DETECTION OF *ESCHERICHIA COLI* FROM SUSPECTED TABLE EGG IN DIFFERENT KITCHEN MARKET OF MOHAMMADPUR AREA OF DHAKA CITY

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DECEMBER, 2021

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A Thesis

Submitted to the Faculty of Animal Science and Veterinary Medicine

Sher-e-Bangla Agricultural University

In Partial Fulfillment of the Requirements for the Degree of

MASTER OF SCIENCE (MS) IN POULTRY SCIENCE
DEPARTMENT OF POULTRY SCIENCE

Semester: July-December, 2021

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CERTIFICATE

*This is to certify that thesis entitled, "**DETECTION OF ESCHERICHIA COLI FROM SUSPECTED TABLE EGG IN KITCHEN MARKET OF MOHAMMADPUR AREA OF DHAKA CITY**" submitted to the Faculty of Animal Science and Veterinary Medicine, Sher-e-Bangla Agricultural University, Dhaka, in partial fulfillment of the requirements for the degree of **MASTER OF SCIENCE (M.S.) in POULTRY SCIENCE** embodies the result of a piece of bona-fide research work carried out by **ROTYLA NAZNIN ROTY**, Registration no. **14-05831** under my supervision and guidance.No part of the thesis has been submitted for any other degree or diploma.*

I further certify that such help or source of information, as has been availed of during the course of this investigation has duly been acknowledged.

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ACKNOWLEDGEMENT

At the beginning, the author bows the grace and mercy of the "Almighty Allah," the omnipotent and omniscient, who enabled her to complete this thesis. The author expresses her grateful acknowledgement and indebtedness to her beloved father Md Mohiuddin Ratan and mother Nurjahan Begum for their ending prayer, sacrifice and dedicated efforts to educate her level.

The author with a sense of respect, expresses her heartfelt gratitude to her honorable supervisor and Professor ***Dr. Md Anwarul Haque Beg****, Department of poultry science, Sher-e-Bangla Agricultural University, Dhaka-1207 for his continuous inspiration, valuable suggestions, constructive criticism, constant guidance and intensive supervision throughout the period of the study. Preparation of this thesis without his intense co-operation would not have been possible.*

Heartfelt gratitude and profound respect to her honorable Co-supervisor ***Dr Amina khatun,*** *Assistant professor, Department of Pathology, Sher-e-Bangla Agricultural University, Dhaka-1207 for her keen interest, valuable advice, creative suggestions, co-operation and encouragement to bring this thesis up to its present standard.*

The author humbly desires to express her profound respect & sincere appreciation to respectable teacher, ***Dr. Maksuda Begum*** *and* ***Dr. MA Mannan,*** *Assistant Professor, Department of Poultry Science; and Microbiology and Parasitology, SAU for their motivation & inspiration to conduct the academic work.*

The author expresses her grateful thanks to all staffs and employees of the Department of poultry science, Sher-e-Bangla Agricultural University, Dhaka 1207, for their co-operation and encouragement to prepare this thesis.

The author expresses her deepest sense of gratitude, indebtedness and profound respect to her beloved sister Rovina Nova, relatives and friends Mehedi Hasan, Md. Moniruzzaman, Abu Raihan, Akhel chandra, kazi jubayedul haque, Sukla sikder, Md Moniruzzaman Mim, Humaira Akter, Sharmin akter for their blessings, encouragement and moral support in all phases of this academic pursuit from beginning to the end.

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LISTS OF ABBREVIATIONS

SAU	Sher-e-bangla Agricultural University
Spp.	Species
E. coli	*Escherichia coli*
NA	Nutrient Agar
EMB	Eosin Methylene Blue
	MacConkey Agar Media
Gm	Gram
%	Percentage
+ve	Positive
-ve	Negative
Gm-ve	Gram negative

ABSTRACT

The *Escherichia coli* are a pathogen that is enteropathogenic and causes infection to human and animal sector. It is also a food safety and sanitary indicator. This study was conducted to investigate the *E. coli* from table chicken egg in Mohammadpur area from January to June 2022. A total of 100 egg samples were collected from different kitchen market of Mohammadpur. Bacteria were isolated & identified based on the colony charactetistics in different media, staining properties and microscopic observation. The colony characteristics of *E. coli* were greenish red colored with faint metallic sheen in EMB agar and whitish colored in Nutrient agar media. The *E. coli* observed in rod shaped, gram negative, single or paired arranged under microscope stained with gram's stain. In physical parameter the prevalence of brown with red spotted color, oval shaped, irregular shaped, cracked, leaked, dirty were 25,% 26.67%, 0%, 0%, 10%, 20%, respectively. The higher prevalence of brown with oval shaped was 26.67% and lower prevalence of leaked egg was 10%. The prevalence of Townhall, Agargoan, Paka Market, Krishi Market, Bihari Camp were 15%, 30%, 0, 10% & 20%, respectively. The higher prevalence of Agargaon was 30% and no positive case found in Paka Market.

Keywords: *E. coli*, Table egg, Mohammadpur, Prevalence

CHAPTER 1

INTRODUCTION

Poultry industry is a promising sector for poverty elevation in Bangladesh. The Bangladesh poultry industry primarily produces chickens, although a few other species like duck, pigeon, quail, goose, turkey, and guinea fowl are available. In Bangladesh two types of chickens have been reared, one for eggs and another for meat purpose. Currently Bangladesh is producing 2057.64 chicken eggs against the current annual demand of121.18crore (Hossain Md Salim *et al*., 2021). Globally chicken eggs are common food and consume in various dishes as a cheap source of protein which considered as the most nutritious foodstuffs for human (Pasquali *et al*., 2014). Egg components have been attributed diverse biological activities including antimicrobial activity, protease inhibitory action, vitamin binding properties, anticancer activity, and immunomodulatory activity. Eggs are also an important source for minerals as phosphorus and irons, and a good source of vitamins like A, D, E, K, B1, B2, B9, B12, choline and selenium. On the other hand, nutrient substances present in eggs create an excellent environment for the growth and multiplication of bacteria.

The amount of interest is considered reasonable since it has a more affordable price and a rather complex filling. As we know, eggs are one of the perishable products of animal origin. The eggs on the market have certainly gone through a fairly length delivery process, starting from the cage to the egg distributor, then going to merchants and finally reaching consumers, of course it took a long time. Retail eggs are usually over 7 days. Duration of dispatch and storage of eggs reduces the quality of the eggs and considerably increases the likelihood of microbial contamination.

It was argued that bacterial damage to eggs can be caused by two factors, namely internal and external factors are those that originate from the inside, namely, the eggs were infected while they are still in the parent's body, for example the parent suffers from collibacilosis, therefore the eggs contain the bacteria *E. coli* spp. Extrinsic factors that

come from outside, including bacteria entering the eggs that occur after leaving the parents body, such as manure, air, tools and the hands of the parent flock. So it is possible the eggs are contaminated with *E. coli* bacteria from chicken feces. *E. coli* bacteria are enteropathogenic and toxic microorganisms harmful to human health. Enteropathogenic bacteria are gram negative bacteria, pathogenic in nature, that attack human digestive system. Certain strains of *E. coli* can cause gastroenteritis in human, where gastroenteritis is a disease of the digestive system such as vomiting and diarrhea caused by infection, which are bacteria that cause digestive disorder such as nausea, vomiting and diarrhea.

Eggs can be contaminated with many bacteria such as *E. coli, Salmonella sp., Proteus sp., Listeria monocytogenes, Staphylococcus sp., Streptococcus sp., and Bacillus sp.* (Lee *et al.,* (2016). The *E. coli* is one of the common microbial floras of the gastrointestinal tract of poultry and human. Eggs contaminated with bacteria may lead to transmission of pathogens which associated with food-borne illness to consumers that has already been established (Osimani *et al.,* 2016, Chousalkar *et al*., 2018). Although most of the *E. coli* strains are harmless, some can cause food poisoning and diarrhea especially in elderly, infants, and those with impaired immune systems (Begum *et al*., 2014). *E. coli* can be primary pathogen, when the infection pressure is high, from heavily contaminated drinking water systems or in alternative housing systems, from high levels of dust (=dried manure). Most times, *E. coli* is secondary pathogen. The door is opened by viral infections, by high levels of ammonia or by problems with the gut wall integrity, caused by enteritis. Stress can also make layers susceptible for diseases. Production stress and stress related to ventilation failures (draught) are the most important ones. Antibiotics can lead to the emergence and dissemination of resistant *E. coli* which can then be passed into human via eggs or direct contact with chickens. The emergence of antibiotic resistance in bacteria has become a serious problem worldwide. Antibiotic resistance is increasing day by day and become a public health hazard globally (Ferri *et al*., 2017). In Bangladesh, antibiotics are used as growth promoters as well as to control infectious poultry diseases (Hasan *et al*., 2014). This misuse or overuse of antibiotics in the poultry industry results the development of an increasing number of antibiotic resistant *E. coli* (Islam *et al*.,

2018). So, identification of *E. coli* from chicken eggs and determination of their antibiotic sensitivity patterns is very much essential for the proper treatment and control purposes.

The extent of egg spoilage due to effect of microorganisms is very high which result in big economic losses (Saif *et al*., 2009; Howard *et al*., 2011). At the beginning, the microbial load is very low but it increases day by day. Besides these egg can be contaminated from different stages like during collection, handling, storage and transportation. Among the various microorganisms, the well-known enteric pathogens particularly *Salmonella, E. coli, Campylobacter spp. and Listeria spp.* were isolated from table eggs and their contents (Adesiyum *et al*., 2005). The transmission of the disease from chicken to humans has been suspected. Risk of egg borne disease strongly increases because of unhygienic conditions of egg production and improper practices of egg handling, distribution including also storage times and temperatures. If all the necessary precautions are not taken during the poultry production, marketing and processing chains, in that case poultry meat and eggs can be contaminated by infectious agents that are harmful to humans. So, this study holds a great importance to understand the present risks of chicken egg borne diseases on human health and will help to take necessary measures to reduce the risk by creating public awareness, improving knowledge in rural women, good hygiene practices, thorough cooking, provision of vaccines and essential medicines and development of linkages with the different agencies.

Table eggs are the best and easy source of food, containing quality protein, essential amino acids, essential vitamins and minerals needed for human good health (MAFF, 2009). Freshly laid eggs are generally devoid of organisms. However, following exposure to environmental conditions (for example, soil, dust and dirty nesting materials), eggs become contaminated with different types of microorganisms (Ellen *et al*., 2000). Eggs are liable to contamination either before laying (congenitally) or after laying when the microorganisms reach the egg contents through penetration the shell and cause low egg quality, low shelf life and safety inducing public health hazards (Board and Fuller *et al.*, 1994), in addition, fecal matter, improper washing, using of contaminated water and bad

handling are the common sources of contamination. Coliforms count is the traditional indicator of possible fecal contamination, microbial quality, wholesomeness and reflects the hygienic standards adopted in the food operation. The bacteria most frequently isolated from eggs are Gram-negative bacteria such as *E. coli, Enterobacter*, and *Klebsiella spp.* (Musgrove *et al*., 2008).

