

COMPARATIVE BACTERIAL CONTAMINATION OF EGGS RAISED UNDER DIFFERENT MANAGEMENT SYSTEMS IN PUNJAB, INDIA

SHUMAILA TASKEEN*, RANDHIR SINGH, JASBIR S. BEDI, A.K. ARORA,
R.S. AULAKH and JASWINDER SINGH

Department of Veterinary Public Health and Epidemiology,
Guru Angad Dev Veterinary and Animal Sciences University (GADVASU), Ludhiana Punjab, India

Received: 04.12.2023; Accepted: 17.02.2024

SUMMARY

A cross-sectional study was designed to investigate differences in contamination rates of eggs surface from the deep litter and caged poultry layers farms across five districts in Punjab, India. Around 24 farms (12 per category) were randomly selected and farmers were surveyed using a well-informed questionnaire. Additionally, a total of 480 freshly laid eggs were aseptically collected and subjected to estimation of total bacterial count (TBC), total Enterobacteriaceae count (TEC) and occurrence of *S. aureus* and *E. coli* (critical pathogens). The analysis revealed that deep litter system harbored eggs with significantly higher TBC, TEC, *S. aureus* and *E. coli* compared to cage system. The association of occurrence of the *S. aureus* and *E. coli* from different systems with respect to the factors present on the farm was confirmed using generalized linear model (GLM) to associate self-formulation of feed and absence of biosecurity with higher occurrence of *S. aureus* and *E. coli* in deep litter farms. Therefore, the study highlights the need for implementation of strict biosecurity and sanitary measures during feed formulation for birds and egg production at farm level to minimize the chances of transmission of bacterial load into the consumer's food chain.

Keywords: Cage system, Deep litter, Egg, *Escherichia coli*, *Staphylococcus aureus*

How to cite: Taskeen, S., Singh, R., Bedi, J.S., Arora, A.K., Aulakh, R.S. and Singh, J. (2024). Comparative bacterial contamination of eggs raised under different management systems in Punjab, India. *Haryana Vet.* 63(2): 231-236.

The presence of all essential amino acids (AAs) and non-essential AAs makes poultry eggs the most nutritious foods of animal origin (Kralik *et al.*, 2017). At the time of production, the microbiological safety of eggs is attributed to the innate protection provided by eggshell and a semi-permeable membrane, however, the risk of contamination mainly bacterial in nature upsurges after laying due to the contact with environmental contaminants. Furthermore, the migration of these surface contaminants inside the egg is facilitated by improper handling and unhygienic storage of eggs (high moisture and temperature) (Pyzik and Marek, 2012). Apart from the spoilage causing organisms, a number of clinically significant and food-borne bacterial species such as *Salmonella enterica*, *Yersinia enterocolitica*, *Listeria monocytogenes*, methicillin resistant *Staphylococcus aureus* and *Escherichia coli* have been previously reported from table eggs (Syed *et al.*, 2018). While the surface bacteria pose a major threat to consumer's health, only limited surface decontamination procedures for the eggs are in use worldwide, including countries like United States of America (USA), Australia and Japan (Hutchison *et al.*, 2004). However, few studies have previously reported the inefficiency of current sanitization techniques employed in the commercial egg industry (Al-Ajeeli 2017). Therefore, it can be presumed that bacterial strains with relatively higher tolerance to disinfectants have higher chances of survival on the eggshell surface and are more likely to enter the food chain. Furthermore, very

limited studies have been conducted exploring the differences in surface contamination over the egg surface with respect to the systems of poultry bird rearing. Therefore, present study was undertaken to examine the difference in contamination rate and occurrence of priority pathogens i.e., *S. aureus* and *E. coli* on eggs raised under deep litter and cage system.

MATERIALS AND METHODS

Survey

A meticulously crafted questionnaire was validated through farmer participation at the Kisan Mela, before its subsequent deployment at selected farm sites. The questionnaire was so designed to assess the distinctive characteristics and disparities in management systems and agricultural practices between deep litter and cage farms.

