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Bacterial contaminants in chicken eggs available from various sources

A.R. KALDATE[1], R.S. GANDGE[1], R.J. ZENDE[2], D.N. DESAI[3] AND R.R. PHARANDE[1]*

[1]Department of Veterinary Microbiology, [2]Department of Veterinary Public Health, [3]Department of Poultry Science, Mumbai Veterinary College, Maharashtra Animal and Fishery Sciences University, Nagpur Maharashtra, India

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ABSTRACT

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Microbial contamination of eggs significant impact on poultry industry and poses a serious public health problem risk globally In present study a total of 40 chicken egg samples, comprising 10 eggs each from a poultry farm, wholesaler shop, retail shop, and directly from a transportation vehicle delivering eggs from farm to wholesaler. The mean Total Viable Count (TVC) on eggshell surfaces was 106.20, 168.00, 165.00 and 250.00×10^4 CFU/ml respectively. The TVC on poultry farms eggsshells was significantly higher (P≤0.01, while wholesaler shop eggs recorded significantly lower TVC (P≤0.01) compared to other sources.The TVC differences between eggs from retailer shops and transportation vehicles were not statistically significant. No microbial growth was detected in the egg contents of the any samples. Among the 40 eggshell samples, 30/40 (75%) yielded various *Staphylococcus* spp isolates including *Staphylococcus lentus* in 12/40 (40%), *Staphylococcus xylosus* in 9/40 (30%), *Staphylococcus kloosii* in 2/40 (7%), *Staphylococcus sciuri* in 7/40 (23%). The remaining 10/40 (25%) isolates were *Proteus* spp 4, *Citrobacter* spp. 2, and *Klebsiella* spp 4 were. All 30 *Staphylococcus* spp isolates tested negative for biofilm production on Congo red agar and presence of the *Nuc* gene via PCR amplification. Antibiotic sensitivity tests on 40 isolates using disc diffusion method revealed that *Staphylococcus* spp isolates were sensitive to Ciprofloxacin, Vancomycin, Gentamicin, but resistance to Penicillin and Erythromycin. *Proteus* spp, *Klebsiella* spp, and *Citrobacter* spp. were sensitive to Ciprofloxacin, Gentamicin, Neomycin and resistant to Penicillin and Erythromycin.

Keywords: Chicken eggs**,** Microbial contaminants, Total Viable Count, Antibiogram

INTRODUCTION

Poultry eggs are the most consumed food globally. In 2018 World Health Organization (WHO) stated that poultry eggs contribute significantly as a readily available source of protein to an average human being. Bacterial contamination of eggs can occur vertically and horizontally (De Reu *et al.*, 2008). Horizontal contamination is more likely associated with dirty shells, cracked eggs, and storage in contaminated environments. The membranes, yolk and albumen, can be directly contaminated through the trans-ovarian pathway and during the laying of the eggs (Messens *et al.*, 2005; Abdullah, 2010). A high number of microorganisms on the eggshell increases the risk of microbial penetration and contamination of the egg content through the shell pores. During transportation, many eggs get cracked, some remain soiled, and some may be dirty when arriving at the wholesaler's market. The retailers then purchase these eggs from the wholesaler and sell them to consumers in the prevailing condition. This may lead to severe health hazards and constitute risk factors for consumers. Therefore, good handling, appropriate storage, and other conditions are essential to minimize egg contamination throughout the chain from farm to consumer. Bacterial contamination of eggs can compromise egg quality, leading to spoilage and the transmission of pathogen. The TVC on eggshells is a crucial measure, as it affects both the safety of eggs and the shelf life of finished goods.

Antibiotics play an essential role in poultry farming. The use of antibiotics in treating and preventing bacterial infection, as well as growth promoters at sub-therapeutic levels, has contributed to the rise of antibiotic resistance bacteria. Given the economic importance and public health implications of egg-borne bacterial pathogens and antimicrobial resistance, the present study aims to detect bacterial contaminants from chicken eggs and analyze the antibiogram pattern of bacterial isolates recovered.

MATERIALS AND METHODS

Determination of total viable counts (TVC)

Processing of chicken eggs was conducted for both eggshell and egg contents separately, adhering to all the aseptic measures within the biosafety facility. A total of 40 chicken eggs, comprising 10 eggs each from a poultry farm, wholesaler shop, retail shop, and directly from the vehicles delivering eggs from farm to wholesaler, were investigated. For evaluating total viable counts, a standard spread plate technique was followed wherein the samples were serially diluted from 10^{-1} to 10^{-10}, and 100 µl of respective dilution was spread on the surface of plate count agar with the help of an L-shaped spreader. After applying the inoculums, the plates were incubated aerobically at 37ºC for 24 hrs hrs. The plates having between 30-300 colonies were counted using colony counter. The Total Viable Count (TVC) was determined

*Corresponding author E mail: rpharande@gmail.com

in CFU/ml using the following formula.

$$CFU/ml = \frac{(\text{Number of colonies}) \times \text{Dilution factor}}{\text{Volume of sample}}$$

Data obtained from this study were analyzed using a completely randomized design in ICAR wasp 2.0 software to determine the total viable count from egg samples.