The *E. coli* is a normal inhabitant of the intestinal tract of both man and animals and can penetrate the shell contaminating the egg contents (Mayes and Takeballi *et al.*, 1983). The *E. coli* is a Gram-negative, facultative anaerobe, rod-shaped bacterium, and the normal habitat in the lower intestine of warm-blooded organisms (Singleton *et al.*, 1999). Most of the *E. coli* strains are harmless, but some pathogenic strains can cause food poisoning as severe abdominal cramps, diarrhea in addition to urinary tract infections, and neonatal meningitis. In rarer cases, virulent strains have a major role in bowel necrosis (Todar *et al*, 2007).

The *E. coli* is a major pathogen of commercial poultry causing colibacillosis with manifestations such as airsacculitis, pericarditis, septicemia, and death of the birds (about 28% death in Sonali birds) (Biswas *et al*., 2006). Enterotoxigenic *E. coli* (ETEC) is a major pathogen of animals, being responsible for diarrhea in calves resulting significant financial losses. Debnath *et al*., (1990) claimed 28% of the total death in calves occurred in first month of life and 50% of death during first week due to *E. coli* infection. It also causes on-farm contamination of different animal species (Fairbrother and Nadeau, 2006). ETEC is the most common cause of food and water-borne human diarrhea worldwide. In developing countries, the incidence of enteric diseases due to ETEC is estimated about 650 million cases per year, resulting in 800,000 deaths, primarily in children of below five years old (Turner *et al*., 2006).

The *E. coli* is an important zoonotic pathogen. *E. coli* O157:H7 was first recognized in 1982 as a human pathogen and cattle have been identified as a major source of *E. coli*

O157:H7 infection of human but it is not pathogenic in cattle and present in the feces of healthy cattle (Elder *et al*., 2000). Moreover, *E. coli* isolation reveals fecal contamination in the combined-sewer outflows (Perez Guzzi *et al*., 2000). So, it is necessary to emphasize the detection of *E. coli* from the table egg that may cause illness in animals and birds as well as in human being.

Considering the above information, this study was conducted to detect *E. coli* from eggs in kitchen market of Mohammadpur. The present study was therefore conducted to determine the prevalence of *E. coli* in chicken eggs in kitchen market of Mohammadpur area of Dhaka city with the following objectives:

- Detection of *E. coli* bacteria from table egg collected from kitchen market of Mohammadpur area of Dhaka city.
- Observation of occurrence of *E. coli* contamination at the same area.

CHAPTER 2

REVIEW OF LITERATURE

Eggs are one of the animal products that come from poultry and have been known as a source of high quality protein food. Chicken eggs contain all the essential components such as proteins, lipids, vitamins, minerals, carbohydrates, and growth factors required by the human being. Despite of their nutritional values eggs can cause health problems through consumption of contaminated eggs with pathogenic microorganisms. This study is mainly based on this.

2.1 Isolation and identification of bacteria

Regita Cahyani Sauring *et al.* (2021) Conducted an investigation to determine the presence of *Escherichia coli* bacteria in eggs that are sold in the central market of Gorontalo city. The research method used is descriptive research type with a quantitative research approach, cross sectional research design, a sampling place for the central market of Gorontalo City and a place for researching samples of the Microbiology Laboratory of Gorontalo State University, the number of samples used was 30 samples of eggs. Quail eggs were positive for *Escherichia coli* bacteria with a percentage of 6.7%, and negative egg yields from *Escherichia coli* bacteria were 28 or 93.3%. It can be concluded that from 30 samples of eggs there were 2 eggs that were positive for *Escherichia coli* bacteria, the bacteria contamination factor in eggs was influenced by external factors.

Mo'ataz *et al*. (2021) Carried out an experiment to determine the presence of coliforms, fecal coliform and *Escherichia coli* in both baladi and farm table eggs in both shells and contents. A total of 100 farms and baladi eggs samples (50 samples of each) were randomly collected from poultry farms, markets, supermarkets, and groceries in Assist governorate. Eggs were microbiologically examined, and isolates were identified by biochemical and (PCR). The obtained results revealed that coliform and fecal coliform

incidences were 62, 56, 54 and 32% for the farm hens' eggshell, egg content, baladi hens' eggshell and egg content, respectively. The biochemical identification of revealed 16 isolates of *E. coli* and these results were complementary with the molecular identification. *E. coli* incidence was 6, 8, 12 and 6% for the farm hens' eggshell, egg content, baladi hens' eggshell and egg content, respectively.

Pratama KA, *et al.* (2020) showed his experiment that *E coli* contamination of consumed eggs around the campus of the agricultural state polytechnic of payakumbuh, Indonesia. Total 30 samples of eggs consumed for food were taken from 5 cooreatives around the campus of the Agricultural state Polytechnic of payakumbauh university campus. Testing the amount of *E. coli* in eggs using the total bacterial count (TPC) method, the average *E. coli* was 1.9 $10^{\wedge 6}$ cfu/mL. The existence of *E. coli* illustrates the contamination of chicken eggs from laying hens.

Rabbani *et al.* (2020) carried out an experiment of duck and hen eggs (clean and dirty) selected from wholesalers for this study from January- June, 2013. After collection and transportation to the laboratory bacteriological analysis was performed under two major principles of assessments. At first microbiological quality was evaluated and then total viable count (TVC), Total coliform count (TCC) and Total *Salmonella* Count (TSC) were performed. A total of 40 egg (20 egg of hens and 20 eggs of duck) samples were subjected to assessment on microbiological quality.. The TVC and TCC showed highly significant correlation with TSC of dirty duck eggs. The highest bacterial load was found in dirty duck eggs and lowest score were found in clean hen eggs. The study indicated that hen eggs are safer than duck eggs.

Methaq Ghalib Abd AL-Rubaiey *et al.* (2020) the main aim of the research was to focus the light on some bacterial contamination on cracked eggshell and egg content plus studying the sensitivity of these bacterial isolates to antibiotics. For this purpose, a total of 50 eggs were collected from the markets in Baghdad city (Iraq) and examined for

bacterial isolation from cracked eggshells and from the egg contents. The bacterial isolates were cultured and purified then transferred to a specific media to study its sensitivity against antibiotics. The results revealed that bacteria isolated from both cracked eggshells (46%) and egg contents (44%). The bacteria isolated include *E. coli*, *Staphylococcus*, *Enterococcus faecalis*, *Enterobacter spp* and *Pseudomonas*. The results of antibiotic sensitivity test showed that all bacteria are resistant to Bactracin. It can be concluded that the consumers should get ride of cracked eggshells and never used for human consumption.

Ema *et al.* (2018) Studied aimed to isolate and identify the egg-borne bacteria from different parts of duck eggs such as egg shell (outer and inner), yolk and albumen, and to assess the anti-biogram profile of the isolated bacteria. *E. coli, Staphylococcus spp*. and *Salmonella spp*. were isolated and identified from the duck egg samples. Prevalence of *E coli* in outer egg shell was 80%, where as in inner egg shell and inner egg content, this prevalence was 20% and 10%, respectively. Similarly, the prevalence of *Staphylococcus spp*. was 75%, 17.5% and 7.5% in outer egg shell, inner egg shell and inner egg content, respectively. The prevalence of *Salmonella spp.* was 82.5% in outer egg shell, 22.5% in inner egg shell and 12.5% in inner content of egg. All these three bacterial isolates were sensitive to Ciprofloxacin and Gentamicin and resistant to Ampicillin and Cephalexin.

Yones, N. Ab. J. *et al*. (2018) Designed to assess the hygienic condition of local and imported table egg-shell at Mosul markets. Two hundred local and imported eggs were randomly collected from the markets and transferred immediately to the scientific research unit in the college of Veterinary medicine / University of Mosul for bacteriological examination. Total counts using four types of Medias were used: Standard Plate Count ager (SPC), MacConkey agar, Violet Red Bile Glucose ager (VRBG) and Tryptone Bile X-glucoronide (TBX) for *Escherichia coli* and fecal coliform bacteria. Results showed that the means of total viable coliform and *E. coli* bacterial count in the local egg-shell at different markets ranged from 0.3 to 3 x107 on SPC agar, 0.08-10x107 on Mac Conkey ager, 0.014-3x105 on (VRBG) and 0.3-7x104 on (TBX)

compared with 0.032-5x107 on SPC agar, 0.03-3.3x105 on MacConkey agar, 0.014-5.9x104 on (VRBG) with no growth on (TBX) for coliform and *E. coli* in the imported table-egg shell at different markets. Biochemical tests for local and imported table egg-shell were performed. It was concluded that the rate of contamination in the local table eggs was more than the imported one and the isolates of *E. coli* bacteria were obtained only from local eggs on the TBX medium.

Vinayanando *et al*. (2017) Reported that occurrence of *E. coli* in different categories of table eggs collected from markets was evaluated. Isolates were analyzed for the presence of virulence genes, antibiotic susceptibility pattern and efficacy of per acetic acid and chlorine for the purpose of decontaminating table eggs. Significant differences were observed in the occurrence of *E. coli* between different groups *viz*. processed (cleaned, washed, sanitized and packed eggs), unprocessed (un-cleaned, un-sanitized and loose eggs) and free range (eggs obtained from backyard poultry) table eggs. Overall, *E. coli* occurred in table eggs at 28.6% with 22.9, 29.2 and 50.0% occurrence in processed, unprocessed and free-range table eggs, respectively.

Lee, Minhwa *et al*. (2016) Found that contamination by food borne pathogens in 475 eggs and 20 feed samples collected from three egg layer farms, three egg-processing units, and five retail markets in Korea. Microbial contamination with *Salmonella species*, *Escherichia coli*, and *Arcobacter species* was examined by bacterial culture and multiplex polymerase chain reaction (PCR). The contamination levels of aflatoxins, ochratoxins, and zearalenone in eggs and chicken feeds were simultaneously analyzed with high-performance liquid chromatography coupled with fluorescence detection after the post-dramatization. While *E. coli* was isolated from 9.1% of eggs, *Salmonella* species were not isolated. Acrobatic species were detected in 0.8% of eggs collected from egg layers by PCR only. While aflatoxins, ochratoxins, and zearalenone were found in 100%, 100%, and 85% of chicken feeds, their contamination levels were below the maximum acceptable levels (1.86, 2.24, and 147.53 μg/kg, respectively). However, no eggs were contaminated with aflatoxins, ochratoxins, or zearalenone. Therefore, the risk of

contamination by mycotoxins and microbes in eggs and chicken feeds is considered negligible and unlikely to pose a threat to human health.

Kumar *et al.* (2015) conducted an experiment of total number of 16 eggs from different farms and markets that 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 egg shells and 2 from egg contents of single egg sample. Gram's positive bacteria (*Staphylococcus)* were found predominantly present on eggshell. The egg shells were also found contaminated with pathogenic bacteria (*Salmonella* and *Escherichia coli).* Whereas no *Escherichia coli* O157:H7 was found on egg shell 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.

Adnan *et al.* (2015) *Escherichia coli* are one of the common microbial floras 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 egg shells, 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*.