Sample collection and processing

The bacterial contamination was expressed as total bacterial count (TBC) and total *Enterobacteriaceae* count (TEC) per unit surface area (mm²) of eggshell. 24 poultry layer farms (12 per category) across five districts of Punjab viz., Ludhiana, Amritsar, Bhatinda, Rupnagar and Mansa were sampled using convenient and purposive sampling technique representing different geographical regions in the state of Punjab. Further, a total of 20 freshly laid eggs were collected from each farm, thus making it to a total of 480 egg samples.

The processing of eggshell and inner content was

*Corresponding author: shumaila.taskeen@gmail.com

followed using method described by Musgrove *et al.* (2005). Initially, each egg was aseptically handled to measure length and breadth using digital vernier calipers (Advance, Mumbai), followed by cracking of egg at air-sac end using sterile glass rod. The inner content was separated and collected in sterile disposable sampling bag in aseptic manner. The remaining eggshell was put in a sterile disposable bag containing 20 ml of buffered peptone water (BPW) and hand crushed before estimation of TBC, TEC and isolation of *Staphylococcus aureus* and *Escherichia coli*, potentially present on the eggshell. The separated inner content of each individual egg was only used for the isolation of *S. aureus* and *E. coli*.

Total bacterial count (TBC) and total *Enterobacteriaceae* count (TEC)

For the determination of total bacterial count (TBC), each eggshell was washed with 20 ml of BPW in a sterile sampling bag (Nasco sampling bag, HiMedia Lab, Mumbai); 10 ml of wash was used for performing TBC. The wash of 5 eggs was pooled to make one lot. Four such lots were used for each farm to estimate TBC. The formulae used for calculation of surface area of egg and estimation of TBC are mentioned ahead (Equations 1-3). For TBC, after dilution of egg wash, 0.1 ml of the sample was spread over the plate count agar (HiMedia lab, Mumbai) and inoculum was spread evenly using 'L' shaped disposable spreader. The plates were incubated at 37° C for 24-48 h. After incubation, the plates depicting colonies ranging from 25-250 were selected and the colonies were counted using colony counter.

Calculation:

Equation 1:

$$\text{TBC or TEC/surface area of eggs (mm}^2\text{)} = \frac{\text{TBC (CFU) or TEC of eggshells from farm}}{\text{Total surface area of eggshells (mm}^2\text{)}}$$

Equation 2:

$$\text{Surface area of the eggshell (S.A) (mm}^2\text{)} = (3.155 - 0.0136L + 0.0115B) L \times B; L = \text{length (mm), } B = \text{breadth (mm)}$$

(Narushin and Romanov, 2002)

$$\text{Equation 3: Colony forming unit (CFU/ml)} = \text{Average no. of colonies} \times \text{reciprocal of dilution factor}$$

For determination of TEC, 1 ml of diluted samples was poured on sterile petriplates in duplicate. Later, 15 ml of molten violet red bile agar (VRBA), priorly equilibrated at 50° C in water bath was poured over the sample and swirled to mix it with the agar. The agar was allowed to solidify and overlaid with 10-15 ml of VRBA followed by incubation at 37° C for 24 h. The purple red colonies with diameter equal to or more than 0.5 mm were suggestive of members of *Enterobacteriaceae*. The plate depicting

colonies not more than 150 were selected and TEC was determined using the formula mentioned in equations 1-3.

The remaining 10 ml of the individual eggshell wash was used for isolation of *S. aureus* and *E. coli*.

Isolation and identification of *S. aureus* and *E. coli*

Egg samples were processed for *S. aureus* isolation following a method outlined by Syed *et al.* (2018). Egg shell wash and inner content were enriched in trypticase soy broth (TSB) with 10% NaCl and sodium pyruvate and then incubated overnight. A loopful from each sample was streaked onto Baird Parker agar (BPA) with egg yolk telurite emulsion and incubated. Typical colonies were picked, streaked onto brain heart infusion (BHI) agar for purification, and subjected to Gram's staining and catalase test. Positive colonies, showing characteristic morphology and catalase reaction, were stored in BHI broth with glycerol at -20° C.

