Isolation and identification of some bacterial isolates from table egg

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Abstract

This paper presents the degree of contamination of table eggs with bacteria of the genus Staphylococcus, E-coli, Salmonella, Streptococcus and Clostridia taking into account the source of the eggs. The results of the study indicate a relatively high degree of contamination of table eggs with Staphylococcus bacteria and enterobacteriaceae both in yolk and on egg shell.

The study included inspection, examination of brown eggs, gathered in winter and summer seasons from local markets, the origin the bacteria diagnosed by cultural, microscopic examination and biochemical tests methods.

Results referred that bacteria were the main organism contaminate the egg 95% and fungi were 5%, we focused here on bacterial growth. Contamination ratio was; Staphylococcus 75%, enterobacteriaceae 20% (E-coli 9% and salmonella 11%) and 4.9% streptococcus and 0.1 clostridium perfringens. Smears are taken from egg shell and yolk, bacterial growth concentrate on shell in winter at 100 % while yolk 0%, in summer the growth was equal 50%-50% in both egg shell and yolk.

عزل وتشخيص بعض العزلات البكتيرية من بيض المائدة

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الخلاصة

تستعرض الدراسة مدى تلوث بيض المائدة بالبكتريا من جنس العنقوديات والاشريشيا القولونية والسالمونيللا والعنقوديات والمكورات السبحية والمطثيات، اخذين بالاعتبار مصدر البيض. أشارت الدراسة لوجود درجة عالية من التلوث لمايكروبي لقشرة وصفار البيض بالجراثيم آنفة الذكر.

تضمنت الدراسة فحص بيض المائدة الجوزي الذي جمع في الصيف والشتاء من السوق المحلية شخصت البكتريا بالطرق المزرعية والمجهرية والفحوصات الكيمياحيوية.

أشارت نتائج الدراسة إلى ان البكتريا هي الميكروب الرئيسي الذي يلوث البيض بنسبة 95% والفطريات كانت 5%، ولقد انصبت دراستنا على النمو البكتيري حيث كانت نسبة التلوث كالآتي: العنقوديات 75%، البكتريا المعوية20% بنسبة 9% الاشريشيا القولونية و11% السالمونيللا ، لمكورات السبحية 4.9% والمطثيات 0.1%.

أخذت العينات من قشرة وصفار البيض وقد تركز النمو على قشرة البيض في الشتاء بنسبة 100% وكان النمو متساوي على القشرة والصفار في الصيف50%.

Introduction

Human illnesses resulting from the consumption of poultry products contaminated by Salmonella can be expensive for the poultry industry, governments, and affected individuals. The total annual costs of medical care and lost productivity resulting from food-borne Salmonella infections of humans in the United States have been estimated at up to $3.5 billion A single serotype, S. enteritidis, may account for as much as $870 million of this total. Negative publicity generated by media reports regarding Salmonella contamination of particular foods can significantly affect consumer demand for those items. International markets for poultry products are increasingly subject to restrictions based on food safety considerations (1). The microflora of the eggshell is dominated by Gram-positive bacteria, whereas Gram-negative bacteria are best equipped to overcome the antimicrobial defences of the egg content. Much of the research on eggshell and egg content contamination focuses on Salmonella, since infection with Salmonella enteritidis, resulting from the consumption of contaminated eggs or egg products, is still a major health problem. Observed Salmonella prevalence on the eggshell and in the egg content vary, depending on the fact whether investigations were based on randomly sampled table eggs or on eggs from naturally infected hens. The limited information available on other pathogens shows that they are exclusively isolated from the eggshell and not from the internal contents(2).

The absence of standard structures and drainage system in the market and the relatively high humidity could have contributed to the high microbial growth. It was also found out that most retailers do not store eggs in refrigerators, thus the eggs are exposed to weather conditions, resulting in their contamination. The isolated microbes could cause severe health problems like, diarrhea, nausea and abdominal pain, since they are pathenogenic (3).

E. coli is a gram-negative bacterium of the family Enterobacteriacae and is a normal inhabitant of intestinal tract of birds (4). It is one of the opportunist pathogen responsible for number of disease conditions such as yolk sac infection, air sac disease, perihepatitis, enteritis, omphalitis, coligranuloma, colibacillosis etc (5).

