good adhesion to the agar. They were situated at least 15 mm from the edge of the dish and sufficiently far apart so the inhibition zones do not overlap. The inhibition zone was measured with a Vernier. The data were referred to the standard table for interpreting inhibition zones furnished by the suppliers of antibiotic disks in order to establish the correlation between the inhibition zone and the minimal inhibitory concentration (M.I.C.).

Results and Discussion

The results obtained in table 1 showed that the bacteria isolated from both cracked eggshells (46%) and egg contents (44%). The most prevalent bacteria species belong to Enterobacteriace (mainly *E. coli*) followed by *Staphylococcus* which represented about (18%) of the total isolates from cracked eggshells, in spite of isolation of same species from egg contents (6%), (14%) respectively table 2. Other bacteria strain isolate from cracked eggshells followed by *Enterococcus faecalis* (6%), *Enterobacter* (4%), while

the bacteria isolated from egg content followed by *Pseudomonas* (14%), *Enterococcus faecalis* (8%), *Enterobacter* (2%) table 2.

Table 3 revealed different results of microbial against the antimicrobial disc. All bacteria showed resistance to Bactircin disc against the antimicrobial disc except *Enterococcus*. The antibiogram study of the pathogens showed that all the isolates were sensitive to CN, DOX, AZM, TC whereas, all the isolates showed intermediate zone of inhibition.

In Sudan, bacteria were isolated from eggs as *Bacillus cereus*, *Escherichia coli*, *Salmonella* spp. and *Listeria monocytogenes, Staphylococcus aureus*, *Staphylococcus epidermidis* and *Staphylococcus xylosus* (Salih *et al*., 2018). In Sudan too, there was another study to isolate bacterial contaminants from eggs which focused on two bacteria *Bacillus* and *Staphylococcus* (Mustafa and Farahat, 2018).

In Egypt, *E.coli*, *Enterobacter* spp., *Enterococcus* spp., *Klebsiella* spp., *Citrobacter*, *Pseudomonas* spp. and *Staph. aureus* were isolated from the eggs (Mansour *et al*., 2015).

(Sabarinath *et al*., 2009) studied 450 table eggs collected from the markets to examine bacterial contamination and they noticed that about half number of the eggs were contaminated.

However, a study was performed to detect Enterobacteriaceae and *Enterococcus* in the eggs collected from the cages of hens which approved higher contamination rates in furnished cages than the conventional cages (Wall *et al*., 2008).

(McWhorter and Chousalkar, 2019) focused on *Salmonella* isolated from eggs and found that the farms were Salmonella free at hatching while an ascending infection was noted in the eggs of the hen.

In addition, the bacteria of Enterobacteriaceae family was screened and isolated at high ratios from the eggs of the hen (Stepien-Pysniak, 2010).

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