# Isolation and Identification of Some Bacterial on the Eggs in Some Regions of Karbala Province

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## Abstract

A total of 70 egg when collected from various regions in Karbala to identification to microorganism that found on shell of the egg. After transported the eggs to laboratory and doing the important diagnostic steps, the results showed the bacteria with gram positive (Staphylococcus) were predominantly found on eggshell. The eggshells were also found contaminated with bacteria Escherichia. Coli, Bacillus, Streptococcus . Also the results of this study showed that the Vancomycin have highly susceptible to the Staphylococcus isolates while this isolates also showed over 90% resistant to penicillin. The presence of these microorganisms on eggshells present a significant threat to consumers. Proper sanitation to reduce microbial contaminations of eggs that usually occur after production through processing of eggs.

***Keywords:*** *Table egg, Contamination, Antibiotic sensitivity, Karbala.*

## Introduction

Table eggs are the safest and simplest food source as they contain proteinswith high-quality, essential amino acids ,mineralsandvitamins that are important for good health [1]. By going through the ventilation window or ventilation system, the surface of eggs (shell) may be contaminated, Moreover, most scientists say that contamination exists mainly after it has been introduced due to contact with polluted surfaces such as chicken stools, nesting products, soil, forage, shipping containers, packaging, handling and animals (rodents, insects and pets) [2]. Staphylococcus aureus is an important part of the microflora and can be isolated from the surface and table egg material. By entering the food channel route it may lead to degradation and injuries to consumers [3,4]. Containers are really essential elements in supplying customers with high quality table eggs. Manufacturing process is planned to shield eggs from cracking when processing by having a cushioning effect, and shielding eggs from microbes, natural pests, lack of moisture, staining and extreme heat which may lead to degradation and to eliminate damage and contamination The packing products and boxes used must then be allowed to enter oxygen as the eggs have to breathe, also the ingredients used to cover table eggs should be permeable, safe and odorless gases [5,6]. Outer shell of egg includes a large number of microorganisms like *Staphylococcus aureus, Escherichia. Coli, Streptococcus spp, Bacillus spp , Salmonella spp* and *Listeria monocytogenes* [7]. *Staphylococcus* is responsible for many diseases that appear in poultry. it form greater percentage of infected to eggshells [3,8]. The lack of effective mechanisms, a market proper drainage and extremely high moisture may have resulted in highly growth of microorganisms. It has also been found that most supermarkets do not keep eggs in refrigerators, thus exposing eggs to weather factors that contribute to their pollution. Isolated microbes can cause serious health issues, such as vomiting , nausea, diarrhea and abdominal pain [9]. *E. Coli* are Gram-negative intestinal family bacteria and are natural Inhabitants of intestinal bird tract. This is one of the opportunistic organisms responsible for a variety of diseases such as yolk sac infection, air sac infections, systemic inflammation of hepatitis, inflammation of the intestines, colonic tumor, etc. [8]**.** The present study aims to Isolation and identification some bacterial cells that contaminated the table egg shell in some regions in Karbala city. Iraq.

## Material and Methods

Sample collection and biochemical test analysis

A total of 70 samples of table eggs from various regions of Karbala were collected. Egg samples were packed in a sterile and clean container and then transported to the laboratory to prepared for microbiological examination. Eggs collected with sterile cotton swabs were displaced in the nutrient broth for overnight at 37 ° C in the incubator and then cultured in the cultural media. Nutrient Agar, MacConkey Agar, Blood Agar, Methylene Blue Eosin Agar (EMB), Minatole Salt Agar and finally use the Muller-Hinton Agar for antibiotic susceptibility tests phenotype of living microorganisms have been identified according to shape, color, size and color changes in different media. Gram stain with oil-immersion optical microscopy have been used . Finally, the biochemical tests also used to confirm genus of microorganism which involve; oxidase, coagulase, catalase tests, on the other hand api 20E (Analytical Profile Index 20 enterobacteria) was used in the study of Enterobacteriaceae [10].