The isolation of *E. coli* from eggs was performed using method described by Mezhoud *et al.* (2016). The eggshell wash and the inner content were enriched in BHI broth prior to selective plating on eosin methylene blue (EMB) agar. The presumptive colonies producing metallic sheen were further confirmed using matrix assisted laser desorption time of flight (MALDI-TOF).

Molecular confirmation of *Staphylococcus aureus*

The presumptive *S. aureus* were confirmed using genus and species specific polymerase chain reaction (PCR), while ATCC 25923 *S. aureus* strain was used as the positive control. The cycling conditions and primer details have been tabulated in table I.

MALDI-TOF

Presumptive *E. coli* colonies were cultured on BHI agar plates and incubated at 37° C for 24 h. 0.1 mg of cell material from each isolate was transferred to a MALDI target site, treated with 70% formic acid and matrix solution (10 mg/mL concentration of α -cyano-4-hydroxycinnamic acid in 50% acetonitrile and 2.5% trifluoroacetic acid) and then analyzed. Scores ranging from 1.7 to 2.3 indicated varying levels of genus and species identification reliability, with scores above 2.3 indicating highly probable species identification.

Statistical analysis

The statistical analysis was conducted using IBM SPSS Statistics v.26.0 (IBM Corporation, Armonk, NY). The normality of data distribution (TBC and TEC) was checked using Kolmogorov-Smirnov test and the comparison of means between different management systems was done using Kruskal Wallis (TBC) and independent 't' test (TEC),

Table 1. Primers used for the confirmation of *S. aureus*

Category	Gene	Primer sequence	Amplicon size (bp)	Reference
Genus specific	16S RNA	F: CAGCTCGTGTCTGAGATGTR: AATCATTTG TCCCACCTTCG	420	(Strommenger <i>et al.</i> , 2003)
Species specific	nuc	F: GCGATTGATGGTGATACGGTTR: AGCCAAGC CTTGACGAACATAAAGC	279	(Brakstad <i>et al.</i> , 1992)

Table 2. Farm characteristics of layer poultry farms based on different system of rearing

Variables	Response	Frequency of farms		
		Deep litter (N=12)	Cage (N=12)	Total (N=24)
Gender	Male	12 (100%)	12 (100%)	24 (100%)
	Female	0	0	0
Type of farming	Only poultry	10 (83%)	12 (100%)	22 (91.6%)
	Mixed	02 (16.6%)	0	02 (8.3%)
Feed purchase	Self-formulated	10 (83.3%)	0	10 (41.6%)
	Commercially purchased	02 (16.6%)	12 (100%)	14 (58.3%)
Any cleaning of eggs practiced	Yes	0	02 (16.6%)	02 (8.3%)
	No	12 (100%)	10 (83.3%)	22 (91.6%)
Use of litter as manure	Yes	12 (100%)	12 (100%)	24 (100%)
	No	0	0	0
Farm biosecurity	Present	05 (41.6%)	12 (100%)	17 (70.8%)
	Absent	07 (58.3%)	0	07 (29.1%)
Commonly reported diseases	Chronic respiratory disease (CRD)	08 (66.6%)	02 (16.6%)	10 (41.6%)
	Gumboro	05 (51.6%)	0	05 (20.8%)
	Coccidiosis	10 (83.3%)	02 (16.6%)	12 (50%)
	Vitamin deficiency	10 (83.3%)	0	10 (41.6%)

Table 3. Total bacterial count on eggshells from different systems of poultry rearing

