Research Article

Isolation, Identification and Antibiogram of *Escherichia coli* from Table Eggs

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Abstract | *Escherichia coli* is one of the common microbial flora of poultry gut. Most of *E. coli* isolates are nonpathogenic but are considered to be an indicator of fecal contamination in food industry. A study was carried-out on the prevalence, incidence, isolation and antibiogram of *E. coli* from table eggs. A total of 100 table eggs were collected from various locations of district Peshawar, Pakistan and divided into three parts *viz.*, the egg-yolk, egg-white and eggshell. These were cultured on different media and identified organism was subjected to antibiogram study using the disk diffusion method. The overall prevalence of *E. coli* was found as 37%. While, incidence was recorded as 15% in eggshells, 12% in egg-whites and 10% in egg-yolks. It was concluded that the table eggs were contaminated with *E. coli* and higher incidence of *E. coli* was recorded in eggshells as compared to other components of the eggs. The antibiotics ciprofloxacin and enrofloxacin were recorded highly active against *E. coli*.

Keywords | *Escherichia coli*, Egg-shell, Egg-yolk, Prevalence, Egg-white, Antibiogram, Peshawar

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INTRODUCTION

Poultry farming is widely adopted in Pakistan and almost every farmstead keeps some poultry mainly for consumption and cash sales. The science and technology have contributed widely for the expansion of poultry industry and a number of strategies have been adopted to modulate the quality of poultry products (Abel et al., 2014). In Pakistan, there are about 25000 poultry farms, providing employment and income for livelihood of fifteen thousand people. In the country, there are 400 hatcheries, 150 feed mills, 8.5 million broiler breeders, 0.428 million layer breeders and their feed consumption is 5.51 million metric tons per year (Anonyms, 2011; FAO, 2011).

E. coli are one of the common microbial flora of gut of farm animals, poultry and human being. Most of *E. coli* isolates are harmless, however, some strains are pathogenic and may cause serious food poisoning in human beings (Begum et al., 2014). A recent survey about prevalence of virulence *E. coli* based on Congo red binding ability have indicated more than 90% isolates as pathogenic (Yadav et al., 2014). In past two decades, severe outbreaks with gastrointestinal symptoms have been occurred by food borne pathogenic *E. coli*, particularly 0157:H7 (Armstrong et al., 1996). *E. coli* and its related species are named as "enteric bacteria"; because they mostly live in the intestinal tracts of human and other animal species (Minnock et al., 2000). About 10 to 15% of intestinal coliforms are opportunistic and pathogenic serotypes and cause a variety of lesions in immuno-compromised hosts including poultry (Daini et al., 2008; Mailk et al., 2013); and may cause omphalitis, yolk sac infection, cellulitis, colibacillosis and swollen head syndrome (Gross, 1994).

Table eggs are the primary source of protein in human diet. These are used in a number of traditional Pakistani dishes from decades. However, the recent studies have declared

that enteric bacteria like *Salmonella*, *E. coli*, *Listeria*, etc., could contaminate these eggs and may cause egg-borne diseases (Adesiyun et al., 2006; Adesiyun et al., 2007). Some global epidemics have also been linked with egg consumption and known to cause egg-borne pathogens present in poultry eggs and their contents (CDC, 1990; Rocourt et al., 2003). Food poisoning associated with egg-borne pathogens may cause severe morbidity or mortality with diarrhea, vomiting, nausea and abdominal cramps (Mitchell, 2005). The present investigation was therefore, designed to study the prevalence and incidence of *E. coli* in table eggs sold in retail market of district Peshawar. Moreover, the antibiogram study of isolated *E. coli* from poultry eggs was also carried out to investigate the susceptibility pattern of various antibiotics.