To determine the TVC from eggshells, a swabbing method was applied for the surface bacterial count as per Loongyai *et al.* (2011). The surface of a whole egg was aseptically swabbed using a sterile wet cotton swab, which was diluted with normal saline. The samples were further diluted in a series (10^{-1} to 10^{-10}), and 100 µl of each dilution was spread on the surface of a plate count agar (Himedia, India). The Petri dishes with the inoculum were incubated at 37°C for 24-48 hrs, and TVC was determined as mentioned above. For the enumeration of bacteria in egg contents, the eggs were first dipped in 75% ethanol for 5 min and allowed to air dry. The narrow end of the egg was flamed for 5–10 sec, and then a wide hole was made with sterilized forceps. The whole egg contents were collected and mixed in a sterile Petri dish and 100 µl inoculated on Plate Count Agar (PCA) after serial dilution (10^{-1} to 10^{-10}) in a normal saline solution for the total bacterial count. The inoculated Petri dishes were incubated at 37ºC for 24–48 hrs, and TVC was determined as mentioned above.

Isolation and identification of bacterial contaminants

Isolation and identification of bacterial contaminants were performed using conventional methods based on morphology, colony characteristics, and biochemical test per Cowan and Steel (1970). To identify *Staphylococcus* at the species-level VITEK 2 systems Version 9.02 (Biomerieux) was used.

Qualitative detection of biofilm production by *Staphylococcus* spp was performed by using the CRA as described by Vasudevan *et al.* (2003). CRA plates were inoculated with *Staphylococcus* spp isolates and incubated aerobically for 24 hrs at 37°C followed by subsequent storage at room temperature for 24 hrs. A positive result was indicated by black colonies with a dry crystalline consistency. Non-slime producers remained red with smooth or dry crystalline colonial morphology.

The amplification of the 270 bp region of the *Nuc* gene of *Staphylococcus* spp was carried out using published oligonucleotide primer sequences (Ali *et al*., 2014). The oligos were manufactured and supplied by M/s Eurofins Genomics India Pvt. Ltd. Bengaluru(India).

Antimicrobial Susceptibility Testing of Bacterial Isolates

Antimicrobial sensitivity of isolates was performed by disc diffusion assay on Mueller-Hinton agar (MHA) using antibiotic discs Ciprofloxacin (5ìg), Penicillin (2ìg), Cotrimoxazole (25ìg), Oxytetracycline (30ìg), Tetracycline (30ìg), Vancomycin (30ìg), Erythromycin (15ìg), Gentamicin (10ìg), Neomycin (10ìg), and Azithromycin (15ìg). Each bacterial isolate's was suspensded in a solution to achieve equivqlent to 0.5 McFarland standards. Antibiotic discs were placed on the surface of the MHA plate, which was subsequently incubated at 37^{O}C for 24 hrs. Based on the diameter of the zones of inhibition around the antibiotic discs, the organisms were classified as susceptible, intermediate, or resistant to a specific antibiotic (CLSI, 2018).

RESULTS AND DISCUSSION

Determination of total viable counts (TVC)

The eggshell and egg contents were processed separately to determine total viable counts (TVC) on the egg surface and the internal contents of eggs to detect horizontal and vertical contamination of eggs.

In the current investigation, the TVC counts from various sources showed 1.06×106 CFU/ml from a wholesaler shop, 1.68×10^{6} CFU/ml in a retailer shop, 1.65×10^{6} CFU/ml from a vehicle, and 2.5×10^{6} CFU/ml on the eggshell surfaces of a chicken farm. Compared to eggs from all other sources, eggs from poultry farms had significantly ($P\leq0.01$) higher total viable counts on shell surfaces. The eggs obtained from wholesaler shops had significantly ($P\leq0.01$) lesser TVC on eggshell surfaces than all other sources. However, there was no statistically significant difference between the TVC counts on the shell surfaces of the eggs collected from retail shops and transportation vehicles. Location-wise analysis of egg roll samples was studied by Nwachukwu and Juliet . (2018), who investigated a total of 60 pieces of egg rolls from hawkers of four central locations in Delta state and screened for pathogenic bacterial contamination and reported total aerobic count ranged from 2.50×10^{6} – 4.80×10^{8} CFU/g. Area-wise analysis of eggshells conducted by Sar *et al.* (2020) revealed CFU ranging between 1.0×10^{6} - 1.1×10^{8}/ml in Jos East, 4.3×10^{5} – 6.0×10^{7}/ml in Jos South, and 3.4×10^{5} – 1.1×10^{8}/ml in Jos North respectively. Ansah *et al.* (2009) assessed the microbial quality of table eggs sold in four selected markets within the Tamale Metropolis.

