

# Isolation of total aerobic and pathogenic bacteria from table eggs and its contents

Siriporn Chaemsanit<sup>1</sup>, Ali Akbar<sup>2</sup> and Anil Kumar Anal<sup>1,\*</sup>

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## Abstract

Total number of 16 eggs from different farms and markets were collected to isolate the total aerobic microbial load and the pathogenic bacteria load on its shell and in contents. The aerobic bacteria 118 were isolated from the samples, out of which 116 from eggshells and 2 from egg contents of single egg sample. Gram's positive bacteria (*Staphylococcus*) were found predominantly present on eggshell. The eggshells were also found contaminated with pathogenic bacteria (*Salmonella* and *Escherichia coli*). Whereas no *Escherichia coli* O157:H7 was found on eggshell and contents. Three eggshell samples from farm layers were found contaminated with *E. coli*. Two samples were found contaminated with *Salmonella* one each from farm and market. Four out of eight (50%) samples from farm layer were found contaminated with pathogenic bacteria, while only one out of eight (12.50%) from market was found contaminated with pathogens. Eggs from market were found less contaminated as compared to farm eggs.

**Keywords:** Food safety, *Salmonella*, *Escherichia coli*, *Staphylococcus aureus*, Table eggs

## 1. Introduction

Foodborne illness is a major public health problem and the main cause of diarrhoeal diseases affecting all developed and developing countries (Akbar and Anal 2014; Akbar and Anal 2013b). Table eggs are the best and easy source of food, containing quality protein, essential amino acids, essential vitamins and minerals needed for a good health (MAFF, 2009). Asia is the largest egg producing region with 65% global outputs (Ernst, 2009). A combined share of egg production from China, India and Japan are more than 46%. However, China itself is the number one of the top 10 countries that have provided 38% of the world's eggs demand in 2011 (Peter, 2011).

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<sup>1</sup> Food Engineering and Bioprocess Technology, Asian Institute of Technology, Klong luang, Pathumthani 12120, Thailand

<sup>2</sup> Department of Microbiology, Faculty of Life Science, University of Balochistan Quetta, Pakistan

\* Corresponding author, e-mail: anil.anal@gmail.com, anilkumar@ait.ac.th

Eggs have natural defense system against the contaminating microbes, such as cuticle, calcium hard shell and shell membrane (Jerzy and Dagmara, 2009). The albumen contains several egg white proteins that have antimicrobial properties, especially the lysozyme. Ovomuroid is another proteinase that inhibits the ability of bacterial to use the protein in albumen. Furthermore, the pH in albumen which is about 9–10 and the viscosities of the egg white are not suitable for microbial growth (Froning, 1998). Egg can be contaminated at both egg shell and egg contents by a variety of microbes with a wide range of pathogens such as *Campylobacter jejuni*, *Listeria monocytogenes*, *Escherichia coli*, *Yersinia enterocolitica* and especially *salmonella* (Ricke *et al.*, 2001; Board and Tranter, 1995). *Staphylococci* are most common bacteria contaminating eggshells. Contamination is more likely linked with cracked egg, dirty shells and storage in contaminated surroundings. It can be contaminated during formation and laying process (Abdullah, 2010). Elliott (1954) revealed that stored or aged eggs have more possibility to become infected than fresh eggs due to the degradation of natural defense mechanisms in egg over time. The eggshell contamination increasing the chances of egg contents contamination by penetration (Messens *et al.*, 2006). Bacterial contamination can happen at three main parts of egg (egg yolk, albumen and shell membrane / egg shell) (Bahrouz, 2005). *Salmonella enteritidis* is able to invade the cells of the follicles before ovulation and multiply themselves after 2 h of infection (Howard *et al.*, 2005). Eggs are considered to be a medium to low risk food for foodborne illness which can become contaminated with bacteria, like *Salmonella* and other enteric pathogens (Chousalkar *et al.*, 2010). The most common foodborne pathogens associated with food of animal origin are *Salmonella*, *Campylobacter*, *Listeria monocytogenes*, *Staphylococcus aureus* and *Escherichia coli* O157 (Akbar and Anal, 2013a; Ghasemian, 2011; Akbar and anal, 2011). In current study a survey was conducted for enumeration of aerobic bacterial load and the pathogens on eggshells and in egg contents. The pathogens were also examined for its antibiogram study.