Maha A.M. *et al*. (2013) A survey of the microbial quality based on enterobacteriaceae counts of the table eggs sold in Egyptian markets was conducted to evaluate their quality and the possibility of presence of Enterovirulent *Escherichia coli* strains. Six hundred random table egg samples were collected from different shops, supermarkets and homes. Each six pooled eggs constituted a composite sample. Higher enterobacteriaceae count/g was recorded in the content ($1.1x10^2$, 7.9x10) and in the shell ($1.2x10^2$, $2.6x10^2$) log10 cfu/g of Baladi hen and Duck eggs respectively. Lower enterobacteriaceae counts in brown shell and white shell hen eggs (4.9x10, 6.3x10 log10 cfu/g) in the content and the shell of both types. Twenty (%) of white shell and brown shell, 20, 36% of baladi hen eggs; and 36, 68% of duck eggs (content and shell) were marginally exceeded the maximum permissible count of enterobacteriaceae by European Communities Standards. *Escherichia coli*, *Enterobacter*, *Citrobacter*, *Klebsiella*, *Protus*, *Providencia* and *Shigella* had been recovered from the content and the shell of different types of table eggs. *E. coli* strains isolated from different types of table eggs were serotype into 7 different serotypes included O44, O111, O114, O125, O126, O127 and O128. Most of these isolates (37/39) were stx2 positive. Interestingly, all stx2 positive isolates were negative for stx1 and eae genes. Enterobacteriacae count limits should be added to the microbiological criteria for fresh table eggs as regulation in Egyptian standards. Hygienic measures should applied in home produced hen and duck eggs to lower bacterial load in egg shell and subsequently in egg content.

Wiriya loongyai *et al.* (2011) studied to detect the prevalence of enteric pathogens those can produce disease in the communities. They collected bacterial isolates from different housing system for laying hens. In the entire experiment, 20 were receiving from the open housing system and 19 eggs were received from the evaporative cooling system. They isolated the bacteria by polymerase chain reaction assay and conventional microbiological methods and *E. coli*, *Salmonella* and were presumptively obtained and then analyzed by PCR (Polymerase chain reaction). They observed enteropathogenic, enterotoxigenic and entero-invasive *E. coli*, also observed from the egg samples. By all of this observation mention that the pathogenic bacteria which are present can cause a serious health risk to consumers.

Abdullah *et al.* (2010) this paper presents the degree of contamination of table eggs with bacteria of the genus *Staphylococcus, E-coli, Salmonella, Streptococcus* and *Clostridia* taking into account the source of the eggs. The results of the study indicate a relatively high degree of contamination of table eggs with *Staphylococcus* bacteria and enterobacteriaceae both in yolk and on egg shell. he study included inspection, examination of brown eggs, gathered in winter and summer seasons from local markets, the origin the bacteria diagnosed by cultural, microscopic examination and biochemical tests methods. Results referred that bacteria were the main organism contaminate the egg 95% and fungi were 5%, we focused here on bacterial growth. Contamination ratio was; Staphylococcus 75%, enterobacteriaceae 20% (*E-coli* 9% and *salmonella* 11%) and 4.9% *streptococcus* and 0.1 *clostridium perfringens*. Smears are taken from egg shell and yolk, bacterial growth concentrate on shell in winter at 100 % while yolk 0%, in summer the growth was equal (50%) in both egg shell and yolk.

M.A. Zinnah *et al*. (2007) *Escherichia coli* from 10 different biological and environmental sources were isolated and characterized in the Department of Microbiology and Hygiene, Bangladesh Agricultural University, Mymensingh during the period from January to May 2007. A total of 100 samples, 10 from each of human feces and urine, rectal swab of cattle, sheep and goat, cloacal swab of chicken, duck and pigeon, drain sewage and soil were collected aseptically and subjected to primary isolation by propagating in nutrient broth followed by culture on different agar media. Gram's staining and hanging drop techniques were also performed. Biochemical properties of the isolates were studied and reaction in TSI agar slant was also observed. Pathogenicity of 10 representative *E. coli* isolates, one from each source were determined by lethality assay in 12 day-old embryonated eggs, in day-old chicks and in day-old suckling mice models. *E. coli* was isolated successfully from all the samples. All the *E. coli* isolates were found to produce bright pink colonies on MacConkey agar, yellowish green colonies surrounded by an intense yellow green zone on BG agar and characteristic metallic sheen colonies on the EMB agar. In case of *E. coli* isolated from cattle, slight variation in colony character on EMB agar was observed showing greenish red colonies with faint metallic sheen. In Gram's staining technique, all the isolates were pink colored,

small rod shaped Gram negative bacilli and in the hanging drop technique they were motile.

2.2. *Escherichia coli*

2.2.1. Growth Conditions

E. coli, a member of the Enterobacteriaceae family, grows optimally at 37°C under aerobic conditions, although it is a facultative anaerobe and can therefore grow under anaerobic conditions. It has also been previously reported that some strains of *E. coli* have been known to grow at temperatures of up to 53°C (Fotadar *et al.*, 2005), although this is not typical nor recommended for commonly used laboratory strains. *E. coli* is a relatively hearty bacterium and can survive at temperatures of 4°C for extended periods of time (up to 3 months) on solid media, although increased storage times at low temperatures may result in decreased viability. *E. coli* can also grow under a wide pH range; typical growth and maintenance is performed at a neutral pH of 7.0. Taking all optimal growth conditions into consideration (i.e., 37°C, aeration, pH of 7.0).

2.2.2. Cultural properties of *E. coli*

M.A. Zinnah *et al* (2007) his study was conducted to all the samples were cultured primarily in nutrient broth at 37ºC for 18-24 h, then sub cultured onto the MacConkey, brilliant green and EMB agar by streak plate method (Cheesbrough, 1985) to observe the colony morphology (shape, size, surface texture, edge and elevation, color, opacity etc). The organisms showing characteristic colony morphology of *E. coli* was repeatedly sub-cultured onto EMB agar until the pure culture with homogenous colonies were obtained.

2.2.3. Microscopic study

Gram's staining method Gram's staining was performed as per procedures described by Merchant and Packer (1969) to determine the size, shape and arrangement of bacteria. The organisms revealed gram negative, pink colored with rod shaped appearance and arranged in single or in pair were suspected as *E. coli.*

Momani *et al;* (2017) Reported that microbial contamination of table eggs has important consequences to the poultry industry and illness from contaminated eggs is a serious worldwide public health problem. Contaminated eggs and their products increase the risk of illness in humans. The significance of these illnesses can vary from mild symptoms to life-threatening conditions. The purpose of this study was to investigate the food-borne pathogen contamination of table eggs sold in Jordanian markets. One hundred eggs were randomly purchased from packed eggs available in the markets, including free-range home eggs, eggs farmed just after cleaning and just before cleaning and delivered to the microbiology lab. The collected swabs were cultured on suitable media and standard microbiological tests were performed to identify the isolated organism. The following bacterial species were isolated from egg shell surfaces: *Staphylococcus*, *Streptococcus spp*., *Pseudomonas spp*., *Proteus spp*., *Klebsiel*la *spp., Escherichia coli, Bacillus spp., Listeria monocytogenes and Salmonella spp*.

CHAPTER 3

MATERIALS & METHODS

This study was conducted to the poultry science laboratory of the Faculty of Animal Science and Veterinary Medicine in Sher-e-Bangla Agricultural University, Dhaka-1207. The whole experiment was performed during the period of January 2022 to June 2022.

3.1. Different types of materials and chemicals

- Collecting vial
- Test tube
- Test tube holder/ rack
- Conical flask
- Spirit lamp
- Cotton, Foal paper
- Petridish
- Measuring cylinder
- Electric balance machine
- Electric stirrer
- Glass Spreader
- Streaking loop
- Incubator
- Laminar air flow
- Refrigerator
- Autoclave
- Staining dye
- Emulation oil

- **Sterilization & preparation of instruments and glass wares**

Glass Petridis were cleaned by using 2% sodium hypochlorite solution & rinse in it. Soaked for overnight in a dish washing detergent and then washed it. Round brush was used for cleaning of test tube, collecting vial etc. & cleaned the other instruments by washing under running tap water. Kept it for air dry. After dried the entire instrument wrapped with the foal paper and got ready for autoclaving. Autoclaved at 121^0 C temperature 15 lbs. per square inch for 15 minutes. After autoclaving the instruments and glass wares were kept for air dry.

3.2. Study area

The study was conducted at the local wet market of Agargaon, Sher-e-Bangla Nagar and Mohamadpur. The samples were collected from different kitchen market of Townhall, Agargoan, Paka Market, Krishi Market & Bihari Camp. There was 5 area of samples collection

Table 1: Number of samples collected from different areas in kitchen market of Mohammadpur, Dhaka city

Sl. No.	Name of Area	Number of Sample
01	Townhall	20
02	Agargaon Bazar	20
03	Paka Market	20
04	Krishi Market	20
05	Bihari Camp	20
	Total	100

3.3. Study period

The study period was January to June, 2022.

3.4. Total sample

Total sample were 100. Twenty eggs samples were collected from each kitchen market of Mohammadpur, Dhaka city.

3.5 Egg collection

A total of 100 table eggs were collected from different areas in kitchen market of Mohammadpur area of Dhaka city. Hygienic and aseptic practices were followed during sample collection.

Fig 1: Selection of sample

Fig 2: Collection of egg sample

3.6 Experimental procedure

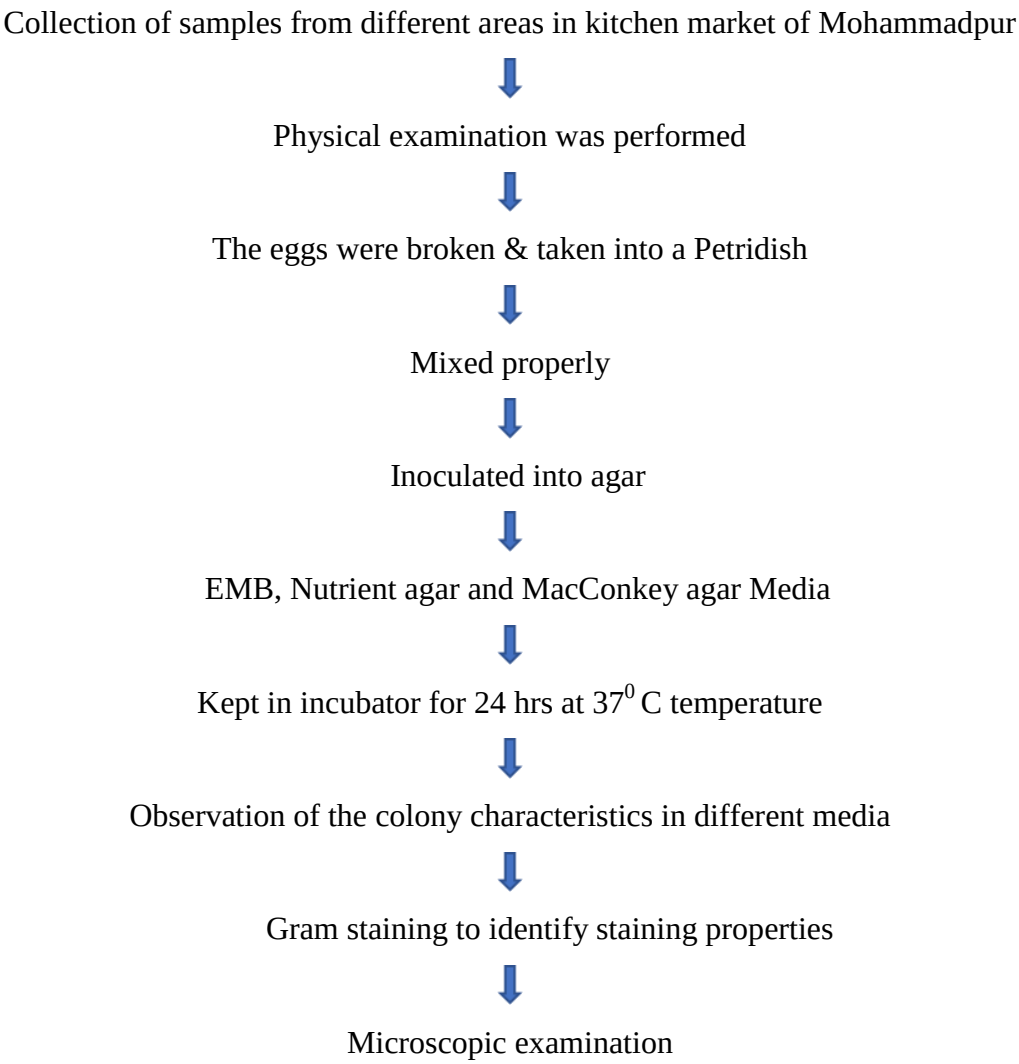

Fig 3: Schematic diagram of the experimental design

3.7. Procedure for isolation of *E.coli* colony

3.7.1 Collection & transportation of egg

Egg samples were collected from following area by using cage transported aseptically to the poultry laboratory in Sher-e-bangla Agricultural University (SAU) campus. Samples were used for observation within 24 hours after collection.

