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Original Article

Effect of storage at room temperature on prevalence of some pathogenic bacteria over egg shells

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Abstract
The study was conducted to determine the effect of storage of table eggs at room temperature (25 ±1˚C) on viability of some pathogenic bacteria. A total of 200 eggs were collected randomly from various shops in Lahore (Pakistan) and divided into two groups equally. One was stored at room temperature for ten days while other was processed on the same day. Enumeration and prevalence of bacteria in terms of colony forming units (CFUs) was worked out following serial dilutions. Mean of bacterial count was obtained as 298 × 104 CFUs/ml of original suspension at zero time on day 1, which diminished to 116 × 104 CFUs/ml at day 10. Most of the samples (91%) were densely populated with *Shigella* (139 CFUs × 104 /ml of original suspension), and least (61%) with *Klebsiella* (43 CFUs × 104ml of original suspension), sandwiching (76%) with E.coli (98 CFUs × 104/ml of original suspension). Number of samples scoring each category of the bacterial contamination as well as the CFUs levels dropped significantly ($P<0.05$) to 61% on day 10 suggesting the positive impact of room temperature storage on the surface hygiene of eggs.
Key words: Pathogens, egg shells, *E.coli, Shigella, Klebsiella.*

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INTRODUCTION

Due to protective outer shell and inner membranes, eggs are thought as clean food. The outer shell surface may get contaminated by various microorganisms under different conditions to cause spoilage (Gantois *et al.,* 2009). Egg shell surface does not only harbor a variety of pathogens like *Salmonella, Listeria, Campylobacter, E.coli* (Ghasemian *et al.,* 2011), but the contaminants also multiply rapidly under suitable conditions (Cook *et al.,* 2003). A total coliform count of 212 CFUs (AL-Bachir and Zeinou, 2006), and 4-20×10^6 CFUs/ml of original suspension (Esther, 2008) on day zero is indicative of the contamination of eggs during production. Caging environment, does not affect the level of microbial contamination which occurs at the time of egg production (Hannah *et al.,* 2011).

The present study was aimed to investigate the effect of storage on intensity and diversity of some pathogenic bacteria contaminating egg shell surface. Antibiotics resistance of the pathogens explained their anthropogenic nature on one hand while on the other hand, handling of such eggs needs precautions as to avoid the associated health risks.

MATERIALS AND METHODS

Two hundred eggs were purchased from different shops in Lahore, among which 100 were processed for bacteriological analysis on day 1while the second set was stored at room temperature for ten days. Both categories of eggs were dipped in 150ml (autoclaved) water, each in a separate container. After dipping, egg surface was rubbed with the help of autoclaved cotton swabs to shift maximum number of microbial flora from surface of egg shell into water. Following this practice, two serial dilutions (1:100 and 1:10000) were prepared from original suspension. The dilutions were subsequently spread in an amount of 0.1 ml/EMB agar plate and incubated at 37˚C. This process was repeated for rest of the eggs on day 10. Plates

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with count of 30-300 colonies were selected and size, shape elevation, margins, surface texture, consistency, pigmentation and optical nature of well separated colonies were noted. Following pure culturing of each isolate, their glycerol stocks were prepared.

Characterization and identification was worked out with pure culture of each isolate by Gram's reaction , endospore staining ,and motility , catalase , oxidase , Indole , Citrate utilization , Methyl red , Voges Proskauer tests. Each isolate was examined for its degree of pathogenicity by growing over the blood agar medium. Ampicillin (25µg), nalidixic acid (30µg), rifampin (5µg) and vanomycin (30µg) were employed to assess antimicrobial susceptibility/resistance by Kirby-Bauer method of disk diffusion(Benson, 2001; Pelczar *et al.,* 1986). Plates were examined after 24 hrs for zone of inhibition around the disks.

Statistical analysis:

One way Analysis of variance (ANOVA) was applied to the obtained results at $p<0.05$.

RESULTS

Average number of 298 and 116 CFUs × 10^4 /ml of first suspension were obtained on day 1 and 10 respectively. In other words the eggs purchased and stored at room temperature for ten days harbored 447×10^6 and 174×10^6 culturable bacterial contents per egg, respectively (Fig. 1).