MATERIALS AND METHODS

Study Design

A total of one hundred poultry table eggs were collected randomly from different markets existed in various localities of district Peshawar, Khyber Pakhtunkhwa, Pakistan. Eggs were collected from four different localities (n= 25 from each locality) i.e., Bacha Khan Chowk, Karkhano Road, Nahaqi and Palossi Markets of Peshawar. Although, the eggs were kept at room temperature at sale outlets, so it was ensured that these should not be older than 24 hours. Moreover, the eggs with visible fecal shell contamination were not taken as samples. The collected eggs were transported to laboratory under cold chain and were kept in refrigerator at 4°C until they were processed for microbial contamination.

Laboratory Procedures

For the isolation of *E. coli*, table eggs were processed according to procedure described by Adesiyun et al., (2006). In brief, using aseptic conditions one sterile swab moistened in normal saline (0.9% NaCl w/v) was applied to the surface of each egg. It was dipped in 1ml saline in universal bottle to form a representative egg shell sample. For egg-yolk and egg-white samples, the eggs were immersed in 75% ethanol for 5 minutes and then pointed end of each egg was disinfected on Bunsen burner flame for 5-10 seconds. Then, a small hole was made on the shell surface and the egg-yolk and egg-white were emptied separately into the sterilized polythene bags. The contents were blended manually. The resultant mixtures and egg shell samples were used for bacteriological culture as described earlier (Nazia et al., 2015). The isolated *E. coli* were then subjected towards different biochemical and sugar fermentation tests for species confirmation like starch test, lipid hydrolysis test, casein hydrolysis test, gelatin hydrolysis, carbohydrate fermentation test, triple sugar iron test, which were based on their capability to breakdown complex molecules in to simpler nutritional elements.

In-vitro Susceptibility of *E. coli* to Antimicrobials

The antibiotics (Difco, Michigan, USA) used during the study were amoxicillin (10ug), colistin (10ug), gentamycin (10ug), enrofloxacin (05ug), kanamycin (10ug), ciprofloxacin (05ug), norfloxacin (10ug), tetracycline (30ug) and doxycycline (30ug). All the *E. coli* isolates of table eggs were investigated for their in-vitro susceptibility pattern to various antimicrobial agents using disk diffusion method as described by Bauer et al. (1966). In brief, the Muller Hinton agar (Difco, Michigan, USA) was prepared, dispensed in Petri dishes and surface was dried by incubating at 37°C for 30 minutes. The isolated colonies were selected and suspended evenly in 4ml sterile normal saline solution (0.9% NaCl w/v; pH: 7.0). A sterile cotton swab was dipped into the suspension and culture was smeared on the surface of Muller Hinton agar in such a way that all agar surfaces would be covered evenly with the bacterial suspension. The plates were then placed in incubator for 30 minutes to get dried. The antibiotic discs were placed on the agar surface with the disc disperser and slightly pressed with sterile forceps to keep it adhere to the surface. The plates were then closed, wrapped in polythene bag, inverted in such a way that medium and discs would be in upward portion and placed in incubator for 24 hours at 37°C. The zones of inhibition were observed as a clear area, free from growth around the discs. Clear zones of inhibition made against organism by the antibiotics were recorded in mm from the centre of disc of zone with the observed annotations.

Data Analysis

All the experimental results were calculated and presented in percentage format using the Excel Spreadsheets.

RESULTS

Of the total 100 table eggs examined, the overall prevalence of *E. coli* was recorded as 37.00%, while 63.00% eggs were found free from *E. coli* contamination (Table 1). Of the 25 eggs examined from Bacha Khan Chowk, the prevalence of the *E. coli* species was noted in 40.00% eggs. Similarly, 25 eggs acquired from Karkhano Road market, the prevalence was observed as 48.00%. When the same number eggs were examined from Nahaqi market, the prevalence of *E. coli* was recorded as 32.00% in eggs. Whereas 25 eggs collected from Palossi market showed the prevalence of *E. coli* as 28.00% (Table 2).