## 2. Material and Methods

### 2.1 Sample collection

A total number of 16 shell eggs were collected from eight different places from farm and markets. Eight shell egg samples were randomly collected from two kind of farms (caged system and caged-free) on the day eggs were laid, whereas eight eggs were purchased from local and super markets aseptically in sterile polythene bags. All commercial eggs purchased were less than 4 days old from its date of production. All the samples were process with in 6 h of its collection.

## 2.2 Total aerobic bacterial count determination

For surface bacterial count, a swab method was applied following (Loongyai *et al.* 2010). The surface of whole egg was swabbed aseptically with sterile cotton swab and then diluted with normal saline. The samples were further diluted serially and 1 mL of respective dilution was poured on the surface of nutrient agar (NA) (Himedia, India).

For the enumeration of bacteria in egg contents, samples were first dipped in 75% ethanol for 5 min and allowed to air dry. The upper end of the egg was flame for 5–10 sec and then holed with sterilized implement. The whole egg contents were mixed in sterile polythene bag and then poured on NA after serial dilution for aerobic bacterial count. The inoculated Petri dishes were incubated at  $35\pm 2^{\circ}\text{C}$  for 24–48 h.

Aerobic bacterial colonies were initially differentiated with the help of its morphology and Gram's staining and then sub-cultured on Blood Agar (Biomark, India), McConkey agar (Biomark) and Mannitol Salt Agar (Himedia). The isolates were further confirmed to genus and species level with the help of biochemical test such as, catalase, oxidase, coagulase, methyl red, voges-proskauer, urease, indole, citrate utilization, motility, Lysine decarboxylase (LDC), Lysine deaminase (LDA), hydrogen sulfide and glucose, lactose fermentation tests. All the isolates were identified according to bergeys manual of determinative bacteriology.

## 2.3. Pathogenic bacteria count determination

### ***Salmonella***

Egg shell surface and contents (1 mL) were transferred to triptic soya broth (TSB) (Himedia) and incubated at  $35\pm 2^{\circ}\text{C}$  for 12–18 h. Amount (0.1 mL) of the pre-inoculated TSB was transferred to Rappaport-Vassiliadis broth (RVB) (Himedia) and incubated at  $35\pm 2^{\circ}\text{C}$  for 24 h. A loopful of RVB was transferred to Xylose Lysine Deoxycholate (XLD) agar (Himedia) and incubated at  $35\pm 2^{\circ}\text{C}$  for 24–48 h. Red-pink colonies with or without black center were presumptively consider *Salmonella* and were subjected to biochemical confirmation following bacteriological analytical manual (FDA 2001).

### ***Escherichia coli* and *Escherichia coli* O157:H7**

Egg shell surface and contents (1 mL) were transferred to eosin-methylene blue agar after serial dilution and incubated at  $35\pm 2^{\circ}\text{C}$  for 24–48 h. Colonies with green metallic-sheen were further confirmed with the help of biochemical tests following bacteriological analytical manual (FDA 2001). The *E.coli* colonies were sub-cultured on sorbitol macConkey agar (Himedia) for *E. coli* O157:H7 strain confirmation.

### ***Staphylococcus* species**

The Gram's positive colonies from blood agar were sub-cultured on mannitol salt agar (Himedia) for *Staphylococcus* species confirmation.