3.7.2 Physical examination

Physical examination was done by necked eye to detect the color, shape, liked & dirty. If any difficulties found, they were discarded for further analysis.

Table 2: Physical parameter observation after sample collection

Sl No	Parameter	Observation
1	Color	Brown with red spotted
2	Shape	Oval, irregular
3	Cracked	Considered
4	Leaked	Considered
5	Dirty	Considered

Fig 4: Brown with red spotted egg

Fig 5: Irregular shaped egg

Fig 6: Cracked egg

Fig 7: Dirty egg

When all the physical parameters found same then the further study were continued otherwise the sample discarded.

3.8 Media used for bacterial colony observation

3.8.1 Solid Media Preparation

3.8.1.1 Nutrient Agar Media

For preparation of Nutrient agar media, in a conical flask 2.8gm powder of Nutrient agar dissolved in 100 ml distilled water and boiled to dissolve completely through electric stirrer. Then sterilized by autoclaving at 121^0 C temperature at 15 lbs./ $inch^2$ for 15 minutes. After autoclaving media was poured into petridises for solidification and the quantity of media for medium size Petridis was 30/ml Petridis. Then inoculated and incubate at 37^0 C temperatures for overnight.

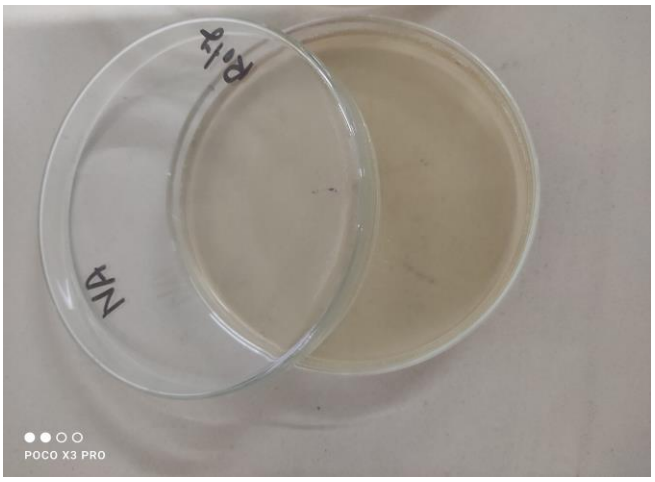

Fig 8: Nutrient Agar Media

3.8.1.2 MacConkey Agar Media

Due to preparation of MacConkey agar media, in a conical flask 51.53 gm powder of MacConkey agar dissolved in 1000 ml distilled water and boiled to dissolve completely. Then sterilized by autoclaving at 121^0 C temperature at 15 lbs./ $inch^2$ for 15 minutes. A fter autoclaving media was poured into petridishes for solidification and the quantity of media for medium size Petridis was 30/ml Petridis. Then inoculated and incubate at 37^0 C temperatures for overnight.

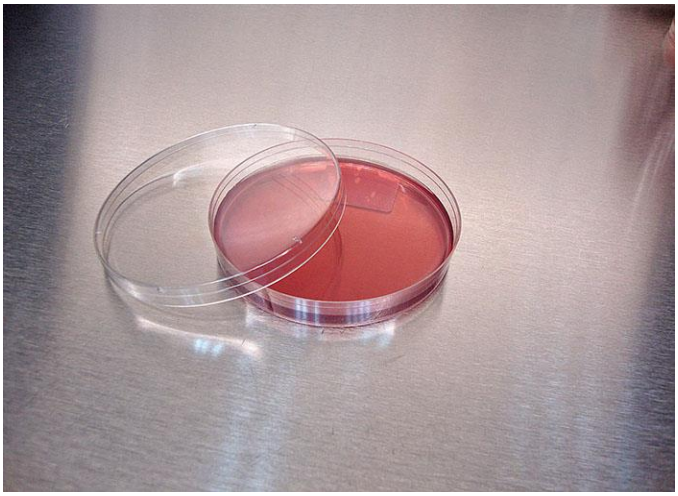

Fig 9: MacConkey agar media

3.8.1.3 Eosin Methylene Blue (EMB) Agar Media

For preparation of EMB agar media, in a conical flask 35.96 gm EMB agar powder dissolved in 1000 ml distilled water and boiled to dissolve completely. Then sterilized by autoclaving at 121^0 C temperatures at 15 lbs. / $inch^2$ for 15 minutes. After autoclaving media was poured into petridishes for solidification and the quantity of media for medium size Petridis was 30/ml Petridish. Then inoculated and incubate at 37^0 C temperatures for overnight.

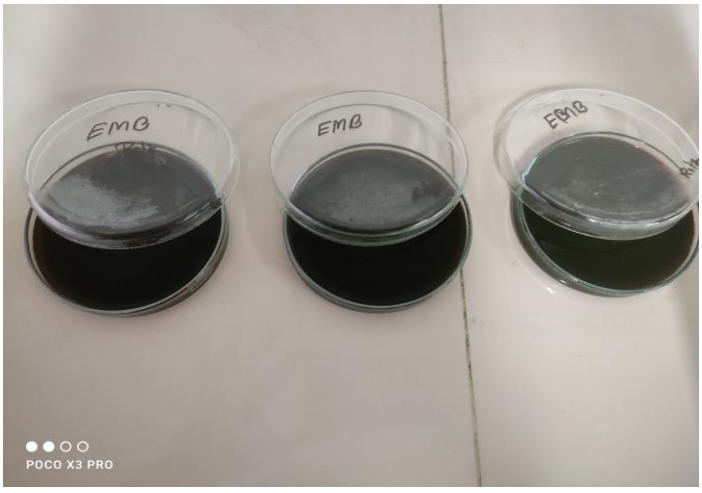

Fig 10: EMB agar media

3.8.2. Inoculate in agar media

Each sample was spread over the EMB, Nutrient Agar & MacConkey Agar media by spread plate method. Cotton ber with smooth edge was used for this spread. After spreading, the petridishes incubated at 37^0 C temperatures for overnight.

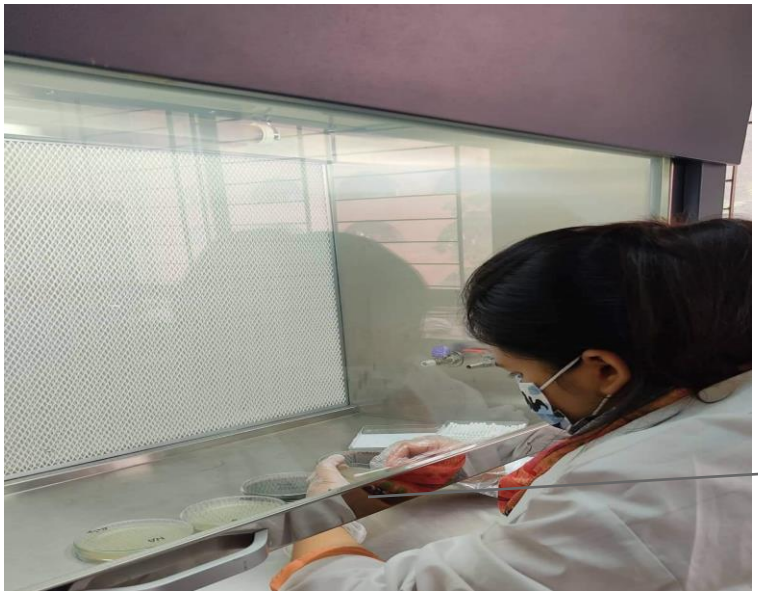

Fig 11: Spread in MacConkey agar media plate

Fig 12: Spread in Nutrient agar media plate

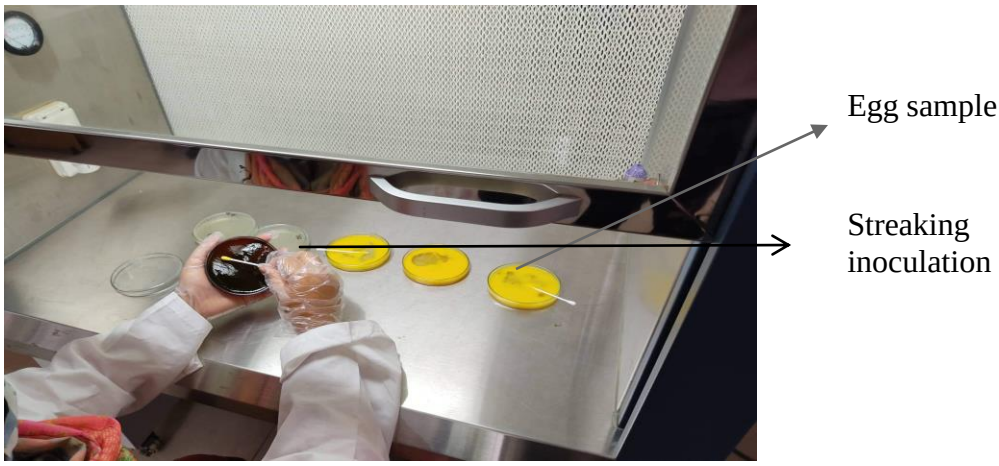

Fig 13: Spreading in EMB agar media plate

3.8.3. Incubation

The inoculated petridishes incubated at 37^0 C temperatures for observing for bacterial media.

3.8.4. Colony morphology

The colony morphology of the isolated *E. coli* was studied as mentioned by Merchant and Packer (1967). Colony morphology such as shape, size, surface texture, edge and elevation, color, and opacity developed after 12 hour of incubation were carefully studied and recorded.