Table I: Colonial characteristics of bacteria isolated from egg shell surface

Isolate	Size (mm) (Color)	Configuration (Elevation)	Margin/ Consistency/ Opacity	Indole test	Gram's and Endospore staining
E.coli	2-3 Metallic Sheen	Round nucleated (Drop like)	Smooth/ Butterious/ Opaque	-ve	-ve
Shigella	1-2 (Colorless)	Round (Raised)	Smooth/ Mucoid/ Opaque	-ve	-ve
Klebsiella	3-4 (Dark pink)	Irregular nucleated (Umbonate)	Smooth/ Mucoid/ Translucent	-ve	-ve

Table II: Biochemical characteristics of bacteria isolated from egg shell surface

Isolate	Catalase/ Oxidase test	MR/ Citrate test	VP-I &II	Hemolysis	Antibiotic sensitivity test (D= mm)			
					AMP	NA	RD	VA
E.coli	-ve/ +ve	+ve/ -ve	-ve	Γ	R 12	R 12	R 5	R 8
Shigella	-ve/ +ve	+ve/ -ve	-ve	Γ	R 18	R 10	R 9	S 12
Klebsiella	-ve/ +ve	+ve/ +ve	+ve	B	R 16	R 12	R 8	R 8

Amp: Ampicillin with disk potency of 25µg, NA: Nalidixic acid with disk potency of 30µg, RD: Rifampin with disk potency of 5µg, VA: Vanomycin with disk potency of 30µg (Kirby-Bauer method).

The bacterial colonies ranged from 1-4mm in size with varying colors, configuration, elevation, margin and consistency (Table I). Isolates were identified as *E.coli, Shigella* and *Klebsiella.* CFUs for *Shigella* sp. were highest as being 139 CFUs × 10^4/ml of original suspension on day 1,

as compared to *E.coli* (98 CFUs × 10^4/ml) and *Klebsiella* (61 CFUs × 10^4/ml). The bacterial contents dropped on day 10 with values of 50, 36, and 30 CFUs × 10^4/ml of original suspension for *Shigella, E.coli* and *Klebsiella* sp. respectively (Fig. 1).

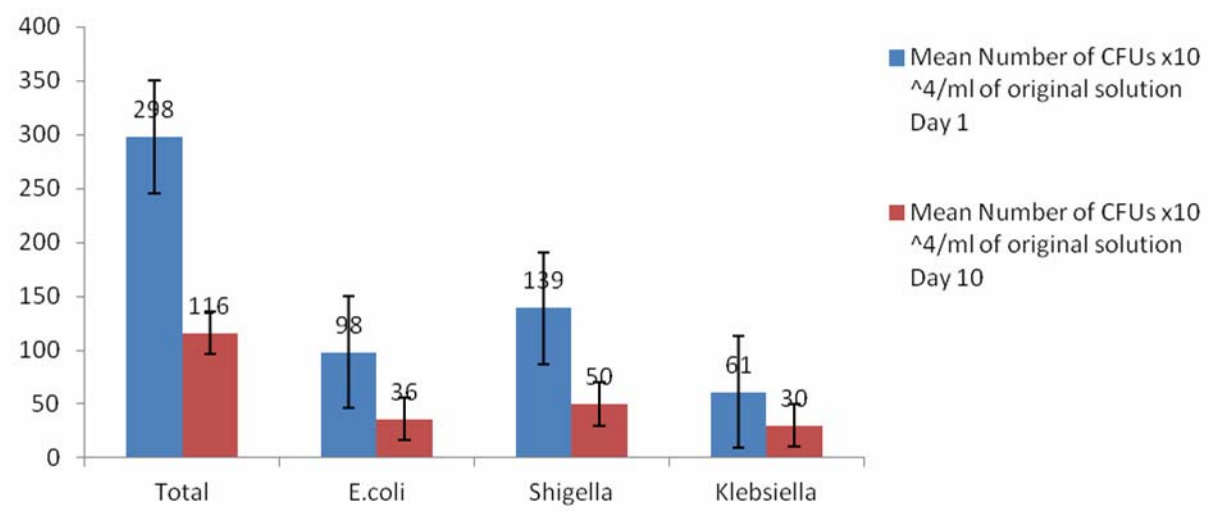

Figure 1: Number of CFUs x10^4/ml of original solution isolated over egg shells at different time periods