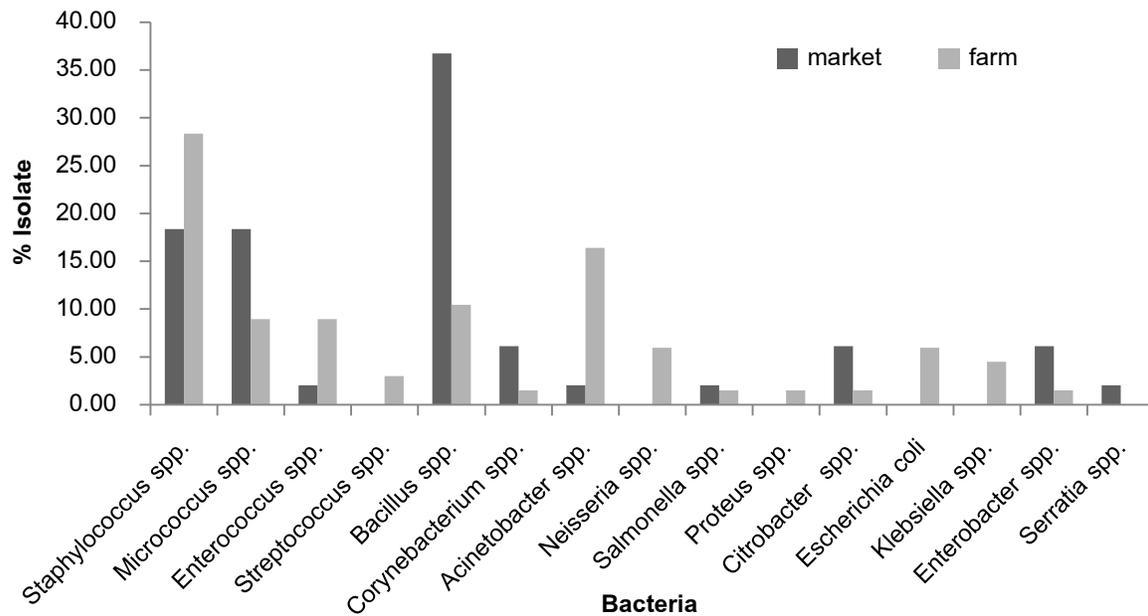
### **2.4 Antibiotic sensitivity test**

Antimicrobial sensitivity of pathogenic bacteria was performed with the help of disc diffusion assay using antibiotic discs, Tetracycline (30 µg), Ampicillin (10 µg), Kanamycin (30 µg), Enrofloxacin (5 µg) and Gentamicin (10 µg) (Oxide, UK). The fresh bacterial culture from non-selective agar were diluted to  $10^8$  CFU/mL and spread over mueller-hinton agar (Himedia) and incubated at  $35\pm 2^\circ\text{C}$  for 16–24 h. Zone of inhibition were interpret as per the clinical laboratory standard institute (CLSI) guideline.

### **3. Results and Discussion**

A total number of 118 aerobic bacterial isolates were isolated from the samples, out of which 116 were from eggshells and 2 from egg contents of single egg sample. The egg contents isolates were identified as belonging to the enterobacteriaceae family. The 67/116 isolates were from farm layer and 49/116 from market layer, respectively, where the 41/67 isolates were Gram's positive and 26 Gram's negative. The 40/49 isolates from market layer were Gram's positive and 9 Gram's negative. Gram's positive bacteria were the predominant flora of eggshells. Gram's positive bacteria can tolerate dry and harsh conditions and is present in dust, soil and feces, which is the major reason of its presence on eggshells (De Reu *et al.* 2007).

The total aerobic count range of bacteria on eggshell was (2.9 to 6.2 log CFU/mL) in market layer, whereas 7.2 to 8.0 log CFU/mL in farm layers samples. Only one egg sample contents from farm layer was found contaminated with 3.0 log CFU/mL of aerobic bacteria. All 116 isolates were belonging to 15 different genus, included *Staphylococcus spp.*, *Micrococcus spp.*, *Enterococcus spp.*, *Streptococcus spp.*, *Bacillus spp.*, *Corynebacterium spp.*, *Acinetobacter spp.*, *Neisseria spp.*, *Salmonella spp.*, *Proteus spp.*, *Citrobacter spp.*, *Escherichia coli*, *Klebsiella spp.*, *Enterobacter spp.* and *Serratia spp.* (Figure 1). *Staphylococcus spp.* (18.40 in market and 28.40% farm layer) and *Bacillus spp.* (36.70 in market and 10.45% in farm layer) was predominantly found associated to eggshell. Rajmani and Verma (2011) and Arathy *et al.*, (2009) reported bacteria of the same genus from eggs in their studies.