3.8.5. Subculture

The colony from the primary culture was sub-cultured into same media for obtaining the colony. The characteristics colonies were observed during sub-culture in different media.

3.8.6. Gram's staining procedure & Microscopic examination

For performing gram's staining procedure, a small colony was picked up with a sterile loop into a glass slide and smeared. Then it was heated gently to fix on it. Applied crystal violet as a primary stain into the smear on glass slide & allowed to stain for two minutes. Then the slide washed out with running tap water. Followed by few drops of gram's iodine as a mordant was added and then washed out with running tap water. After washing rapidly added alcohol for decolonization. After this added safranin as a counterstaining and kept it for two minutes. Again it washed out with running tap water.

CHAPTER 4

RESULT & DISCUSSION

Result

The result of this study has illustrated the quality of table egg supplied in the selected areas of Mohammadpur, Dhaka city such as Townhall, Agargaon Bazar, Paka Market, Krishi Market, Bihari Camp. Total Quality of egg was studied considering the. *E. coli* existed in the supplied sample.

4.1 Physical examination

It is evident from table -3 that in physical examination where number of 5 cases found positive (*E.coli*) out of 20 cases showing brown with red spotted color number of 4 cases in oval shaped egg out of 15 sample, number of 2 cases in leaked out of 20 and number of 4 cases found in dirty egg out of 20 sample. The higher prevalence found in oval shaped egg (26.67%) whereas, lowest found in leaked egg (10%). But no prevalence found in case of irregular shaped and cracked egg.

Table 3: Prevalence of physical parameter observation

Sl No.	Parameters	Result	No of eggs	No of *E. coli* positive	Prevalence (%)
1	Color	Brown with red spotted	20	5	25
2	Shape	Oval shape	15	4	26.67
		Irregular shape	05	00	00
3	Cracked	Considered	20	00	00
4	Leaked	Considered	20	2	10
5	Dirty	Considered	20	4	20
	Total		**100**	**15**	**15%**

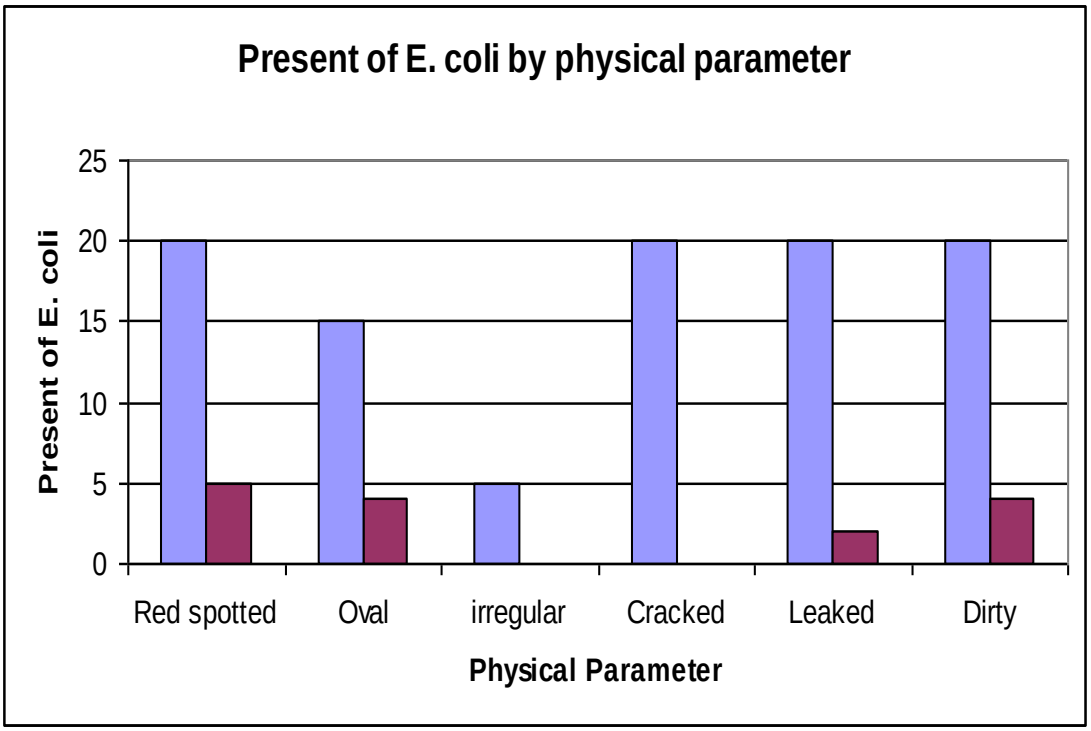

Fig 14: Graphical representation of the present *E. coli* in physical parameter

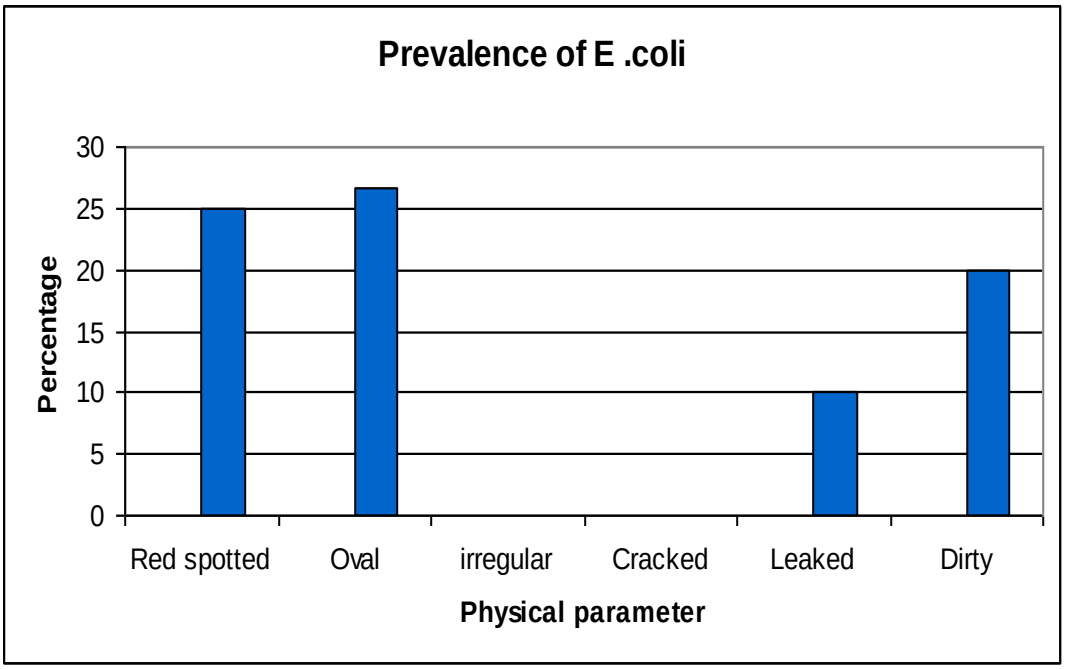

Fig 15: Graphical representation of the prevalence of *E. coli* in physical examination

Table 4: Comparison of egg sample in different market.

Sl no	Parameters		Market Area				
			Agargoan	**Paka market**	**Townhall**	**Bihari camp**	**Krishi market**
1	Color (Brown with red spotted)		√	√	√	√	√
2	Shape	Oval	√	–	√	–	√
		Irregular	–	√	–	√	–
3	Cracked		–	√	–	–	√
4	Leaked		√	–	–	√	√
5	Dirty		√	√	√	√	√

4.2. Bacteriological examination

4.2.1. Colony characteristics in EMB agar, Nutrient agar & MacConkey agar

In EMB the E. coli bacteria produced Greenish colony with metallic sheen & In Nutrient agar it producedwhitish colony. There were no observations of the colonies in MacConkey agar media.

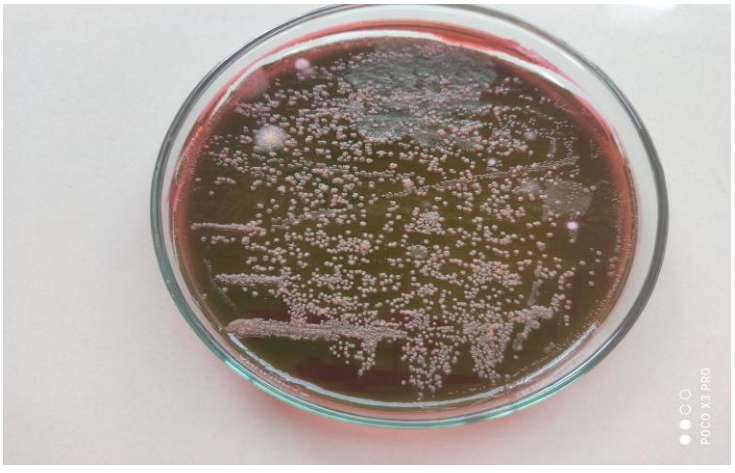

Fig 16: Greenish red color colony with faint metallic sheen (EMB agar)

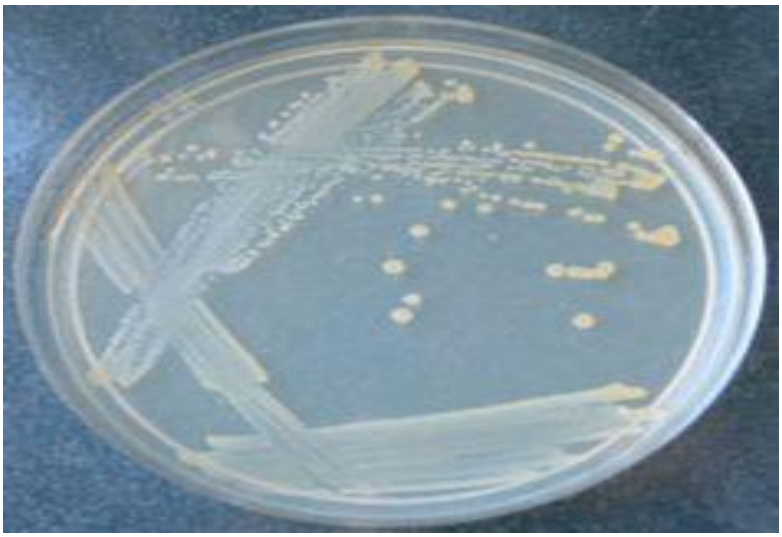

Fig 17: Whitish color colony in Nutrient agar media

4.3. Grams staining and microscopic observation of *E. coli*

After gram's staining, the slide were observed under light microscope and found gram negative, rod shaped, pink color colony arranged as single or paired which indicated the presence of *E. coli* during observation.