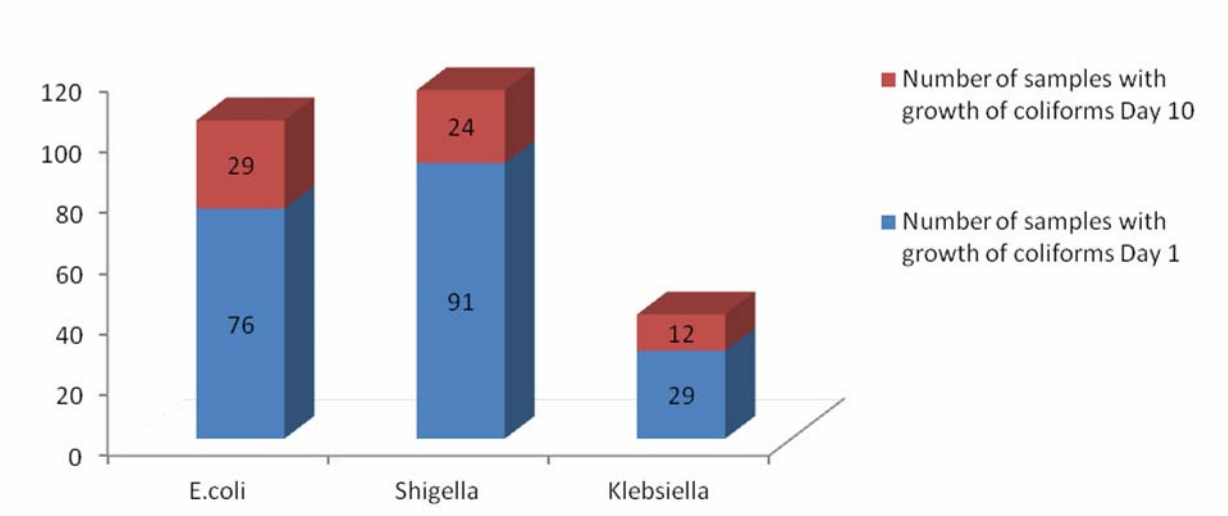

Figure 2: Number of samples showing growth for different pathogens over egg shells at different time periods

On day 1, 91% samples were populated with *Shigella,* while on day 10, only 24% samples appeared positive. *E.coli* appeared on 76% samples on day 1 and on 29% of the samples on day 10. *Klebsiella* was isolated from 29% and 12% samples on day 1 and 10 respectively. *E.coli* and *Shigella* showed γ-hemolysis while only *Klebsiella* showed β-hemolysis on the blood agar (Table II). Each isolate was found resistant to all the drugs tested, except *Shigella* which were susceptible to Vanomycin (Table II).

DISCUSSION

A significant decrease of 61% in the bacterial count over the eggs' shells was observed following 10 days of room storage.

Reduction in the bacterial content of eggs' shell with the passage of time is known. Another study reported 2.7×10^6 and 1.3×10^7CFU coliforms/g from shells of cracked eggs on 2nd and 4th day of storage at room temperature of 29±1˚C representing (Edema and Atayese, 2006).

From the results of present and earlier studies it may be concluded that coliform bacteria show diminishing rate of survival with time (Omeira *et al.,* 2006). However, storage at room temperature for more than15 days is not recommended because quality of eggs is deteriorated with the increase in storage time and temperature decreased rate of hatchability (Tabidi, 2011). Birds' eggs have a limited shelf life under natural conditions, and may get occurrence of trans-shell infection (Cook *et al.,* 2003). As cuticle composition of egg depends upon hen age and egg freshness, hence with age, a decrease in glycosylation of cuticle leads to depletion of lipids, and increase in permeability of the egg shell which supports trans-shell contamination (Omeira *et al.,* 2006). Although, cuticle is quite resistant to bacterial shell penetration but not when partially or entirely removed (De Reu *et al.,* 2006). The eggshell characteristics such as surface area, shell thickness and number of pores did not influence the bacterial eggshell penetration(De Reu *et al.,* 2006), however porosity of egg shell and membranes facilitate rapid penetration. Storage of the eggs with organic material adhering to egg shell, allows microbial growth (Knape *et al.,* 2002) and water in addition to it serves as a good medium for penetration of microbes across the shell pores (Board and Halls, 1973) and therefore such storage conditions may deteriorate the egg contents as reported by(Jones *et al.,* 2004). These authors documents that 36.7% of the samples expressed an average value of less than 0.1 log CFU of *Enterobacteriaceae* /ml for the eggs' suspension. Cortes *et al.* (2004) showed that 45% of eggs were contaminated with *E. coli*. and had reported that the mean of total viable bacteria and coliform in the egg contents were 3.95×10^4 CFU/g and 4.94×10^3 CFU/g, respectively.