System of farming			
Deep litter		Cage farms	
Farm ID	Average log ₁₀ CFU/mm ²	Farm ID	Average log ₁₀ CFU/mm ²
LD-1	6.39	AC-1	5.21
LD-2	5.06	AC-2	3.92
LD-3	4.69	AC-3	3.87
LD-4	5.02	AC-4	5.13
MD-1	5.0	BC-1	3.69
MD-2	5.11	BC-2	4.95
MD-3	5.34	BC-3	3.90
MD-4	5.92	BC-4	3.87
RD-1	5.32	LC-1	4.02
RD-2	6.29	LC-2	4.09
RD-3	5.38	LC-3	3.95
RD-4	5.37	LC-4	5.32
Mean log ₁₀ CFU/mm ² ±S.E	^a 5.40±0.152	^b 4.32±0.179	

a,b different superscripts denote significant difference in between two different farming systems (p<0.05)

Table 4. Total Enterobacteriaceae count per unit (mm²) eggshell surface from different systems of poultry rearing

System of farming			
Deep litter		Cage farms	
Farm ID	Average log ₁₀ CFU/mm ²	Farm ID	Average log ₁₀ CFU/mm ²
LD-1	2.65	AC-1	2.53
LD-2	2.70	AC-2	2.56
LD-3	2.78	AC-3	2.58
LD-4	2.69	AC-4	2.31
MD-1	2.73	BC-1	2.39
MD-2	2.80	BC-2	2.35
MD-3	2.74	BC-3	2.47
MD-4	2.67	BC-4	2.45
RD-1	2.68	LC-1	2.46
RD-2	2.68	LC-2	2.58
RD-3	2.75	LC-3	2.41
RD-4	2.69	LC-4	2.51
Mean log ₁₀ CFU/mm ² ±S.E	^a 2.71±0.013 S.E	^b 2.46±0.025 S.E	

a,b different superscripts denote significant difference in between two different farming systems (p<0.05)

Table 5. *Staphylococcus aureus* contamination of egg samples collected from different systems of poultry rearing

System of farming	Sample	Sample size	Sample (+) for <i>S. aureus</i>	Occurrence %	Overall occurrence %
Deep litter	Eggshell	240	37	15.4	^a 30
	Inner content		35	14.5	
Cage	Eggshell	240	39	^a 16.25	^b 20
	Inner content		09	^b 3.75	

a, b different superscripts indicate significant difference in *S. aureus* contamination in sample from different farming system ($p < 0.05$).

Table 6. Generalized linear model (GLM) estimates and odds ratio for occurrence of *S. aureus* with the predictor variables

Predictor	Odds ratio	p value	95% Wald Confidence interval
Farm type (Deep litter)	13.815	0.000	1.826 to 3.425
Feed formulation (self-formulated)	0.198	0.000	-2.46 to -.775
Biosecurity (present)	0.295	0.000	-1.894 to -0.544
Medication (self-prescribed)	0.992	0.000	-0.612 to 0.596

respectively. The results were expressed as significant if $p < 0.05$.

To analyze the association of response of farmers and contamination percentage of *S. aureus* and *E. coli* in different management systems, generalized linear model (GLM) was employed; wherein farm type, feed formulation, biosecurity were taken as predictors and contamination of *S. aureus* and *E. coli* was taken as dependent variable. To model the occurrence of *S. aureus* and *E. coli*, binomial family distribution with logit function (link = "logit") was used. The difference in occurrence of *S. aureus* and *E. coli* between the management systems was assessed using chi-square statistic, with $p < 0.05$ taken as significant.

RESULTS AND DISCUSSION

Survey

Of all the characteristics surveyed, the major difference in between the two management systems were reportedly; (i) self-formulated feeds were utilized by majority {83.3% (10/12)} of the deep litter farms, while all the caged farms used commercialized feed (12/12; 100%) (ii) None of the deep litter farms engaged in cleaning of eggs, however only 2 out of 12 (16.6%) caged farms practiced cleaning of soiled eggs (iii) With respect to farm biosecurity; 41.6% (5/12) of the deep litter farms and all the caged farms allowed only authorized farm workers to enter inside the farms.