Fig 18: Microscopic observation

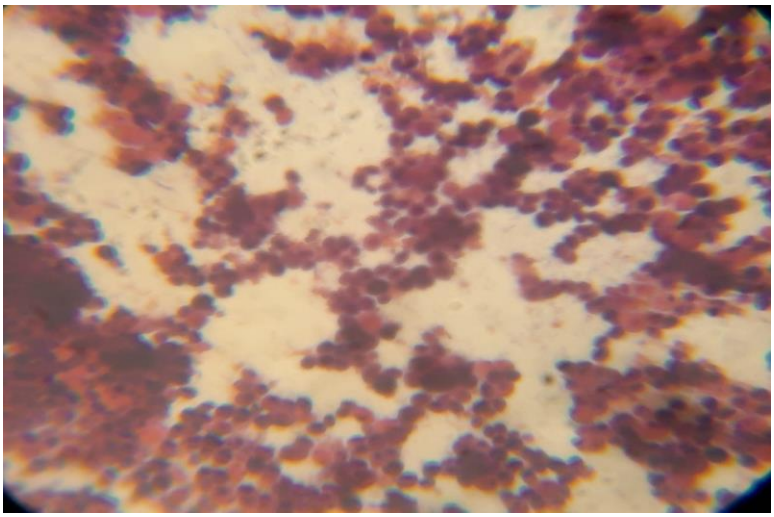

Fig 19: *E. coli* under microscope (100X)

Table 5: Growth properties, staining properties, microscopic observation of the *E. coli*

Properties	Observation
Growth Properties	Produced greenish red color colony with faint metallic sheen in EMB agar & Produced whitish colony in Nutrient agar
Staining Properties	Gram negative bacteria
Microscopic Observation	Rod shape, pink color, arranged single or paired.

Growth properties, staining properties & microscopic observation are give in table-4. In growth properties where observed greenish red color colony with faint metalllic sheen in EMB agar and whitish colony in Nutrient agar. In microscopic observation where rod shape, pink color single or paired arranged**.**

Table 6: Prevalence of *E. coli* in table eggs collected from retail market

Sl no	Name of area	Total No. of eggs examined	No. of *E. coli* positive	Occurrence (%)
1.	Townhall	20	3	15
2.	Agargoan	20	6	30
3.	Paka market	20	0	0
4.	Krishi market	20	2	10
5.	Bihari camp	20	4	20
	Overall	100	15	15%

Prevalance of *E.coli* in table eggs collected from retail market of different areas of mahammadpur are presented in table-5. Prevalence of *E.coli* were 15, 30, 0, 10, & 20 in townhall, Agargaon, Paka Market, Krishi Market & Bihari camp respectively. The higest prevalence found in Agargaon (30%) whereas, lowest found in Krishi market (10%). But no prevalence recorded in paka market. This maybe due to unhygienic condition of Agargoan & good hygienic condition of paka market.

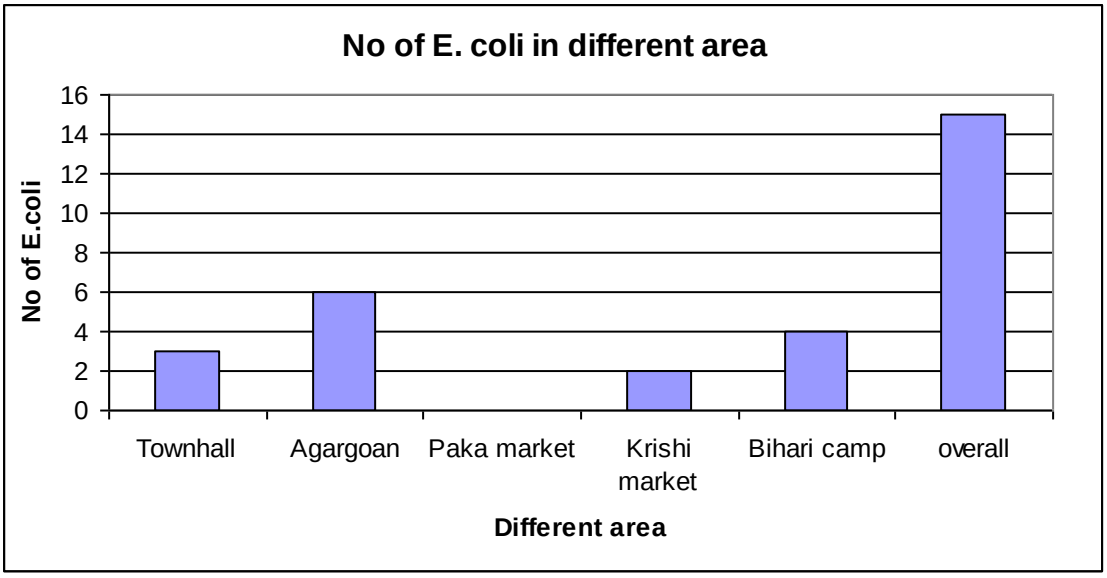

Fig 20: Graphical representation of Number of *E. coli* in different areas.

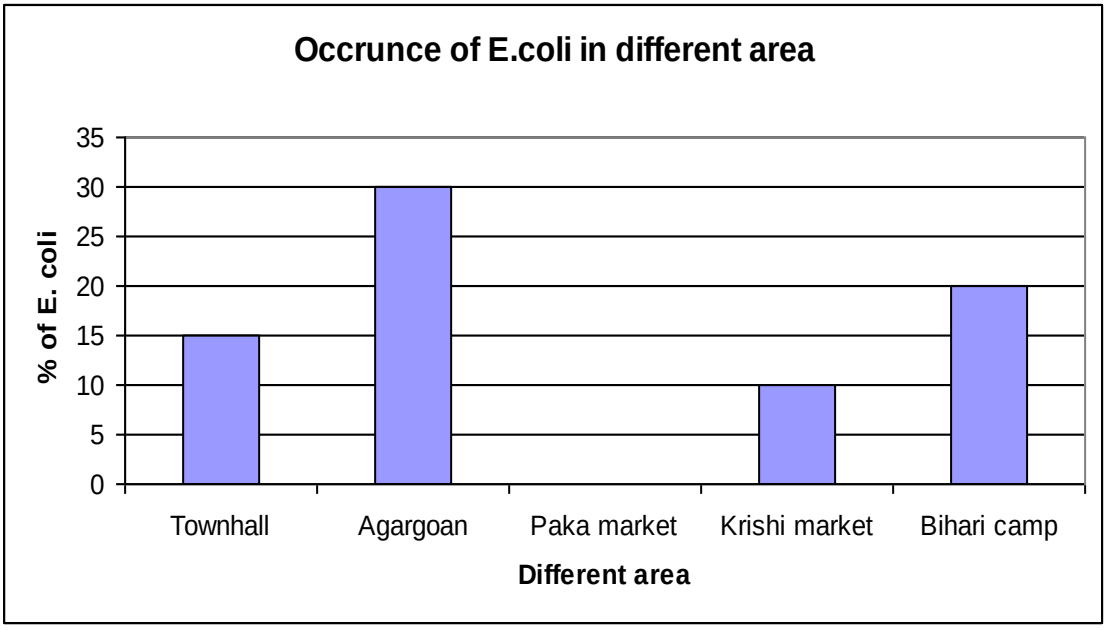

Fig 21: Graphical representation of the prevalence of *E. coli* in different areas.

Table 7: Percentage of *E. coli* and mixed contamination

Total sample	*E. coli* positive	Mixed contamination
100	15	5
Prevalence	15%	5%

The table-6 showed that out of 100 samples there was 5 mixed contamination.

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 microorganism. Out of the total 100 samples of consumption table eggs collected in and around the Mohammadpur area and tested in this study, the 15 samples were contaminated with *E. coli*. The presence of the bacterial contamination indicates that the consumer eggs have been contaminated with pathogenic agents. *Escherichia coli* can infect consumer eggs through infected broodstock, fecal contamination, packaging and transport systems, which can lead to cracked or broken egg shells, long storage times and environmental pollution.

The prevalence of *E. coli* was 15% in chicken eggs in the present study. This finding was in agreement with the findings of Hasan *et al*. (2021) who reported that 38.89% chicken eggs were contaminated with *E. coli* in Rajshahi city of Bangladesh. Our finding was lower than the finding of Hasan *et al*. (2021) who reported that the prevalence of *E. coli* was 38.89% in chicken eggs in Rajshahi because of maintain hygienic condition in present study area. Similarly Islam *et al.* (2018) who reported that 34.64% chicken eggs were contaminated with *E. coli* in Dhaka city of Bangladesh. However, Akond *et al.* (2009) reported that 42% chicken eggs surfaces were contaminated with *E. coli* in poultry and poultry farms environments in Bangladesh. Aurangzeb *et al*. (2015) was demonstrated an overall 37.00% *E. coli* contamination in table eggs in pakisthan. In the current study, the prevalence *E. coli* was 58.33% in commercial layer farm eggs *E. coli* in farms in Trinidad. The prevalence of *E. coli* was 27.78% on chicken egg shells in our study. Similar reports were published previously by Adesiyun *et al*. (2005) and Eman *et al.* (2015). Adesiyun *et al*. (2005) reported that 28.3% chicken egg shells were contaminated with *E. coli* in Trinidad. Eman *et al.* (2015) stated that 28.58% chicken egg shells were *E. coli* contaminated in Egypt.

Similarly, El-kholy *et al*. (2014) reported that the prevalence of *E. coli* was 11.76% in chicken egg contents in Beni-suef city, Egypt. However, our finding is higher than the findings of Adesiyun *et al.* (2005), Sabrinath *et al*. (2009). Adesiyun *et al*. (2005) reported that 3.8% chicken egg contents was positive for *E. coli*. Sabrinath *et al*. (2009) reported that the prevalence of *E. coli* was 13.

CHAPTER 5

CONCLUSION

The prevalence of *E. coli* is 15%.Among the kitchen market occurrence of *E. coli* was highest in Agargoan 30% and lowest in Paka market. The presence of *E. coli* bacteria in table egg particularly in the inner content of egg is alarming as they cause public health hazards. Findings of this study indicate the importance of improving hygienic measures and increasing public awareness of sanitation during egg production, handling, transportation and processing to prevent the spread of resistant bacteria and food-borne pathogen.

The higher incidence of microorganisms may be attributed to unhygienic and improper handling during collection, transportation and storing of the eggs. Constant microbiological monitoring is therefore essential for maintaining the hygienic measures that should be followed during handling, transportation and storage to minimize the contamination of eggs.

The following principles may be suggested to ensure the quality assurances and quality control of eggs and egg products:

Buying

- Buy eggs only if sold from a refrigerator or refrigerated case.
- Open the carton and make sure that the eggs are clean and the shells are not cracked.

Storing

- Store promptly in a clean refrigerator at a temperature of 40^0 F or below. Use a refrigerator thermometer to check.
- Store eggs in their original carton and use them within 3 weeks for best quality
- Use or egg hard cooked eggs.

Preparing

- Wash hands, utensils, equipments and work surfaces with hot water, soapy water before and after they come in contact with raw eggs and raw egg-containing foods.
- Appropriate cleaning and disinfections of the poultry houses.

RECOMMENDATION

This study was conducted to identify the microbial quality of table egg in the study area. Some samples were found with *E. coli.* It can cause serious health hazard for consumer. So, further analysis should be done to identify the source of contamination, degree of pathogenicity of bacteria and to take precautionary measure.

LIMITATION

Due to Corona virus outbreak in 2019, the research work became hampered when it declared as a pandemic in all over the world. Strict lockdown was declared in our country. In this critical situation it couldn't continue. The entry of lab wasn't available; it was a huge problem to keep the continuation of work.

REFERENCE

Adesiyun A, Offiah N, Seepersadsingh N, Rodrigo S, Lashley V, Musai L and Georges K Microbial health risk was posed by table eggs in Trinidad (2005). Epidemiology and Infection, 133: 1049-1056.