**Antimicrobial Susceptibility Test (Disk Diffusion Test):**

The method used for this test, called Kirby-Bauer, is focused on the susceptibility of antibiotics on the surface of Muller-Hinton agar. This has been done by inoculums can be used in this test were made by dissolving 5 isolated colonies to 5 ml of nutrient broth and incubated for (2 hours) at (37oC) after that compared with (0.5) the standard McFarland tube, A sterile swab has been used to generate a bacterial suspension inoculum, this inoculum was distributed on a Mueller-Hinton agar plate and allowed to dry, the discs of antibiotic were put on the medium surface at randomly spaced intervals with flaming forceps or a disc applicator and incubated at 37oC for 24 hours, a zone of inhibition have been measured with a ruler and comparing the results with inhibition zones defined by [11].

**Results and discussion**

The results of this study after doing culturing and identification by gram stain and some biochemical tests as mention above and API20E (Table1),(Figure1) the results showed the *Staphylococcus* is most gram positive bacteria found on egg shell about (39 isolates) 44% from isolates its *Staphylococcus* and this results agree with [3] and [5] as well as [12] and [13] was reported that Gram-positive *Staphylococcus* spp have major natural eggshell contamination of table eggs.

Also researchers found that the major contaminants of egg were Gram-negative bacteria species as *Escherichia coli* [14], [15] and [16]. all these agree with this study were found about (17 isolates) 24% from isolates is *Escherichia coli* as Gram-negative bacteria.

Also the bacillus and streptococcus in this study occupied 13% and 11% of isolates respectively . [17] also found these types of bacteria from eggs.

Only 7% of isolates were non growth because numerous factors that related with the improper handling, inadequate bacterial suspension, improper inoculation, defect in incubation and other environmental factors.