Table 7. *Escherichia coli* contamination of egg samples collected from different system of poultry rearing

System of farming	Sample	Sample size	Sample (+) for <i>S. aureus</i>	Occurrence %	Overall occurrence %
Deep litter	Eggshell	240	59	^a 24.5%	^a 31.25
	Inner content		16	6.6%	
Cage	Eggshell	240	34	^b 14.1%	^b 18.75
	Inner content		11	4.5%	

a,b different superscripts indicate significant difference in *E. coli* contamination in sample from different farming system ($p < 0.05$).

Table 8. Generalized linear model (GLM) estimates and odds ratio for occurrence of *E. coli* with the predictor variables

Predictor	95% Wald Confidence interval	p value	Odds ratio
Farm type (Deep litter)	0.588 to 0.5318	0.03	1.769
Feed formulation (self-formulated)	0.104 to 1.089	0.069	0.337
Biosecurity (present)	0.215 to 0.850	0.015	0.427
Medication (self-prescribed)	0.866 to 2.771	0.140	1.549

TBC and TEC

The TBC of eggshell composite lots ranged from 5 \log_{10} to 6.39 \log_{10} in deep litter farms and from 3.69 \log_{10} to 5.32 \log_{10} in cage systems. A significant difference ($p < 0.05$) was found between the mean TBC of both farming systems, with deep litter farms showing higher contamination levels. Similarly, the TEC ranged from 2.65 \log_{10} to 2.80 \log_{10} in deep litter farms and from 2.31 \log_{10} to 2.58 \log_{10} in cage systems. The mean TEC of deep litter farms was significantly higher compared to cage systems ($p < 0.05$). The environmental bacterial contaminants cause contamination while the egg passes through the vent; therefore, the types of rearing and management (deep litter or cage) could play a significant role in surface microbial load and safety of eggs. The present study reported that total bacterial load (TBC) and total *Enterobacteriaceae* count (TEC) from the eggshells in deep litter system was significantly higher than in eggshells from cage system. These findings were similar to reports by previous researchers (Samiullah *et al.*, 2017, Fahim *et al.*, 2021). The probable reason for increased contamination in deep litter could be due to higher chances of contact with litter, soil and dirt of the farm in the deep litter system, while in cage system, eggs are physically segregated from the hens after laying thus reducing the probability of contamination (Hannah *et al.*, 2011).

Occurrence of *S. aureus*

The occurrence of *S. aureus* in egg samples from deep litter farms was 30% (72/240) and from cage farms was 20% (48/240). In deep litter farms, *S. aureus* was found in 15.4% (37/240) of eggshells and 14.5% (35/240) of inner content, while in cage farms, it was present in 16.25% (39/240) of eggshells and 3.75% (9/240) of inner content. The occurrence was significantly higher in deep litter farms ($p < 0.05$). The presence of *S. aureus* was influenced by farming system, feed formulation, and biosecurity, with deep litter farms using self-formulated feed and lacking biosecurity showing higher contamination. Odds of *S. aureus* contamination were 13 times higher in deep litter farms compared to cage farms, while biosecurity presence reduced contamination frequency (odds ratio: 0.295). These findings were compared with the previously reported studies where the *S. aureus* contamination on eggshells ranged from 5.5% to 14.5% (Pondit *et al.*, 2018, Parveen *et al.*, 2017, Eid *et al.*, 2015, Pyzik *et al.*, 2014, Evêncio-Luz *et al.*, 2012). The difference in the level of contamination of the eggshell surface could be attributed to the difference in geographical location of the farms, farm management practices and hygienic measures at the farm and variation in laboratory detection methods. The incidence of *S. aureus* contamination over the eggshell and inner egg content *viz.*, yolk and albumen pose a potential risk of zoonotic transmission of the pathogen to the consumers (Salihu *et al.*, 2015). Previously, multiple studies have documented the occurrence of *S. aureus* on chicken meat (Abolghait *et al.*, 2020, Bernier-Lachance *et al.*, 2020, Fox *et al.*, 2017, Hanson *et al.*, 2011 and Kitai *et al.*, 2005) however, the studies exploring the incidence of *S. aureus* on eggs are relatively much lower compared to the other food products including meat.