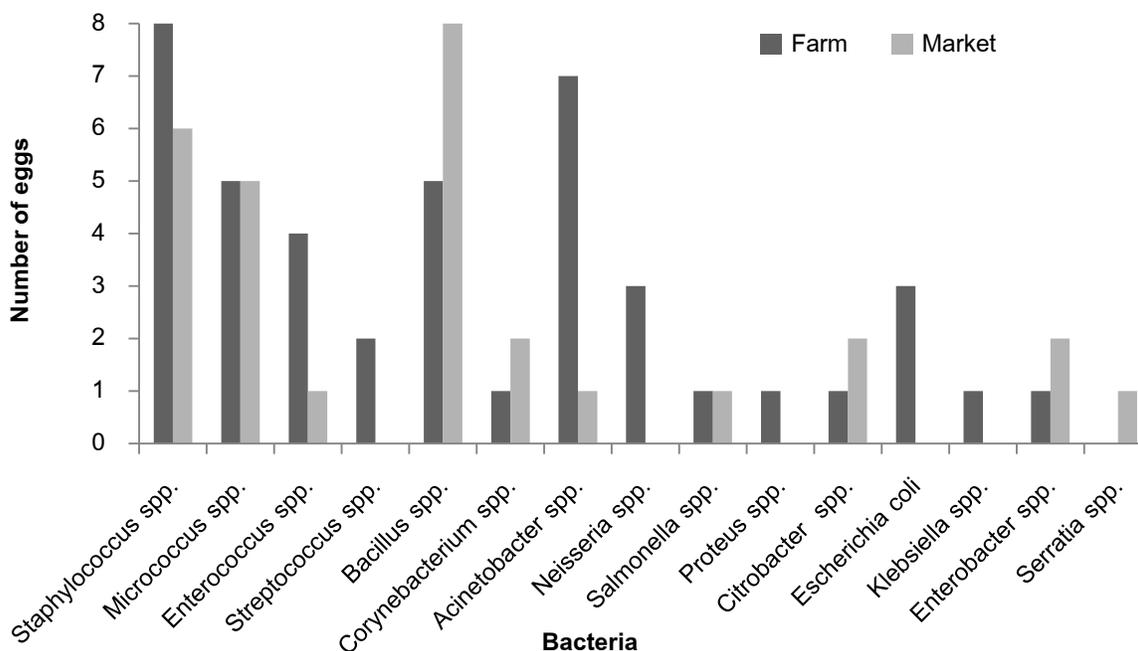


**Figure 1** Percentage of total aerobic bacterial species isolated from market and farm eggs

Abdullah (2010) reported the highest degree of eggshell contamination with Gram-positive bacteria particularly *Staphylococcus* spp. Eggs laid in dirty environment contained more bacteria than eggs laid in clean environment (Bruce and Drysdale, 1994). The surrounding environment and storage condition including temperature and storage duration can influence the level of bacterial contamination (Stepien, 2010). Board and Tranter (1995) reported that the level of contamination on egg shells have a wide range of variation from log 2 to log 7 colony forming unit (CFU) of bacteria per shell. In this study the samples from cage farm were found predominantly contaminated with aerobic bacteria. Our results are in agreement with the results of (Nordenskjöld, 2010). No contamination of *Escherichia coli*, *Escherichia coli* O157:H7 and *Salmonella* spp. was found in contents in this study.

Three eggshell samples from farm layers were found contaminated with *E.coli*. The pathogen *Salmonella* was isolated from two samples one each from farm and market, whereas no *E.coli* was found in market eggs (Figure2). No contamination of *E. coli* O157:H7 was found in the samples from farm and market. Four out of eight (50%) samples from farm layer were found contaminated with pathogenic bacteria, while only one out of eight (12.50%) from market was found contaminated with pathogens. It was confirmed that the farm eggs are more contaminated compare to market eggs. The lower contamination rate of market eggs are may be

because of the cleaning process of eggs before marketing and its hygienic handling. Adesiyun *et al.*, (2005) reported higher prevalence of *E.coli* on eggshells from farms compared to eggs from markets. The antibiogram study of the pathogens from eggshells showed that all the isolates were sensitive to gentamicin, enrofloxacin, tetracycline and ampicillin whereas, all the isolates showed intermediate resistance to kanamycin (Table 1) Musgrove *et al.*, (2006) isolated *Salmonella* and *E.coli* from eggs and analyzed for their antibiogram against 16 antibiotics including ampicillin, tetracycline, gentamicin, and kanamycin. They reported that *E.coli* (73.20%) and *Salmonella* serotypes (34.10%) were susceptible to all antimicrobial used. They reported that some *Salmonella* (63.40%) and *E.coli* (29.90%) isolates exhibited resistance against tetracycline.