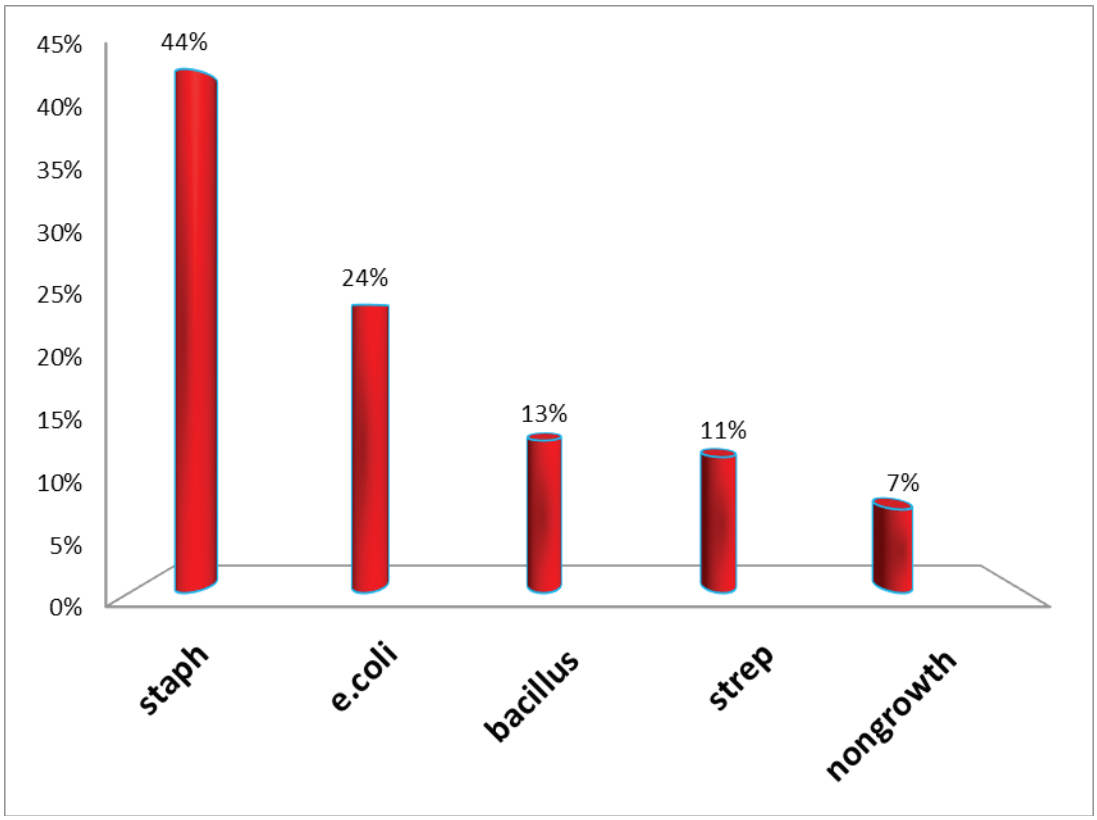


**Figure (1) Prevalence of bacteria that isolated from egg shell.**

**Table (1) showed bacterial samples, ratio, culture media, biochemical test, staining and the shape of bacteria.**

| Bacteria | Gram stain | Biochemical reaction | Morphology |
|---|---|---|---|
| Staphylococcus (39 isolates) 44% | G+ve | Coagulase–ve, Catalase +ve. | Cocci,irregular clusters |
| Escherichia coli (17 isolates) 24% | G-ve | API-20E oxidase–ve | Rod |
| Bacillus (9 isolates) 13% | G+ve | Catalase +ve | Rod |
| Streptococcus (8 isolates) 11% | G+ve | Catalase –ve. | Cocci, long or short chains |

**Analytical Profile Index(API ) 20 E test**

When suspension of bacteria is inoculated in each of the wells and incubated. An after that read or compartment the results as numbers with standard numbers , The results of this test suggest the isolates are *E.coli* depending on the reactions or numbers that found as shown in Table (2), Figure (2)

**Table (2) The different reactions of API 20E test with its results .**

| Identification code | Reaction |
|---|---|
| o-nitrophenyl-b-D-galactopyranoside (ONPG) | + |
| Arginine dihydrolase(ADH) | - |
| Lysine decarboxylase (LDC) | + |
| Ornithine decarboxylase (ODC) | + |
| Citrate (CIT) | - |
| hydrogen sulfide (H2S) | - |
| Urease (URE) | - |
| Tryptophan deaminase (TDA) | - |
| Indole (IND) | + |
| Voges-Proskauer reaction (VP) | - |
| Gelatinase (GEL) | - |
| Glucose fermentation (GLU) | + |
| Mannose fermentation (MAN) | + |
| Inositol fermentation (INO) | - |
| Sorbitol fermentation (SOR) | - |
| Rhamnose fermentation (RHA) | + |
| Sucrose fermentation (SAC) | + |
| Melibiose fermentation (MEL) | + |
| Amygdalin fermentation (AMY) | - |
| Arabinose fermentation (ARA) | + |

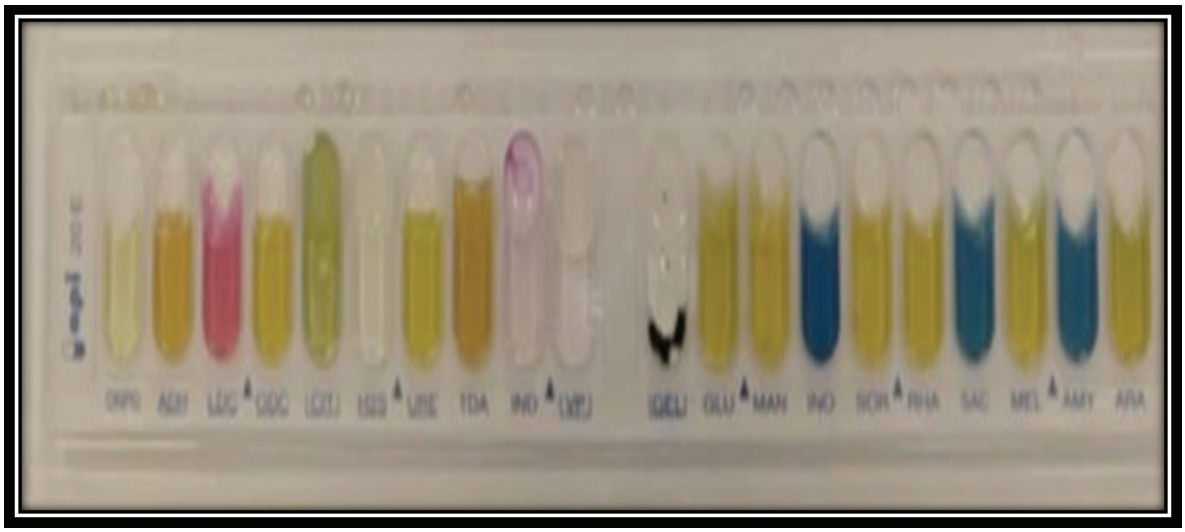

**Figure (2) The results of biochemical reactions in API20 E test.**

**Antimicrobial Susceptibility Test**

All 39 isolates from *Staphylococcus* of chicken eggs were performed Antibiotic sensitivity tests against six Antibiotics are commonly used as shown in Table(3). The results showed that the Vancomycin was susceptible to 79.4% of the isolates as shown in figure(3). also the ciprofloxacin and tetracycline showed sensitivity 61.5% and 51.2% respectively .these result agree with [3] and [18].