Contaminated poultry meat and eggs consistently have been among the most frequently implicated sources of human Salmonella outbreaks. Controlling PT infections in poultry flocks has become an important objective from both the economic and public health perspectives.

Outbreaks of non typhoidal Salmonella infections and sporadic illness have been associated with a variety of causes, particularly foods of animal origin (e.g., beef, poultry, eggs, and dairy products) (1).

Avian Pathogenic E. Coli. Most APEC isolated from poultry are pathogenic only for birds and represent a low risk of disease for people or other animals. However, chickens are susceptible to colonization with E. coli O157:H7, an important Shiga toxin-producing, enterohemorrhagic pathogen of humans, and a low occurrence of natural infection has been found in both chickens and turkeys in different geographic areas (6).

Staphylococcus. In addition to being a major disease-producing organism for poultry, approximately 50% of typical and atypical S. aureus strains produce enterotoxins that can cause food poisoning in human beings). Poultryassociated food poisoning can occur due to enterotoxin producing S. aureus strains, which contaminate poultry carcasses at processing. S. aureus strains from processed poultry are thought to be human strains endemic to the processing plant or from the hands of workers in the plant. The literature varies as to the

origin of processing plant strains with biotyping indicating the passage of human staphylococcal strains to poultry in processing plants; plasmid profiling indicates that endemic strains in the processing plant are introduced by incoming birds

Streptococcosis in avian species is worldwide in distribution, occurring as both acute septicemic and chronic infections with mortality ranging from 0.5%—50%. Infection is considered secondary, because streptococci may form part of the normal intestinal and mucosal flora of most avian species, including wild birds Streptococci are ubiquitous in nature and commonly found in various poultry environments (5).

Clostridial infections associated with 4 disease conditions in poultry or game birds Clostridium colinum is the cause of ulcerative enteritis; C. perfringens and C. septicum have been isolated from cases of necrotic enteritis or gangrenous dermatitis; and C. botulinum is the etiology of botulism (5).

Salmonella In Eggs. Much of the evidence of a link between Salmonella and eggs comes from the table egg industry. breaks of Salmonella have been reported, usually involving the consumption of raw or undercooked eggs (7, 8. 9). In the table egg industry, the strain of concern is Salmonella enteritidis. Henzler et al., (10) traced egg association in outbreaks back to layer houses. Upon sampling, the same phage type of S. enteritidis seen in the outbreak was cultured from eggs gathered at the farm. When most people think of salmonellae and eggs they think about S. enteriditis and table eggs. However, human salmonellosis can also be traced to broilers.

Origin of salmonella - contaminated table eggs and fertile hatching eggs. There are two main schools of thought regarding entry of salmonellae into hatching eggs. The vertical transmission theory states that salmonellae come from an infected hen. The horizontal transmission theory states that salmonellae invade the egg through the shell after the egg is laid. In truth, both routes are probably involved salmonella (11).

Materials and Methods

100 samples were gathering, each one represented set of 30 eggs, gathered in winter and summer seasons by two divisions from yolk and shell. Origin of brown egg was imported from Syria. Samples from yolk are taken by sterilized swap, surface of each of the eggs was first disinfected with 70% ethanol. With a sterilized hammer or hard object, each of the eggs to be cultured was broken. A sterilized cotton wool was then introduced into the content, and transferred onto media plates and from shell by sinking in 5 ml distilled water and handling with sterilized surgical gloves then then serial dilution and take 0.1 ml of it accourding to Cruickshank et al (12) half specimens put in nutrient broth to grow bacteria then culture them in cultural media; nutrient agar, mac Conkey agar, blood agar, xylose-lysine deoxycholate agar xld, s. s agar, and minatol salt agar (other half spread directly in media). All the media were prepared following the manufacturers instruction and sterilized by autoclaving at 121°C for 20 minutes.