In the present study three species *i.e, E.coli, Klebsiella* and *Shigella* were recognized over the egg shell surface. In similar study, Mahdavi *et al.* (2012) isolated 68.28% of Enterobacteriaceae like *E.coli* and *Klebsiella* over egg shells. The highest count was observed for *Shigella* which appeared in most of the samples (91%) on day 1, whereas *Klebsiella* was isolated only from 29 of the samples. *Shigella* has been isolated from cracked eggs (Edema and Atayese, 2006) and reported to become multidrug resistant by 1953 since an outbreak in Japan (Todar, 2012). However, resistance of Shigella against ampicillin have been found to reduce continuously since 1978 to 2011. Perhaps this is the reason that annual number of Shigella diarrhea and dysentery has been reported upto 165 million leading to 1 million deaths in developing countries (Mead *et al.,* 1999). Resistance against ampicillin lead to discovery of nalidixic acid (Bennish *et al.,* 1992). *E.coli* isolates in this study were found resistant against all the drugs tested. This finding may be due to capability of *E.coli* of facing the challenges of stressful environment resulting into mutations. *E.coli, Enterobacter* and *Klebsiella* have been isolated from egg shell in various percentages with high resistance to streptomycin , tetracycline and kanamycin (Adesiyun *et al.,* 2006)http://www.sciencedirect.com/science/article/pii/S0963996905001675. *Klebsiella* has been declared as ESBLs (Extended spectrum beta- lactamase) which are multidrug resistant (Todar, 2012), and had been found resistant against cephalosporins and monobactams (Gundogan *et al.,* 2011).

A group of enzymes, known as KPC residing on transmissible plasmids (Miriagou *et al.,* 2003), has been known to be responsible to induce resistance in several pathogens, including *E.coli* (Hong *et al.,* 2003), *Klebsiella* sp. (Smith *et al.,* 2003) and *Enterobacter* sp.((Bratu *et al.,* 2005). *E.coli* and *Shigella* were found non-pathogenic being gamma hemolytic. Non-hemolytic strain of *E.coli* being resistant to all antibiotics, may be due to antibiotic resistance development through natural selection (Harrigan, 2008).In another study, non-hemolytic strains of *E.coli* were found 100% and 40% resistant against ampicillin and nalidixic acid respectively (Olowe *et al.,* 2008). Beta hemolytic *Klebsiella* was found susceptible for the drugs used in the present study, as also reported on human erythrocytes in an earlier study (Sekowska *et al.*, 2006).

Dropped bacterial count over eggs' shell surface in present study favors the storage of eggs at room temperature in option to washing. (Theron et al., 2003)recommended the storage of eggs at 25˚C after a cold shock of 4 h to increase their shelf life. As the public health

sector is much weak in developing countries and unhygienic handling of such contaminated food items, especially during cooking etc., when hands might be wet enough to dislocate the surface bacteria, might be associated with the risk of enteric interactions.

The present study is suggestive for formulating recommendations/laws by local public health officials to ensure a suitable range of period of storage of eggs before they are marketed. Obviously the storage period ranges have to be worked out for different seasons and localities of the country. Such simple regulation will add to the efforts to eradicate enteric routed infectious diseases among the masses.

REFERENCES

ADESIYUN, A., OFFIAH, N., SEEPERSADSINGH, N., RODRIGO, S., LASHLEY, V. AND MUSAI, L., 2006. Frequency and antimicrobial resistance of enteric bacteria with spoilage potential isolated from table eggs. *Food Research International,* **39**(2): 212-219.