The results regarding the incidence of *E. coli* in different components of eggs has been summarized in Table 3. Of the 100 egg shells examined, the incidence was recorded as 15.00%. Similarly, among 100 egg-whites, the incidence of *E. coli* was noted as 12.00%, whereas within 100 egg-yolks *E. coli* was detected in 10.00% egg-yolks.

Table 1: The overall prevalence of *Escherichia coli* in table eggs collected from retail markets of Peshawar

Total No. of eggs examined	No. of eggs positive	% of eggs positive	No. of eggs negative	% of eggs negative
100	37	37	63	63

Table 2: The number and percentage prevalence of *Escherichia coli* in table eggs collected from different localities of Peshawar

S. No	Name of area	Total No. of eggs examined	Total No. of positive eggs	% of positive eggs	Total No. of negative eggs	% of negative eggs
1	Bacha Khan Chowk	25	10	40	15	60
2	Karkhano Road	25	12	48	13	52
3	Nahaqi	25	8	32	17	68
4	Palossi	25	7	28	18	72

Table 3: The number and percentage incidence of *Escherichia coli* in different components of table eggs

Egg components	Total No. of egg components examined	Number of positive components	Percentage of positive components	Number of negative components	Percentage of negative components
Egg-yolk	100	10	10.00	90	90.00
Egg-white	100	12	12.00	88	88.00
Egg-shell	100	15	15.00	85	85.00

Table 4: Antibiogram results of *Escherichia coli* isolates of table eggs

Antibiotic discs used	Zone around discs	Indication of sensitivity	Degree of sensitivity
Amoxicillin (10ug)	2 mm	+	Weakly sensitive
Colistin (10ug)	8 mm	+++	Quite sensitive
Gentamycin (10ug)	10 mm	+++	Quite sensitive
Enrofloxacin (05ug)	14 mm	++++	Highly sensitive
Kanamycin (10ug)	2 mm	+	Weakly sensitive
Ciprofloxacin (05ug)	14mm	++++	Highly sensitive
Norfloxacin (10ug)	4 mm	++	Moderately sensitive
Tetracycline (30ug)	0 mm	-	Not sensitive
Doxycycline (30ug)	3 mm	++	Moderately sensitive

-: Absence of clear zones around disc; +: clear zone up to 2mm; ++: clear zone with >2-5 mm; +++: clear zone with >5-10 mm; ++++: clear zone with >10-15mm

During present experiments, nine different antibiotics were tested to demonstrate the in-vitro susceptibility of *E. coli* isolates recognized from the table eggs and results were given in Table 4. The antibiotics ciprofloxacin and enrofloxacin were recorded as highly active against *E. coli* isolates and inhibited its growth, while antibiotics colistin and gentamycin were recorded as quite active against *E. coli*. Whereas, drugs norfloxacin and doxycycline showed moderate sensitivity against *E. coli*, as these drugs inhibited the growth of the organisms and showed small zones of inhibition (>2-5mm) around the discs. Furthermore, the antibiotics amoxicillin and kanamycin were marked as weakly active against the organism. However, the antibiotic tetracycline failed to inhibit the growth of bacterial organism on agar plate and was recorded as completely resistant against *E. coli*.

DISCUSSION

It has been estimated that many nutrient substances found in table eggs create an excellent environment for the growth and development of potential spoilage or infectious microorganisms. Present study has demonstrated an overall 37.00% *E. coli* contamination in table eggs. This finding is in agreement with a study conducted by Adesiyun et al. (2006) in Trinidad. The researchers reported a 71/184 (38.6%) table eggs positive for enteric microbes including E. *coli*, *Salmonella*, etc. Likewise another Polish study reported a 40.30% bacterial contamination in table eggs with *E. coli* as most dominant contaminant (Stępień-Pyśniak, 2010).