Adesiyun A, Ofah N, Seepersadsingh N, Rodrigo S, Lashley V and Musai L (2007). Antimicrobial resistance of *Salmonella spp*. and *Escherichia coli* isolated from table eggs. Food Control, 18 (4): 306-311.

Adesiyun A, Offiah N, Seepersadsingh N, Rodrigo S, Lashley V, Musai L, Georges K. Microbial health risk posed by table eggs in Trinidad. Epidemiological Infection. 2005; 133:1049–1056.

Adnan Yousuf, (2015). Isolation, identification and antibiogram of Escherichia coli from table eggs. Journal of Animal health and production. Vol 4, p 1.

Akhtar, F., H. Afzal, M. Ashfaque and Z. Nasim (1982). Egg-shell contaminations of human importance with reference to *Salmonellae*. J. Pak. Vet., 2: 10-11.

Aurangzeb Khan, Rahmatullah Rind, Muhammad Shoaib, Asghar Ali Kamboh, Gulfam Ali Mughal, Shakeel Ahmad Lakho, Kanwar Kumar Malhi, Ali Raza Nizamani, Adnan Yousaf (2015). Isolation, Identification and Antibiogram of Escherichia coli from Table Eggs. J. Anim. Health Prod. 4 (1): 1 -5

Begum S, Hazarika GC and Rajkhowa S (2014). Prevalence of *Escherichia coli* from pigs and cattle. Journal of Animal Health and Production, 2(3): 38-39.

Biswas PK, Uddin GM, Barua H, Roy K, Biswas D, Ahad A and Debnath NC (2006). Causes of loss of Sonali chickens on smallholder households in Bangladesh. Preventive Veterinary Medicine 76 (3-4): 185-195.

Brasher, C., G. Panicker and A.K. Bej, (2002). Evaluation of PCR amplification based detection of heat-killed Escherichia coli and cell-free DNA in shellfish. Mol. Biol. Today, 3: 85-90.

Bulte, M. and P. Jakob, (1995). The use of a PCRgenerated invA probe for the detection of Salmonella sp. in artificially and naturally contaminated foods. Int. J. Food Microbial., 26: 335- 344.

Bonetta, S., E. Borelli, S. Bonetta, O. Conio, F. Palumbo and E. Carraro, (2010). Development of a PCR protocol for the detection of *Escherichia coli* O157:H7 and *Salmonella sp*. in surface water. Environ. Monit. Assess., (In press).

Cebula, T.A., W.L. Payne and P. Feng, (1995). Simultaneous identification of strains of *Escherichia coli* serotype O157:H7 and their Shiga-like toxin type by mismatch amplification mutation assay-multiplex PCR. J. Clin. Microbiol., 33: 248-250.

C. O. vinayananda, nadeem fairoze, C.B Madhavaprasad, S M Byregowda, C S Nagarj, prashanth bagalkot, (2017). Studies on occurrence, characterisation and decontamination of emerging pathogenic Escherichia coli (STEC, ETEC and EIEC) in table eggs. British poultry science, vol 58.

Daly, P., T. Collier and S. Doyle, (2002). PCR-ELISA detection of *Escherichia coli* in milk. Lett. Appl. Microbiol., 34: 222-226.

Darwin, K.H. and V.L. Miller, (1999). Molecular basis of the interaction of *Salmonella* with the intestinal mucosa. Clin. Microbiol. Rev., 12: 405-428.

De Reu, K., K. Grijspeerdt, W. Messens, M. Heyndrickx, M. Uyttendaele, J. Debevere and L. Herman, (2006a). Eggshell factors influencing eggshell penetration and whole egg contamination by different bacteria, including Salmonella enteritidis. Int. J. Food Microbiol., 112: 253-260. and L. Herman, (2006b). Bacterial eggshell contamination in the egg production chain and in different housing systems. Br. Poult. Sci., 47: 163- 172.

Debnath NC, Sil BK, Selim SA, Prodhan MAM and Howlader MR (1990). A respective study of calf morbidity and mortality in small holder traditional farms in Bangladesh. Preventive Veterinary Medicine 9: 1-7

Desmarchelier, P.M., S.S. Bilge, N. Fegan, L. Mills, J.C. Jr. Vary and P.I. Tarr, (1998). A PCR specific for *Escherichia coli* O157 based on the rfbE locus encoding O157 lipopolysaccharide. J. Clin. Microbiol., 36: 1801-1804.

Doran, J.L., S.K. Collinson, J. Burian, G. Sarlos, E.C.D. Todd, C.K. Munro, C.M. Kay, P.A. Banser, P.I. Peterkin and W.W. Kay, 1993. DNA-based diagnostic test for Salmonella species targeting agfA, the structural gene for thin aggregative fimbriae. J. Clin. Microbiol., 31: 2263-2273.

Doyle, M.P. and J.L. Schoeni, (1987). Isolation of *Escherichia coli* O157:H7 from retail fresh meats and poultry. Appl. Environ. Microbiol., 53: 2394- 2396. 62.

Elder RO, Keen JF and Siragusa GR. (2000). Correlation of enterohemorrhagic *Escherichia coli* O157 prevalence in feces, hides, and carcasses of beef cattle during processing. Proceedings of the National Academy of Sciences 97: 2999-3003.

Ellen, H. H., Bottcher, R. W., von Wachenfelt, E. and Takai, H. (2000). Dust levels and control methods in poultry houses. Journal of Agricultural Safety and Health 6: 275-282.

Fairbrother JM and Nadeau E (2006). *Escherichia coli*: On-farm contamination of animals. Review of Science and Technology 25 (2): 555-569.

Fateha Akther Ema , Mohammad Arif1, Md. Ariful Islam and Mst. Minara Khatun, (2018). Isolation and identification of duck egg-borne bacteria and their antibiogram profile. Journal of Advanced Veterinary and Animal Research, 5(2): 110-116.

Ferretti, R., L. Mannazzu, L. Cocolin, G. Comi and F. Clementi, (2001). Twelve-hours PCR-based method for detection of *Salmonella sp*. In Food. Appl. Environ. Microbiol., 74: 977-978.

Ferri M, Ranucci E, Romagnoli P and Giaccone V (2017). Antimicrobial resistance: a global emerging threat to public health systems. Critical Reviews in Food Science and Nutrition, 57: 2857-2876.

Gannon, V.P.J., M. Rashed, R.K. King and E.J.G. Thomas, (1993). Detection and characterization of the eae gene of shiga-like toxin-producing *Escherichia coli* using polymerase chain reaction. J. Clin. Microbiol., 31: 1268-1274.

Gebreyes, W.A., P.R. Davies, W.E.M. Morrow, J.A. Funk and C. Altier, (2000). Antimicrobial resistance of *Salmonella* isolates from swine. J. Clin. Microbiol., 38: 4633-4636.

Guo, L., J. Killefer, P.B. Kenney and J.D. Mick-Morris, (1999). Use of arbitrarily primed polymerase chain reaction to study *Salmonella* ecology in a turkey production environment. Poult. Sci., 78: 24-31.

Haines, R.B., (1938). Observation on the bacterial flora of the hen's egg with a discription of new species of *Proteus* and *Pseudomonas* causing rot in eggs. J. Hyg., 38: 338-355.

Hassan MM, Amin KB, Ahaduzzaman M, Alam M, Faruk MSA and Uddin I (2014). Antimicrobial resistance pattern against *E. coli* and *Salmonella* in layer poultry. Research Journal for Veterinary Practitioners, 2(2): 30-35.

Harry, E.G., (1963). The relationship between egg spoilage and the environment of the egg when laid. Br. Poult. Sci., 4: 91-100.

Hashimoto, Y., Y. Itho, Y. Fujinaga, A.Q. Khan, F. Sultana, M. Miyake, K. Hirose, H. Yamamoto and T. Ezaki, (1995). Development of nested PCR based on the ViaB sequence to detect *Salmonella typhi*. J. Clin. Microbiol., 33: 775-777.

Heininger, A., M. Binder, S. Schmidt, K. Unertl, K. Botzenhart and G. Doring, (1999). PCR and blood culture for detection of *Escherichia coli* Bacteremia in rats. J. Clin. Microbiol., 37: 657-661.

Heijnen, L. and G. Medema, 2009. Method for rapid detection of viable *Escherichia coli* in water using real-time NASBA. Water Res., 43: 3124-3132.

MS Hossain, W Rahman, MS Ali, T Sultana and KMM Hossain(2021). Identification and antibiogram assay of Escherichis coli from isolated from chicken eggs. J. Bio-Sci. 29(1): 123-133.

Howard ZR, O'Bryan CA, Crandall PG, Ricke SC. *Salmonella enteritidis* in shell eggs: Current issues and prospects for control. Food Research International. (2011); 45:755–764.

Holland, J.L., L. Louie, A.E. Simor and M. Louie, (2000). PCR Detection of *Escherichia coli* O157:H7 Directly from stools: Evaluation of commercial extraction methods for purifying fecal DNA. J. Clin. Microbiol., 38: 4108-4113.

Iida, K., A. Abe, H. Matsui, H. Danbara, S. Wakayama and K. Kawahara, (1993). Rapid and sensitive method for detection of *Salmonella* strains using a combination of polymerase chain reaction and reverse dot-blot hybridization. FEMS Microbiol. Lett., 114: 167-172.

I. N Abdulla (2010), Isolation and Identification of some bacterial isolates from table egg. Al-Anbar J.Vet.Sci., Vol: 3 No (2)

Islam MJ, Sultana S, Das KK, Sharmin N and Hassan MN (2008). Isolation of plasmid-mediated multidrug resistant *Escherichia coli* from poultry. International Journal of Sustainable Crop Production, 3(5): 46-50.

Islam M, Sabrin MS, Kabir MHB and Aftabuzzaman M (2018). Antibiotic sensitivity and resistant pattern of bacteria isolated from table eggs of commercial layers considering food safety issue. Asian Journal of Medical and Biological Research, 4(4): 323-329.

Jones, F.T., D.V. Rives and J.B. Carey, (1995). *Salmonella* contamination in commercial eggs and an egg production facility. Poult. Sci., 74: 753-757.

Jothikumar, N. and M.W. Griffiths, (2002). Rapid detection of *Escherichia coli* O157:H7 with multiplex real-time PCR assays. Appl. Environ. Microbiol., 68: 3169- 3171.

Lee M, Seo DJ, Jeon SB, Ok HE, Jung H, Choi C and Chun HS (2016). Detection of foodborne pathogens and mycotoxins in eggs and chicken feeds from farms to retail markets. Korean Journal for Food Science and Animal Resources, 36: 463-468.

Mahon, J. and A. Lax, (1993). A quantitative polymerase chain reaction method for the detection in avian faeces of Salmonella carrying the spvR gene. Epidemiol. Infect., 111: 455-464.

Mahon, J., C.K. Murphy, P.W. Jones and P.A. Barrow, (1994). Comparison of multiplex PCR and standard bacteriological methods of detecting Salmonella on chicken skin. Lett. Appl. Microbiol., 19: 169-172.

Maha A.M. AL-Ashmawy, (2013).Prevalence of Enterobacteriace in table eggs with particular reference of Enterovirulent Escherichia coli strains. International Journal of poultry science, 12: 430-435.