AL-BACHIR, M. AND ZEINOU, R., 2006. Effect of gamma irradiation on some characteristics of shell eggs and mayonnaise prepared from irradiated from irradiated eggs. *Journal of food safety*, **26**(4): 348-360.

BENNISH, M.L., SALAM, M.A., HOSSAIN, M.A., MYAUX, J., KHAN, E.H., CHAKRABORTY, J., HENRY, F. AND RONSMANS, C., 1992. Antimicrobial resistance of *Shigella* isolates in Bangladesh, 1983-1990: increasing frequency of strains multiply resistant to ampicillin, trimethoprim-sulfamethoxazole, and nalidixic acid. *Clin. Infect. Dis.,* **14:** 1055-1060.

Benson, H., 2001. Microbiological Applications: A laboratory manual in general microbiology. McGraw Hill.

BOARD, R.G. AND HALLS, N.A., 1973. The cuticle: a barrier to liquid and particle penetration of the shell of the hen's egg. *British Poultry Science,* **14**(1): 69-97.

BRATU, S., LANDMAN, D., ALAM, M., TOLENTINO, E. AND QUALE, J., 2005. Detection of KPC carbapenem-hydrolyzing enzymes in Enterobacter spp. from Brooklyn, New York. *Antimicrob. Agents Chemother.,* **49**(2): 776-778.

COOK, M.I., BEISSINGER, S.R., TORANZOS, G.A., RODRIGUEZ, R.A. AND ARENDT, W.J., 2003. Trans-shell infection by pathogenic micro-organisms reduces the shelf life of non-incubated bird's eggs: a constraint on the onset of incubation. *Proc. Biol. Sci.,* **270**(1530): 2233-2240.

CORTES, C.R., TELLEZ, I.G., LOPEZ, C.C., VILLASECA-FLORES, J.M., ANDERSON, R.C. AND ESLAVA, C.C., 2004. Bacterial isolation rate from fertile eggs, hatching eggs, and neonatal broilers with yolk sac infection. *Rev. Latinoam. Microbiol.,* **46**(1-2): 12-16.

DE REU, K., GRIJSPEERDT, K., MESSENS, W., HEYNDRICKX, M., UYTTENDAELE, M., DEBEVERE, J. AND HERMAN, L., 2006. Eggshell factors influencing eggshell penetration and whole egg contamination by different bacteria, including *Salmonella enteritidis*. *International journal of food microbiology*, **112**(3): 253-260.

EDEMA, M. AND ATAYESE, A., 2006. Bacteriological Quality of cracked eggs sold for consumption in Abeokuta, Nigeria. *Int. J. Poult. Sci,* **5**(8): 772-775.

ESTHER, S.T., 2008. Isolation and identification of bacteria from chicken eggshells from poultry, wholesales and retail sources. Micrbiology, Federal University of Agriculture Abeokuta.Ogun State.

GANTOIS, I., DUCATELLE, R., PASMANS, F., HAESEBROUCK, F., GAST, R., HUMPHREY, T.J. AND VAN IMMERSEEL, F., 2009. Mechanisms of egg contamination by Salmonella Enteritidis. *FEMS microbiology reviews,* **33**(4): 718-738.

GHASEMIAN, S.H., JALALI, M., HOSSEINI, A., NARIMANI, T., SHARIFZADEH, H. AND RAHEIM, E., 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 Microbilogy,* **4**(4): 249-253.

GUNDOGAN, N., CITAK, S. AND YALCIN, E., 2011. Virulence properties of extended spectrum beta-lactamase-producing

Klebsiella species in meat samples. *J. Food Prot.,* **74**(4): 559-564.

HANNAH, J.F., WILSON, J.L., COX, N.A., CASON, J.A., BOURASSA, D.V., MUSGROVE, M.T., RICHARDSON, L.J., RIGSBY, L.L. AND BUHR, R.J., 2011. Comparison of shell bacteria from unwashed and washed table eggs harvested from caged laying hens and cage-free floor-housed laying hens. *Poult. Sci.,* **90**(7): 1586-1593.