Bacteria identified by culturing, incubation, the morphology of organisms was studied for size, shape, outline, color, and changes on various media. Bacteria were stained using Gram stain and examined using light microscope of X100 with oil immersion. Then biochemical tests to correct diagnosis include coaglase, catlase, oxidase tests according to (13). Then confirm diagnosis by api 20E (Analytical Profile Index 20 enterobacteria). API 20 E is a standardized identification system for Enterobacteriaceae and other non-fastidious, Gram negative rods (14).

Among the simplified biochemical test kits sold for the identification of bacteria is the API system. Different API kits have been designed for variousgroups of bacteria-for example, enterobacteria, lactobacilli, and anaerobes. These kits have incommon the same form of construction. The individualtests consist of dehydrated chemicals in a set of plastic cupules (moulded to a strip of plastic) which are inoculated with a bacterial suspension.The development of this system of cupules has beendescribed by (15). Three API kits areavailable for identifying enterobacteria-a screeningkit of 10 tests (1OS), a basic set of 20 tests (20E), and for further characterisation of an organism a kit of50 tests (50E). The 20E kit contains all the tests of the1OS but only a few tests are in both the 20E and 50E. The API 20E became available in the United Kingdom in 1971. The tests included in the kit have not been changed since nor, so far as is known, have their biochemical specifications (16). The identification scheme provided by the manufacturer for use with the kit, however, has undergone considerable development. The original identification chart which gave the expected reactions for each taxon in a plus and minus form was replaced by schemes in which the results of an organism are converted to a numerical code, the 'profile' of the organism.

Results and Discussion

Results were staphylococcus 75%, enterobacteriaceae 20% (E-coli 43% and salmonella 57%) and 4.9% streptococcus and 0.1%clostridia. (Table1) Bacterial growth concentrate on shell in winter at 100% while yolk 0%, in summer the growth was equal 50%-50% in both. The bacteria diagnosed by cultural, microscopic and biochemical tests methods and that results accepted with (17) they proven a degree of egg contamination with staphylococcus both inside egg contents (yolk+white) and on egg shell.

Several factors have been implicated in egg contamination. Among these are faeces of the birds, litter material, egg crates, packing and storage. Others are cloths and hands of poultry workers, dust, the environment, weather conditions, transporting and marketing. Among the common contaminant organisms pathogenic to human beings are Salmonella spp, Staphylococcus spp, Gram positive Bacillus, Mucor, Corynebacteria, Aspergillus, Escherichia coli and Diplococci (18). Osei-Somuah (18) also isolated and identified similar microorganism from the southern part of the country confirming that these organisms can survive under different conditions i.e. 4°C and 60°C. However, Salmonella was not isolated and this suggested that all the eggs were Salmonella free. This may be attributed to the fact that poultry farmers practice strict medication and care (19).

Microorganisms that were isolated and identified from the sampled eggs from the market include Staphylococcus, Streptococcus, enterococcus clostridium and fungi. From both the shell and in the content. This may be due to the fact that the eggs were improperly stored for a long time. Etches (20), reported that, as eggs stay longer, their resistance reduced enabling these organisms to penetrate into the egg content. Warm and moist litters, poor condition in the farm houses and s were reported to be sources of fungi growth and sporulation (21).

With the introduction of alternative housing systems for laying hens in the EU, recent research has focussed on the bacterial contamination of table eggs, e.g. eggshell and egg content contamination. Contamination of eggshells with aerobic bacteria is generally higher for nest eggs from non-cage systems compared to nest eggs from furnished cages or eggs from conventional cages. Studies indicate limited or no systematic differences in eggshell contamination with aerobic bacteria between eggs laid in the nest boxes of furnished cages

and eggs laid in conventional cages. The major differences found in experimental studies between cage- and non-cage systems are less pronounced under commercial conditions. The effect of housing system on eggshell contamination with specific groups of bacteria is variable. Limited information is available on the influence of housing system on egg content contamination. Recent research does not indicate large differences in egg content contamination between eggs from cage- and non-cage systems (ignoring outside nest and floor eggs). The microflora of the eggshell is dominated by Gram-positive bacteria, whereas Gram-negative bacteria are best equipped to overcome the antimicrobial defenses of the egg content. Much of the research on eggshell and egg content contamination focuses on Salmonella, since infection with Salmonella enteritidis, resulting from the consumption of contaminated eggs or egg products, is still a major health problem. Observed Salmonella prevalence on the eggshell and in the egg content vary, depending on the fact whether investigations were based on randomly sampled table eggs or on eggs from naturally infected hens. The limited information available on other pathogens shows that they are exclusively isolated from the eggshell and not from the internal contents (2). Sanitation is very poor as all sorts of animals enter and leave the market freely. Retailers store eggs in an open room without any standard storage facilities. These conditions favour microbial growth because microbes grow within a range of 4°C and 60°C.