Occurrence of *E. coli*

In the deep litter poultry farming system, 31.25% (75/240) of egg samples were contaminated with *E. coli*, with 24.5% (59/240) and 6.6% (16/240) contamination rates for eggshells and inner content, respectively. In contrast, in cage systems, 18.75% (45/240) of egg samples were contaminated with *E. coli*, with 14.16% (34/240) and 4.5% (11/240) contamination rates for eggshells and inner content, respectively. Eggshells from deep litter systems showed significantly higher contamination compared to cage systems ($p < 0.05$).

The occurrence of *E. coli* was correlated with farm characteristics, revealing that farming system and biosecurity significantly influenced *E. coli* occurrence ($p < 0.001$). Higher occurrence was noted in deep litter systems without biosecurity; presence of biosecurity

reduced *E. coli* occurrence, indicated by a low odds ratio (0.427, 95% C.I: 0.20-0.83). Deep litter systems harbored significantly more *E. coli* compared to cage farms ($p < 0.05$), with odds of *E. coli* occurrence 1.769 times higher in deep litter compared to cage systems. These results were compared to previously documented studies where *E. coli* contamination on eggs varied across different geographical regions round the world *viz.*, 37% in Pakistan (Khan *et al.*, 2016); 50% in Southern India (Vinayananda *et al.*, 2017), 34.26% in Zambia (Kapena *et al.*, 2020), 48.7% in Gauteng province of South Africa (Adesiyun *et al.*, 2020), 38.89% in Bangladesh (Hossain *et al.*, 2021); 19.8% in Western Australia (Sodagari *et al.*, 2021), 23% in Nigeria (Olopade *et al.*, 2022) and 11.7% in Punjab, India (Sharan *et al.*, 2023). Since, the major focus of surveillance in majority of the previous studies had been cloacal swabs, chicken meat and poultry litter, present study stands out to be an elaborately conducted study on eggs acknowledging the differences in contamination percentage due to difference in poultry rearing systems.

CONCLUSION

The eggs produced under deep litter and cage farms could be a source of surface bacterial contaminants; however, the risk of such contamination increases with the exposure of eggs with infected floor surface, poultry litter material and droppings. Therefore, effective biosecurity measures and controlling hazards at the farm level will help in eliminating the risk of contaminated eggs entering the food chain.

REFERENCES

- Abolghait, S.K., Fathi, A.G., Youssef, F.M. and Algammal, A.M. (2020). Methicillin-resistant *Staphylococcus aureus* (MRSA) isolated from chicken meat and giblets often produces staphylococcal enterotoxin B (SEB) in non-refrigerated raw chicken livers. *Int. J. Food Microbiol.* **328**: 108669.
- Adesiyun, A.A., Nkuna, C., Mokgoatheng Mamogobo, M., Malepe, K. and Simanda, L. (2020). Food safety risk posed to consumers of table eggs from layer farms in Gauteng Province, South Africa: Prevalence of *Salmonella* species and *Escherichia coli*, antimicrobial residues and antimicrobial resistant bacteria. *J. Food Saf.* **40**(3): e12783.
- Al-Ajeeli, M.N.H. (2017). Evaluation of Performance of Hy-line Brown Laying Hens Fed Soybean and Soybean-free Diets Using Cage and Free-range Rearing Systems (Doctoral dissertation).
- Al-Jaff, B. (2005). The risk of bacterial contamination in hen eggs of Sulaimani poultries. *JZS-A.* **8**(1): 63-71.
- Bernier-Lachance, J., Arsenault, J., Usongo, V., Parent, É., Labrie, J., Jacques, M. and Archambault, M. (2020). Prevalence and characteristics of livestock-associated methicillin-resistant *Staphylococcus aureus* (LA-MRSA) isolated from chicken meat in the province of Quebec, Canada. *PLoS One.* **15**(1): e0227183.
- Brakstad, O.G., Aasbakk, K. and Maeland, J.A. (1992). Detection of