In the current study, 40 eggs processed for TVC of egg contents revealed none of the egg samples had any detectable bacterial counts. According to Mayes *et al.* (1983), absent of bacteria when the eggs are laid; the eggs get contaminated by the fractured shells. The viscous nature of the egg white proteins, their pH, and the bactericidal qualities of the lysozyme and conalbumin present in egg contents all impede the growth of bacteria.

Isolation and identification of bacteria from eggshell surfaces and contents

In order to isolate aerobic bacteria, the eggshell surfaces of 40 chicken eggs, comprising 10 eggs each from a poultry farm, wholesaler shop, retail shop, and a transportation vehicle conveying eggs from farm to wholesaler, were treated. A total of 40 bacterial isolates

were recovered, proving that all 40 eggshell surfaces were infected. The collected bacterial isolates were in the Enterobacteriaceae and Staphylococcus spp.

Staphylococcus spp. were identified at the higher frequency of 30/40 eggs (75.0%) from wholesaler stores, retailer shops, and poultry farms. Whereas 10/40 (25%) of the eggshell surfaces of the transporting vehicle egg were from the Enterobacteriaceae family. *Staphylococcus* spp, included *Staphylococcus lentus* 12 (40%), *Staphylococcus xylosus* 9 (30%), *Staphylococcus loose* 2 (7%), *and Staphylococcus sciuri* 7 (23%) species. Enterobacteriaceae isolates belonged to *Proteus* 4 (40%), *Citrobacter* 2 (20%), and *Klebsiella* 4 (40%) species. In the current investigation, none of the samples from any source was positive for Salmonella, *E. coli*, and *Mycoplasma* spp.

The higher incidence rate of *Staphylococcus* spp (75%) is similar to the observations reported by Fardows *et al.* (2016), who conducted a study of 150 eggshells and observed that 120 (80%) yielded growth of different bacteria; of them, 80 (66.67%) were *Staphylococcus* spp. Akhi *et al.* (2019) collected a total of 75 samples; among them, 53 samples showed *Staphylococcus* spp (*S.aureus*) isolates from 21/25 (84%) egg samples. In the present study *Staphylococcus* spp viz. *Staphylococcus lentus* in 12 (30%), *Staphylococcus xylosus* in 9 (22%), *Staphylococcus kloosii* in 2 (5%), *Staphylococcus sciuri* in 7 (18%) samples were isolated. Syed *et al.* (2018) isolated a total of 21.3% (64/300) Staphylococci from the table eggs, the eggshell contained 59% (38/64) *S. aureus*. They observed a distribution of the remaining 26 isolates of Staphylococci were *S. haemolyticus* (1/64; 1.6%), *S. simulans* (7/64; 10.9%), *S. simulan/S. haemoliticus* (13/64; 20.3%), *S. vitulinus* (2/64; 3.1%), *S. sciuri* (1/64; 1.6%), and *S. lentus* (2/64; 3.1%). Similar findings of *Staphylococcus sciuri* and *Staphylococcus lentus* isolates as of present investigation was observed by Syed *et al.* (2018).

All 30 isolates of *Staphylococcus* spp from eggshell surfaces were observed to be non-slime red with smooth or dry crystalline colonial morphology indicating an absence of biofilm-forming ability of *Staphylococcus* spp. Bakheet *et al.* (2019) studied the characterization of 37 *Staphylococcal* isolates, of which 21 (56.8%) were identified as coagulase-positive *Staphylococci* (CoPS) and 16 (43.2%) were identified as coagulase-negative *Staphylococci* (CoNS). By using Congo Red Agar (CRA) method, 76.2 % of 21 CoPS were positive for biofilm production with variable degrees. Aziz *et al.*, (2019) studied the biofilm formation of 66 strains of *S. aureus* using the Congo red method, 25/66 (37.9%) showed weak biofilm formation whereas 12/66 (18.2%) and 5/ 66 (7.6%) exhibited medium and strong biofilm formation capacity respectively.

Out of 30 bacterial isolates of *Staphylococcus* spp subjected to PCR for amplification of Nuc gene, none of the isolates showed positive results for the presence of Nuc gene and only the positive control of *Staphylococcus* yielded an amplicon of 270 bp. The extracellular thermostable nuclease (TNase) is produced by *Staphylococcus* spp strains. The TNase protein producing *Nuc* gene is used in many laboratories for identification *Staphylococcus* spp. In the present study, the *Nuc* genes in PCR were used for identification of the 30 *Staphylococcus* spp from eggshell surfaces, however none of isolates showed amplicon of 270 bp. Whereas the known positive, standard *S. aureus* yielded amplicon of 270 bp of *Nuc* gene. Bi *et al.* (2018) investigated the microbiological profile/safety of street vended foods and associated environmental samples collected from Mumbai and Delhi. A total of 80 *Staphylococcus* spp. isolates were screened by *Nuc* genes PCR, and 23 (28.75%) were found to be positively indicated as *S. aureus*. Pondit *et al.* (2018) conducted a study prevalence and characterization of *Staphylococcus aureus* from 220 chicken and 80 quail eggshells and they obtained 27 isolates of *S. aureus* out of which they screened 4/7 isolates positive for *Nuc* gene PCR.