The Staphylococcus, Streptococcus which were isolated from the samples are often implicated with fecal contamination. These could be of great health concern since species of these bacteria cause diarrhoea and fever, in the hosts (21,22).

Escherichia coli is known to contaminate the surface of egg while mechanical process can spread the bacteria through eggs and meat. Contamination with the pathogen while in the field, occur through improperly decomposed manure, contaminated water and poor hygienic practices of the farm workers. Escherichia coli causes mastitis, urinary tract infection, meningitis, pneumonia and peritonitis (23).

We conclude from this study that eggs are exposed to contamination due to bad storage conditions in storehouse, wrong show in market, dirty table, high temperature, dust, hand touching, and all other surrounding pollution state, also consumers should keep egg in refrigerator and cooked egg well to kill bacteria. Finally the trade people must be transport egg from good source and good hen farms because the type of rearing (cage or floor) greatly effect on quality of egg and also from countries empty from dangerous zoonotic diseases.

Table (1) Represented bacterial samples, ratio, differential culture media, biochemical test and staining

Total bacteria	%	Media	Biochemical test	Stain
Staphylococcus	75%	blood agar	Coagulase –ve	G+ve
E-coli	9	XLDa gar	API-20 E	G-ve
salmonella	11	XLD agar	catalase +ve oxidase – ve	G-ve
streptococcus	4.9%	Blood gar.	Catalase –ve.)	G+ve
Clostridium perfringens	0.1%	Bloodagar	EndosporeOval/Sub-Terminal] Lactose(+)Sucrose(+)	G+ve

Table (2) Winter and summer contamination

Staph.	E.coli	Sal.	Str.	Clos.	Site	
-	-	-	-	-	Yolk	Winter
+	+	+	+	-	Shell	
+	+	+	+	-	Yolk	Summer
+	+	+	+	+	Shell	

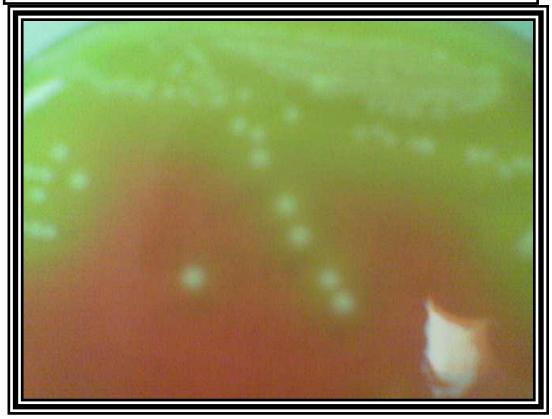
Table (3) Biochemical reaction in the API 20 E test

Identification code	Reaction	E. coli
ONPG	o-nitrophenyl-β-galactosidase	+
ADH	Argininedehydrolase	-
LDC	Lysinedecarboxylase	+
ODC	Ornithinedecarboxylase	+
CIT	Citrate	-
H_2S	H_2S	-
URE	Urease	-
TDA	Tryptophanedeaminase	-
IND	Indole	+
VP	Voges-Proskauer reaction	-
GEL	Gelatinase	-
GLU	Glucose fermentation	+
MAN	Mannitol fermentation	+
INO	Inositol fermentation	-
SOR	Sorbitol fermentation	-
RHA	Rhamnose fermentation	+
SAC	Saccharose fermentation	+
MEL	Melibiose fermentation	+
AMY	Amygdalin fermentation	-
ARA	Arabinose fermentation	+

Pictures

Salmonella

E . coli



Streptococcus

Staphylococcus

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