Over 90% of the *Staphylococcus* isolates appeared penicillin resistant, these results agree with [19] were found Over 66% of the *Staphylococcus* isolates appeared penicillin resistant

**Table (3) Percentage of antibiotic sensitivity of *Staphylococcus* isolates .**

| **Antibiotics** | **Sensitive %** |
|---|---|
| Amoxicillin | 8% |
| Penicillin | 15% |
| Gentamycin | 48.7% |
| Tetracycline | 51.2% |
| Vancomycin | 79.4% |
| Ciprofloxacin | 61.5% |

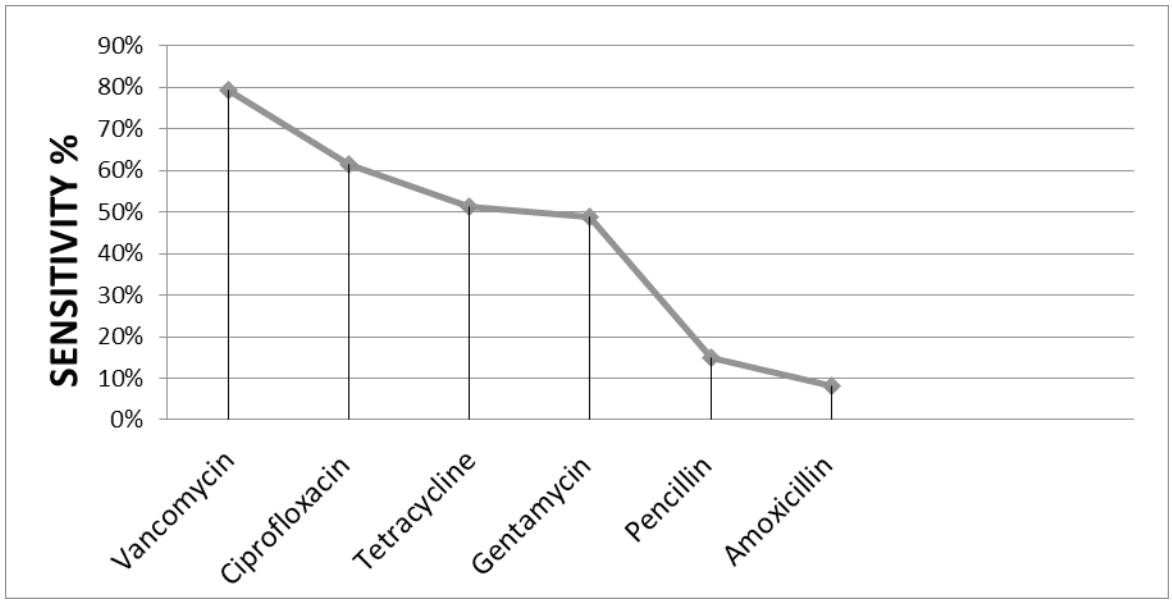


**Figure (3) Showed the *Staphylococcus* isolates have highly sensitivity to vancomycin.**

They conclusion from this study that eggs are vulnerable to contamination due to improper storing conditions , poor market access, dusty surfaces, extreme heat, smoke, hand interaction and all other pollution situations, and that customers must maintain eggs in the refrigerate and also well-cooked eggs. It's to destroy bacteria.Eventually, the persons trading have to be transported Good source eggs also from good hen farms and even in countries free from harmful zoonotic conditions.The properly washed and antiseptic techniques must be applied in every steps of handling, collected, traveling, marketing of eggs to reduce the bacterial contaminations.

**Financial Disclosure:** There is no financial disclosure.

**Conflict of Interest:** None to declare.

**Ethical Clearance:** All experimental protocols were approved under the college of pharmacy and all experiments were carried out in accordance with approved guidelines.

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