- Staphylococcus aureus* by polymerase chain reaction amplification of the nuc gene. *J. Clin. Microbiol.* **30**: 1654-60.
- Eid., S., Nasef, A., Soad, M. and Erfan, Ahmed (2015). Multidrug resistant bacterial pathogens in eggs collected from backyard chickens. *Assiut Vet. Med. J.* **61(144)**: 87-103.
- Fahim, K.M., Khalf, M.A.M., Nader, S.M. and Ismael, E. (2021). Impacts of housing and storage environments on physical quality and the potential public health risks of chicken table eggs. *Adv. Anim. Vet. Sci.* **9(8)**: 1176-1189.
- Fox, A., Pichon, B., Wilkinson, H., Doumith, M., Hill, R.L.R., McLauchlin, J. and Kearns, A.M. (2017). Detection and molecular characterization of Livestock Associated MRSA in raw meat on retail sale in North West England. *Lett. Appl. Microbiol.* **64(3)**: 239-245.
- 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.
- Hanson, B.M., Dressler, A.E., Harper, A.L., Scheibel, R.P., Wardyn, S.E., Roberts, L.K., Kroeger, J.S. and Smith, T.C. (2011). Prevalence of *Staphylococcus aureus* and methicillin-resistant *Staphylococcus aureus* (MRSA) on retail meat in Iowa. *J. Infect. Public Health.* **4(4)**: 169-174.
- Hossain, M.S., Rahman, W., Ali, M.S., Sultana, T. and Hossain, K.M.M. (2021). Identification and antibiogram assay of *Escherichia coli* isolated from chicken eggs. *J. Biosci.* **29(1)**: 123-133.
- Hutchison, M.L., Gittins, J., Walker, A., Sparks, N., Humphrey, T.J., Burton, C. and Moore, A. (2004). An assessment of the microbiological risks involved with egg washing under commercial conditions. *J. Food Prot.* **67(1)**: 4-11.
- Kapena, M.S., Muma, J.B., Mubita, C.M. and Munyeme, M. (2020). Antimicrobial resistance of *Escherichia coli* and *Salmonella* in raw retail table eggs in Lusaka, Zambia. *Vet. World.* **13(11)**: 2528.
- Khan, A., Rind, R., Shoaib, M., Kamboh, A.A., Mughal, G.A., Lakho, S.A., Malhi, K.K., Nizamani, A.R. and Yousaf, A. (2016). Isolation, identification and antibiogram of *Escherichia coli* from table eggs. *J. Anim. Health Prod.* **4(1)**: 1-5.
- Kitai, S., Shimizu, A., Kawano, J., Sato, E., Nakano, C., Uji, T. and Kitagawa, H. (2005). Characterization of methicillin-resistant *Staphylococcus aureus* isolated from retail raw chicken meat in Japan. *JVMS.* **67(1)**: 107-110.
- Kralik, I., Tolušić, Z. and Bošnjaković, D. (2017). Production of poultry meat and eggs in the Republic of Croatia and in the European Union. *Ekonom. Vjesnik.* **30(1)**: 85-96.
- Mezhoud, H., Chantziaras, I., Iguer-Ouada, M., Moula, N., Garmyn, A., Martel, A., Touati, A., Smet, A., Haesebrouck, F. and Boyen, F. (2016). Presence of antimicrobial resistance in coliform bacteria from hatching broiler eggs with emphasis on ESBL/AmpC-producing bacteria. *Avian Pathol.* **45(4)**: 493-500.
- Musgrove, M.T., Jones, D.R., Northcutt, J.K., Harrison, M.A., Cox, N.A., Ingram, K.D. and Hinton, Jr. A.J. (2005). Recovery of *Salmonella* from commercial shell eggs by shell rinse and shell crush methodologies. *Poult. Sci.* **84(12)**: 1955-1958.