The egg contents of all 40 eggs were found to be free of bacterial contamination. The similar findings were made by Chousalkar and Roberts (2012) when they examined the incidence of *Salmonella* spp. in the interior contents of unwashed eggs collected from commercial caged layer farms in Australia. Eggs' internal components, such as the yolk and albumen, were examined for *Salmonella* spp., but none were found.

Antibiogram pattern of bacterial isolates from eggs

The results of an antibiotic sensitivity test using the disc diffusion method were reported for all 40 recovered isolates. So, of the 40 bacterial isolates found on eggshell surfaces, 30 were Gram-positive *Staphylococcus* species, and they all showed a pattern of antibiogram resistance to Penicillin and Erythromycin and sensitivity to Ciprofloxacin, Vancomycin, and Gentamicin. Ten Gram-negative bacterial isolates, however, were discovered to be susceptible to the antibiotics Ciprofloxacin, Gentamicin, and Neomycin and resistant to Penicillin and Erythromycin. *Staphylococcus* spp. isolates ranged in sensitivity to Ciprofloxacin, Vancomycin, and Gentamycin from 28, 28, and 27, respectively. While 25 and 18, respectively, of the *Staphylococcus* spp. isolates were resistant to Penicillin and Erythromycin. All 10 *Proteus, Citrobacter* and *Klebsiella* spp. isolates were susceptible to Ciprofloxacin, Gentamycin and Neomycin. In contrast, 10 and 7 isolates showed resistance to Penicillin and Erythromycin, respectively.

Staphylococcus spp bacterial isolates showed sensitivity to Gentamicin, Ciprofloxacin, and Vancomycin resistance to Erythromycin. The Gram-positive bacteria have the highest sensitivity to Gentamicin, Ciprofloxacin,

and Vancomycin. However, resistance to Erythromycin was observed by Adabara *et al.* (2020), Fardows *et al.* (2016), and Bakheet *et al.* (2019). The highest sensitivity rate (100%) was recorded against Gentamicin and Ciprofloxacin by Jain *et al*. (2017) from the eggshells. *Staphylococcus* spp. being resistant to Penicillin, Erythromycin, Tetracycline, Amoxicillin, and Ampicillin but sensitive to Ciprofloxacin and Gentamicin, as observed by Haque *et al.* (2021). Vancomycin has highly susceptible to the Staphylococcus spp isolates, these isolates also showed over-resistance to Penicillin, as observed by Kadhim *et al.* (2020). The present study also recorded similar observations in *Staphylococcus* spp isolates from eggshells, showing resistance to Penicillin, Tetracycline, and Erythromycin and sensitivity to Vancomycin. All 10 Gram-negative isolates in the current study content, 4 *Proteus* spp., 2 *Citrobacter* spp., and 4 *Klebsiella* spp, showed sensitivity to Ciprofloxacin. According to Ghohar *et al.* (2017), all isolates were Ciprofloxacin-sensitive, and Jain *et al.* (2017) observed that different gram-negative bacteria had the highest Ciprofloxacin sensitivity rates.

CONCLUSIONS

All 40 eggshell surfaces were found contaminated with bacteria; in contrast, egg contents of all 40 chicken eggs investigated indicated the absence of bacterial contaminants. The TVC was significantly ($P \leq 0.01$) higher in egg shells from poultry farms, whereas TVC was significantly ($P \leq 0.01$) lower in egg shells from wholesaler shops. The eggshell surfaces were predominantly contaminated with *Staphylococcus* spp. followed by *Proteus, Klebsiella* and *Citrobacter* spp. None of *Staphylococcus* spp. isolates was positive for biofilm production and Nuc gene. *Staphylococcus* spp. showed highest sensitivity to Ciprofloxacin, Vancomycin, and Gentamicin, whereas *Proteus* spp., *Klebsiella* spp. and *Citrobacter* spp. were sensitive to Ciprofloxacin, Gentamicin and Neomycin. All isolates from the egg were found resistant to Penicillin and Erythromycin. Hence, it is concluded that eggs were exposed to surface contamination from different sources. Therefore, looking into the consumers' safety, it is advised to reduce the bacterial contamination of egg surfaces by taking proper care at the farm level, while transporting and handling the eggs.

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