We got 28-48% contamination of bacterial organism in table eggs collected from different localities of Peshawar. However, another study reported the 36.3 to 69.6% contamination in poultry eggs collected from different points i.e., supermarket, mall and farm (Adesiyun et al., 2006). These differences might be due to difference in management, handling and hygienic conditions used at farm and/or sale outlets. The poultry eggs can get contamina-

tion either horizontally (through the shell) or vertically (trans-ovarial), and could serve a potential source of pathogens participating in the etiology of foodborne diseases (Stępień-Pyśniak, 2010). Indar et al. (1998) reported trans-ovarial transmission of *Salmonella spp.* in table eggs collected from commercial poultry farms in Trinidad. Although, egg-yolk contains maternal immunoglobulin IgG (also called IgY), but its level could be influenced by various factors like, functional quality of immunological system and/or antibiotics exposure to fowl (Tokarzewski, 2002). Moreover the quantitative contamination of eggs depends upon bacterial load in the environment where eggs laid and/or handled (Stępień-Pyśniak, 2010).

The results of the present study indicated the bacterial contamination level as 15, 12 and 10% on eggshells, egg-whites and egg-yolk respectively. Adesiyun et al. (2006) reported in their investigation the contamination level as 19% and 13% in eggshell and egg contents respectively. It has been suggested that temperature, and/or storage conditions provided to the eggs at retail outlets significantly impact the bacterial load of eggs without affecting the bacterial prevalence (Suresh et al., 2005). In consistent with this study, Stępień-Pyśniak (2010) also reported a high contamination level of eggshells as compared to other internal contents. This is probably due to exposure of eggshell with the environment.

The results about *in-vitro* susceptibility of *E. coli* isolated from table eggs of poultry birds recorded during present investigation were in line to the findings reported by previous studies. Like, Akond et al. (2009) isolated and identified *E. coli* from poultry sources of different poultry markets and sensitivity to antimicrobials was recorded as 86, 80, 60, 36, 30 and 26% to norfloxacin, gentamicin and chloramphenicol, neomycin, tetracycline, streptomycin and ampicillin, respectively. Raji et al. (2007) observed ciprofloxacin as highly active (85-100%) antibiotic against *E. coli* isolates. Adesiyun et al. (2007) studied the resistance of bacterial species to seven antimicrobial agents using the Disc Diffusion Method. An overall, 131 bacterial isolates of *E. coli* and *Enterobacteriaceae* were tested, and 125 (95.4%) exhibited resistance to one or more antimicrobial agents. The high resistance was recorded against streptomycin (90.1%), tetracycline (51.9%) and kanamycin (30.5%).

In present investigation we have found 3/9 (33.33%) antimicrobial agents as resistant or weakly sensitive to *E. coli* isolated from table eggs. This finding is in agreement with a study conducted by Musgrove et al. (2006). The study indicated that most (73.2%) of *E. coli* isolated from eggshells were susceptible to all antimicrobial agents. Moreover, the *E. coli* isolates showed 29.9, 6.2 and 3.1% resistance to tetracycline, streptomycin and gentamicin respectively. Similar results were also reported by Ansari et al. (2014).

CONCLUSIONS

It could be concluded from present investigation that table eggs sold in retail market of district Peshawar contained *E. coli*, hence may pose a health hazard to human beings if consumed improperly cooked or raw eggs. Eggshells contained more bacterial contaminants as compared to egg contents. Antimicrobial agent tetracycline was found completely resistant to *E. coli* isolates, whereas, amoxicillin and kanamycin were observed as weakly sensitive. There is a need to educate the people to adopt significant hygienic measures in handling of table eggs and should not be consumed inadequately cooked eggs or egg products.

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CONFLICT OF INTEREST

There is no conflict of interest.

AUTHORS' CONTRIBUTION

Mr. Aurangzeb was the main researcher, Dr. Rahmatullah Rind was his supervisor, Dr. Asghar Ali Kamboh revised the article, Muhammad Shoaib did all the correspondence, Gulfam Ali Mughal, Shakeel Ahmad Lakho, Kanwar Kumar Malhi, Ali Raza Nizamani and Adnan Yousaf contributions in statistics, and other activities related to the research.

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