HARRIGAN, W.F., 2008. Laboratory methods in Food microbiology. Acedemic Press: San Diego, California, USA.

HONG, T., MOLAND, E.S., ABDALHAMID, B., HANSON, N.D., WANG, J. & SLOAN, C. 2003. Program and Abstracts of the 43rd Interscience Conference on Antimicrobial Agents and Chemotherapy. American Society for Microbiology, 75.

JONES, D.R., CURTIS, P.A., ANDERSON, K.E. AND JONES, F.T., 2004. Microbial contamination in inoculated shell eggs: II. Effects of layer strain and egg storage. *Poult. Sci.,* **83**(1): 95-100.

KNAPE, K.D., CHAVEZ, C., BURGESS, R.P., COUFAL, C.D. AND CAREY, J.B., 2002. Comparison of eggshell surface microbial populations for in-line and off-line commercial egg processing facilities. *Poult. Sci.,* **81**(5): 695-698.

MAHDAVI, M., JALALI, M., SAFAEI, H.G. AND SHAMLOO, E., 2012. Microbial quality and prevalence of *Salmonella* and *Listeria* in eggs. *International Journal of Environmental Health Engineering,* **1**(1): 48.

MEAD, P.S., SLUTSKER, L., DIETZ, V., MCCAIG, L.F., BRESEE, J.S., SHAPIRO, C., GRIFFIN, P.M. AND TAUXE, R.V., 1999. Food-related illness and death in the United States. *Emerg. Infect. Dis.,* **5**(5): 607-625.

MIRIAGOU, V., TZOUVELEKIS, L.S., ROSSITER, S., TZELEPI, E., ANGULO, F.J. AND WHICHARD, J.M., 2003. Imipenem resistance in a *Salmonella clinical* strain due to plasmid-mediated class A carbapenemase KPC-2. *Antimicrob. Agents Chemother.,* **47**(4): 1297-1300.

MOREKI, J.C. AND DITSHUPO, T., 2012. Effect of storage time on hatchability of guinea fowl eggs. *J. Anim. Sci. Adv.,* **2**(7): 631-636.

OLOWE, O.A., OGUNGBAMIGBE, T.O., KOLAWOLE, S.O., OLOWE, R.A. AND OLAYEMI, A.B., 2008. Hemolysis production and resistance to fluoroquinolones among clinical isolates of *Escherichia coli* in Osogbo Metropolis, Southwest, Nigeria. *New York Science Journal*, **1**(1): 13-16.

OMEIRA, N., BARBOUR, E.K., NEHME, P.A., HAMADEH, S.K., ZURAYK, R. AND BASHOUR, I., 2006. Microbiological and chemical properties of litter from different chicken types and production systems. *Sci. Total Environ.,* **367**(1): 156-162.

PELCZAR, M.J.JR., CHAN, E.C.S. AND KREIG, N.R., 1986. Microbiology. McGraw Hill Book Co.New York USA.

SEKOWSKA, A., GOSPODAREK, E., JANICKCA, G., JACHNA-SAWICKA, K. AND SAWICKI, M., 2006. [Hydrolytic and haemolytic activity of *Klebsiella pneumoniae* and *Klebsiella oxytoca*]. *Med. Dosw. Mikrobiol.,* **58**(2): 135-141.

SMITH, M.E., HANSON, N.D., HERRERA, V.L., BLACK, J.A., LOCKHART, T.J., HOSSAIN, A., JOHNSON, J.A., GOERING, R.V. AND THOMSON, K.S., 2003. Plasmid-mediated, carbapenem-hydrolysing beta-lactamase, KPC-2, in Klebsiella pneumoniae isolates. J. Antimicrob. *Chemother.,* **51**(3): 711-714.

TABIDI, M.H., 2011. Impact of storage period and quality on composition of table egg. *Advances in Environmental Biology,* **5**(5): 856-861.

THERON, H., VENTER, P. AND LUES, J.F.R., 2003. Bacterial growth on chicken eggs in various storage environments. *Food Research International,* **36**(9): 969-975.

Todar, K. (2012) Todar's Online Textbook of Bacteriology, *Shigella* and *Shigellosis.*