**Figure 2** Number of eggs from farm and market contaminated with specific bacterial species

#### 4. Conclusion

It was concluded in the study that eggshells are predominantly contaminated with Gram's positive bacteria. The contamination was mostly from farm environment and storage conditions. It was found that the market eggshells were less contaminated as compared to farm eggs. Pathogenic bacteria such as *Salmonella* and *E.coli* was found in both farm and market eggs. The egg contents of one egg were also found contaminated with some Gram's negative bacteria. It was found that the pathogenic bacteria were not widely resistance to common antibiotics. It was

concluded that good hygiene practice and proper cleaning of the egg before storage and less storage timing can minimize the risk of contamination in eggs.

**Table 1** Antibiogram of pathogens isolated from table eggs

| Isolate                 | Resistance | Sensitive          |
|-------------------------|------------|--------------------|
| <i>Salmonella</i> (S4)  | Kan        | Gen, Enr, Tet, Amp |
| <i>Salmonella</i> (S11) | Kan        | Gen, Enr, Tet, Amp |
| <i>E.coli</i> (E9)      | Kan        | Gen, Enr, Tet, Amp |
| <i>E.coli</i> (E10)     | Kan        | Gen, Enr, Tet, Amp |
| <i>E.coli</i> (E15)     | Kan        | Gen, Enr, Tet, Amp |

**Note:** Gen: Gentamicin, Enr: Enrofloxacin, Kan: Kanamycin, Tet: Tetracycline, Amp: Ampicillin.

(S4), (S11) = Identification codes for *Salmonella* spp.

(E9), (E10), (E15) = Identification codes for *Escherichia coli*

## References

- Abdullah, I.N. 2010. Isolation and identification of some bacterial isolates from table egg. *Journal of Veterinary Science*. 3(2): 59–67.
- Adesiyun, A., Offiah, N., Seepersadsingh, N., Rodrig, S., Lashley, V., Musai, L. and Georges, K. 2005. Microbial health risk posed by table eggs in Trinidad. *Epidemiology and Infection*. 133: 1049–1056.
- Akbar, A. and Anal, A.K. 2011. Food safety concerns and food-borne pathogens, *Salmonella*, *Escherichia coli* and *Campylobacter*. *FUUAST Journal of Biology*. 1(1): 5–17.
- Akbar, A, and Anal, A.K.,. 2013a. Prevalence and antibiogram study of *Salmonella* and *Staphylococcus aureus* in poultry meat. *Asian Pacific Journal of Tropical Biomedicine*. 3(2): 163–168.
- Akbar, A. and Anal, A.K. 2013b. Occurrence of *Staphylococcus aureus* and evaluation of anti-staphylococcal activity of *Lactococcus lactis* subsp. *lactis* in ready-to-eat poultry meat. *Annals of Microbiology* (online first). DOI 10.1007/s13213-013-0641-x
- Akbar, A. and Anal, K.A. 2014. Zinc oxide nanoparticles loaded active packaging a challenge study against *Salmonella typhimurium* and *Staphylococcus aureus* in ready- to-eat poultry meat. *Food Control*. 38: 88–95.