- Narushin, V.G. and Romanov, M.N. (2002). Egg physical characteristics and hatchability. *J. World's Poult. Sci.* **58(3)**: 297-303.
- Olopade, A., Bitrus, A.A., Momoh-Zekeri, A.H.H. and Bamaïyi, P.H. (2022). Multi-drug resistant phenotypes of extended-spectrum -lactamase (ESBL)-producing *E. coli* from layer chickens. *IJVS.* **36(4)**: 945-951.
- Parveen, A., Rahman, M.M., Fakhruzzaman, M., Akter, M.R. and Islam, M.S. (2017). Characterization of bacterial pathogens from egg shell, egg yolk, feed and air samples of poultry houses. *AJMBR.* **3(2)**: 168-174.
- Pondit, A., Haque, Z.F., Sabuj, A.A.M., Khan, M.S.R. and Saha, S. (2018). Characterization of *Staphylococcus aureus* isolated from chicken and quail eggshell. *JAVAR.* **5(4)**: 466.
- Pyzik, E. and Marek, A. (2012). Characterization of bacteria of the genus *Staphylococcus* isolated from the eggs of Japanese quail (*Coturnix coturnix japonica*). *Pol. J. Vet. Sci.* **15(4)**: 767-772.
- Pyzik, E., Marek, A. and Hauschild, T. (2014). Characterisation of *Staphylococcus aureus* and *Staphylococcus aureus*-like strains isolated from table eggs. *J. Vet. Res.* **58(1)**: 57-63.
- Salihu, M.D., Garba, B. and Isah, Y. (2015). Evaluation of microbial contents of table eggs at retail outlets in Sokoto metropolis, Nigeria. *Sokoto J. Vet. Sci.* **13(1)**: 22-28.
- Samiullah, S., Omar, A.S., Roberts, J. and Chousalkar, K. (2017). Effect of production system and flock age on eggshell and egg internal quality measurements. *Poult. Sci.* **96(1)**: 246-258.
- Sharan, M., Dhaka, P., Bedi, J.S., Singh, R. and Mehta, N. (2023). Characterization of chicken eggs associated *Escherichia coli* and *Staphylococcus aureus* for biofilm production and antimicrobial resistance traits. *Anim. Biotechnol.* **34(8)**: 3533-3544.
- Sodagari, H.R., Wang, P., Robertson, I., Abraham, S., Sahibzada, S. and Habib, I. (2021). Antimicrobial resistance and genomic characterisation of *Escherichia coli* isolated from caged and non-caged retail table eggs in Western Australia. *Int. J. Food Microbiol.* **340**: 109054.
- Strommenger, B., Kettlitz, C., Wener, G. and Witte, W. (2003). Multiplex PCR assay for simultaneous detection of nine clinically relevant antibiotic resistance genes in *Staphylococcus aureus*. *J. Clin. Microbiol.* **41(9)**: 4089-94.
- Syed, M.A., Shah, S.H.H., Sherafzal, Y., Shafi-ur-Rehman, S., Khan, M.A., Barrett, J.B., Woodley, T.A., Jamil, B., Abbasi, S.A. and Jackson, C.R. (2018). Detection and molecular characterization of methicillin-resistant *Staphylococcus aureus* from table eggs in Haripur, Pakistan. *Foodborne Pathog Dis.* **15(2)**: 86-93.
- Vinayananda, C.O., Fairoze, N., Madhavaprasad, C.B., Byregowda, S.M., Nagaraj, C.S., Bagalkot, P. and Karabasanavar, N. (2017). Studies on occurrence, characterisation and decontamination of emerging pathogenic *Escherichia coli* (STEC, ETEC and EIEC) in table eggs. *Br. Poult. Sci.* **58(6)**: 664-672.