- Arathy S., Vanpee G., Belot G., Vanessa M., Claude D., Ravindra N.S. 2009. Bacterial contamination of commercial chicken eggs in Grenada, West Indies. *West Indian Veterinary Journal*. 9 (2): 4–7.
- Bahrouz, M.A. and Al-Jaff. 2005. The risk of bacterial contamination in hen eggs of Sulaimani poultries. *Journal of Zankoy Sulaimani*. 8A (1): 63–71.
- Board, R.G. and Tranter, H.S. 1995. The microbiology of eggs. In: W. J. Stadelman and Coterill O. J. (eds). *Egg Science and technology*. 4<sup>th</sup> ed. Haworth Press Inc. New York.
- Bruce J. and Drysdale E.M. 1994. Trans-shell transmission. *Microbiology of the avian egg* (Board RG and Fuller R, eds), Chapman and Hall, London. UK. 63–91.
- Chousalkar K.K., Flynn P., Sutherland M., Roberts J.R. and Cheetham B.F. 2010. Recovery of *Salmonella* and *Escherichia coli* from commercial egg shells and effect of translucency on bacterial penetration in eggs. *International Journal of Food Microbiology*. 142: 207–213.
- De Reu K., Heyndrickx M., Grijspreedt K., Rodenburg B., Tuytens F., Uyttenaere M., Debevere J. and Herman L. 2007. Estimation of the vertical and horizontal bacterial infection of hen's table eggs. XVIII European symposium on the quality of poultry meat & XII European symposium on the quality of eggs and egg products-Conference proceedings, Prague. Czech Republic: 55-56.
- Elliott R.P. 1954. Spoilage of shell eggs by pseudomonads. *Applied Microbiology*. 2: 158–163.
- Ernst C. 2009. Asia dominates in egg consumption. Sanovo Staalkat group, Sjanghai, China. <http://www.worldpoultry.net/Layers/Eggs/2009/8/Asia-dominates-in-egg-consumptionWP006947W/>
- FDA. 2001. Bacteriological analytical manual (BAM) online. <http://www.fda.gov/Food/ScienceResearch/LaboratoryMethods/BacteriologicalAnalyticalManualBAM/default.htm>
- Froning G.W. 1998. Recent advances in egg products research and development. University of Nebraska-Lincoln.
- Ghasemian S. 2011. The prevalence of bacterial contamination of table eggs from retails markets by *Salmonella* spp., *Listeria monocytogenes*, *Campylobacter jejuni* and *Escherichia coli* in Shahrekord, Iran. *Jundishapur Journal of Microbiology*. 4(4): 249–253.
- Howard Z.R., Moore R.W., Zabala-Diaz I.B., Landers K.L., Byrd J.A., Kubena L.F., Nisbet D.J., Birkhold, S.G., Ricke, S.C. 2005. Ovarian laying hen follicular maturation and in-vitro *Salmonella* internalization. *Veterinary Microbiology*, 108: 95–100.

- Jerzy R. and Dagmara, S.P. 2009. Antimicrobial defense mechanisms of chicken eggs and possibilities for their use in protecting human and animal health. Division of avian diseases, Institute of biological bases of animal diseases University of life sciences in Lublin.
- Loongyai W., Wiriya B. and Sangsawang N. 2011. Detection of *Salmonella* and *Escherichia coli* in egg shell and egg content from different housing systems for laying hens. *International Journal of Poultry Science*. 10(2): 93–97.
- Messens W., Grijspeerdt K. and Herman L. 2006. Eggshell penetration of hen's eggs by *Salmonella enteric* serovar *enteritidis* upon various storage conditions. *British Poultry Science*. 47: 554–560.
- Ministry of Agriculture, Fisheries and Food (MAFF, UK). 2009. The Egg Quality Guide. Department for Environment Food and Rural Affairs (DEFRA), <http://archive.defra.gov.uk/foodfarm/food/industry/sectors/eggspoultry/eggs.htm>.
- Musgrove M.T., Northcutt J.K., Jones D.R., Cox N.A. and Harrison M.A. 2008. Enterobacteriaceae and related organisms isolated from shell eggs collected during commercial processing. *Poultry Science*, 87: 1211–1218.
- Nordenskjöld, J. 2010. Study of microflora on egg shells in egg production in Jordan. Independent project/degree project in food science Uppsala Biocenter University of agricultural sciences.
- Peter B. 2011. Majority of eggs worldwide produced by 15 countries. *Executive Guide to world poultry trends*. 31–35.
- Rajmani R.S. and Verma S.P. 2011. Microbial flora of eggs and egg contents from organized and unorganized poultry farms. *Indian Journal of Veterinary Research*. 20(1): 73–76.
- Ricke S.C., Birkhold S.G. and Gast R.K. 2001. Eggs and egg products. In *compendium of methods for the microbiological examination of foods*, 4<sup>th</sup> ed. Downes F. P. and Ito K. eds. American Public Health Association, Washington, D.C. 473–479
- Stepien P.D. 2010. Occurrence of Gram-negative bacteria in hens' eggs depending on their source and storage conditions. *Polish Journal of Veterinary Sciences*. 13(3): 507–513.