M. A. Zinnah , M. R. Bari , M. T. Islam &, M. T. Hossain, M. T. Rahman , M. H. Haque , S. A. M. Babu , R. P. Ruma and M. A. Islam, (2007) . Characterization of Escherichia coli isolated from samples of different biological and environmental sources. J. Vet. Med. (2007). 5 (1 & 2): 25–32

Mayes, F.J. and M.A. Takeballi, (1983). Microbial contamination of the hen's egg: A review. J. Food rot., 46: 1092-1098.

Methaq Ghalib Abd AL-Rubaiey , Noor A. ALtaai , Moutaz A.W.A. Mounam and Majid Mohammed Mahmood (2020). Study of Bacterial Contamination of Defected Eggshells and Egg Contents in Baghdad City. Plant Archives Vol. 20 Supplement 1, 2020 pp. 2306-2308 e-ISSN:2581-6063 (online),ISSN:0972-5210.

Muhammad Ali Akond, Saidul Alam, S. M. R. Hassan, Momena Shirin (2009). Antibiotic Resistance of Escherichia Coli Isolated from poultry and poultry Environment of Bangladesh. Internet journal of food safety, vol.11, p-19-23.

Oberst, R.D., M.P. Hays, L.K. Bohra, R.K. Phebus and J.M. Sargeant, (2003). Detection of *Escherichia coli* O157:H7 in cattle feces using a polymerase chain reaction based fluorogenic 59 nuclease (TaqMant) detection assay after secondary enrichment. J. Vet. Diagn. Invest., 15: 543-552.

Osimani A, Aquilanti L and Clementi F (2016). Salmonellosis associated with mass catering: A survey of European Union cases over a 15-year period. Epidemiology and Infection, 144(14): 3000-3012.

Pasquali F, Decesaro A, Valero A, Olsen JE and Manfreda G (2014). Improvement of sampling plan for *Salmonella* detection in pooled table eggs by use of real-time PCR. International Journal of Food Microbiology, 184: 31-34

Paton, A.W. and J.C. Paton, (1998). Detection and characterization of shiga toxigenic *Escherichia coli* by using multiplex PCR assays for stx1, stx2, eaeA, enterohemorrhagic *E. coli* ehxA, rfbO111 and rfbO157. J. Clin. Microbiol., 36: 598-602.

Pratama K A, et al (2020). Evaluation of Escherichia coli contamination of consumed eggs around the campus of the agricultural state polytechnic of Payakumbuh, Indonesia. J food safe & hyg; 6 (3): 122-126

Perez Guzzi JI, Folabella A, Miliwebsky E, Rivas M, Fernandez Pascua C, Gomez D, Zamora A, Zotta C and Cordoba M (2000). Isolation of *Escherichia coli* O157:H7 in storm drains in the city of Mar del Plata with bacterial contamination of fecal origin. Revista Argentina de Microbiologia 32 (3): 161-164.

Rabbani G M, Haque S M, Jalil M. A , Rahaman M M, Nabi R M, Siddique B. A (2020). Isolation and Identification of bacteria from shell surface of eggs in local market of sylhet. International journal of natural and social science, 7(4): 81-93

Rahn, K., S.A. De Grandis, R.C. Clarke, S.A. McEwen, J.E. Galan, C. Ginocchio, R. Curtiss and C.L. Gyles, (1992). Amplification of an invA gene sequence of *Salmonella typhimurium* by polymerase chain reaction as a specific method of detection of Salmonella. Mol. Cell. Probes., 6: 271-279.

Rahman, M.M., Z.U.M. Khan, A.N.M. Rahman and M.S. Islam, (2006). Recovery of *Salmonella* from incubated eggs, newly hatched chicks and contaminated environments. J. Anim. Vet. Adv., 5: 598-601.

Rajashekara, G., E. Harverly, D.A. Halvorson, K.E. Ferris, D.C. Lauer and K.V. Nagaraja, (2000). Multidrug resistant *Salmonella typhimurium* DT104 in poultry. J. Food Prot., 63: 155-161.

Regita Cahyani Sauring , Syam S. Kumaji , and Erfan AR. Lainjong (2021). Identification of Escherichia coli Bacteria in Quail Eggs that for Sale in the Central Market of the City of Gorontalo. Journal of Health, Technology, and Science (JHTS) E-ISSN: 2746-167X, Vol. 2, No. 1.

Robles, M.A.G., A.M. Loredo, G.A. Ojeda, J.A.O. Garcia, I.O. Martinez, L.H.M. Ramos and P. Fratamico, (2009). PCR detection and microbiological isolation of *Salmonella sp.* from fresh beef and cantaloupes. J. Food. Sci., 74: 37-40. apid molecular detection of *Salmonella* isolated from poultry farm. The 19th Veterinary Association Malaysia Congress, 3-5 August 2007, pp: 201-203.

Saif YM, Calnek BW, Barnes HJ, Beard CW, McDougald LR. Diseases of Poultry.10th edition, Iowa State University Press, Iowa, USA. (2009).

Sahilah, A.M., L.Y.Y. Audrey, S.L. Ong, W.N. Wan Sakeenah, S. Safiyyah, A.S. Norrakiah, A. Aminah and A.A. Ahmad, 2010. DNA profiling among egg and beef meat isolates of *Escherichia coli* by enterobacterial repetitive intergenic consensusPCR (ERIC-PCR) and random amplified polymorphic DNA-PCR (RAPD-PCR). Int. Food Res. J., 17: 853-866.

Salehi, T.Z., M. Mahzounieh and A. Saeedzadeh, (2005). Detection of InvA gene in isolated *Salmonella* from broilers by PCR method. Int. J. Poult. Sci., 4: 557- 559.

Sama'a M. Mo'ataz , Nagah M. saad and Tawfik A. ELBassiony (2021). Detection of Coliforms in table eggs, NVVJ., Vol. 1, Issue (2).

Santos, L.R., V.P. Nascimento, S.D. Oliveira, M.L. Flores, A.P. Pontes, A.R. Ribeiro, C.T.P. Salle and R.F.F. Lopes, (2001). Polymerase Chain Reaction (PCR) for the detection of Salmonella in artificially inoculated chicken meat. Rev. Inst. Med. Trop. Sao. Paulo., 43: 247-250.

Schneder, A., C. Gronewald, M. Fandke, B. Kurth, S. Barkowski and K. Berghof-ager, (2002). Real-time detection of the genus *Salmonella* with the light cycler system. Biochemica, 4: 19-21.

Schoeni, J.L., K.A. Glass, J.L. McDermot ong, (1995). Growth and penetration of *Salmonella enteritidis, Salmonella* heidelberg and *Salmonella typhimurium* in eggs. Int. J. Food Microbiol., 24: 385-396.

Schrank, I.S., M.A.Z. Mores, J.L.A. Costa, A.P.G. Frazzon, R. Soncini, A. Schrank, M.H. Vainstein and S.C. Silva,(2001). Influence of enrichment media and application of a PCR based method to detect Salmonella in poultry industry products and clinical samples. Vet. Microbiol., 82: 45-53.

Sharma, V.K. and S.A. Carlson, (2000). Simultaneous detection of *Salmonella* strains and *Escherichia coli* O157:H7 with fluorogenic PCR and singleenrichment-broth culture. Appl. Environ. Microbiol., 66: 5472-5476.

Siriporn Chaemsanit , Ali Akbar and Anil Kumar Anal, (2013). Isolation of total aerobic and pathogenic bacteria from table eggs and its contents. Food and Applied Bioscience Journal, 3 (1): 1–9.

Touron, A., T. Berthe, B. Pawlak and F. Petit, (2005). Detection of *Salmonella* in environmental water and sediment by a nested-multiplex polymerase chain reaction assay. Res. Microbiol., 156: 541-553. Characterization of *Escherichia coli* isolates from Peritonitis lesions in commercial laying hens. Avian Dis., 51: 840-844.

Turner SM, Scott-Tucker A, Cooper LM and Henderson IR (2006). Weapons of mass destruction: virulence factors of the global killer enterotoxigenic *Escherichia coli.* FEMS Microbiology Letters 263 (1): 10-20.

Waleed Al Momani, Sana Janakat and Moawiah Khatatbeh, 2018. Bacterial Contamination of Table Eggs Sold in Jordanian Markets. Pakistan Journal of Nutrition, 17: 15-20.

Wang, H., B.W. Blais and H. Yamazaki, (1995). Rapid confirmation of polymyxin-cloth enzyme immunoassay for group D salmonellae including *Salmonella enteritidis* in eggs by polymerase chain reaction. Food Control., 6: 205-209

Wiriya loongyai et al (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,

Yones, N. Ab. J. and Jwher, Dh. M. Contamination of table egg shell with coliform bacteria in mosul city markets. Basrah Journal of Veterinary Research,Vol.17, No.3.

APPENDIX

Composition of different media

- **Nutrient Agar**

Peptone	5.000 gm.
Sodium chloride	5.000 gm.
HM peptone B#	1.500gm.
Yeast extract	1.500gm.
Agar	15.000gm.

- **MacConkey Agar**

Peptones (meat and casein)	3.000 gm
Pancreatic digest of gelatin	17.000 gm
Lactose monohydrate	10.000 gm
Bile salts	1.500 gm
Sodium chloride	5.000 gm
Crystal violet	0.001 gm
Neutral red	0.030 gm
Agar	13.500 gm
pH after sterilization (at 25°C)	7.1±0.2

- **Eosin Methylene Blue Agar**

Peptic digest of animal tissue	10.000 gm.
Dipotassium phosphate	2.000 gm.
Lactose	5.000 gm.
Sucrose	5.000 gm.
Eosin – Y	0.400 gm.
Methylene blue	0.065 gm.
Agar	13.500gm.
Final pH (at 25°C)	7.2±0.2

Photo Gallary

Fig 1: Study Area during research work

Fig 2: Break the eggs

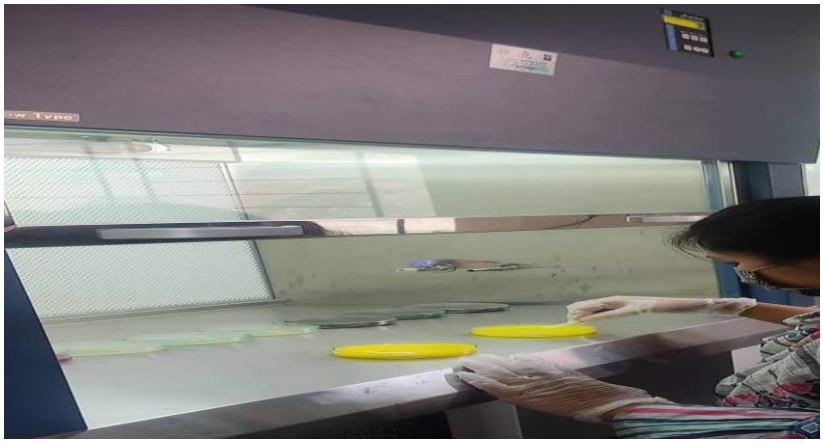

Fig 3: Egg mixing time

Fig 4: Autoclave the petridish

Fig 5: Slide Preparation

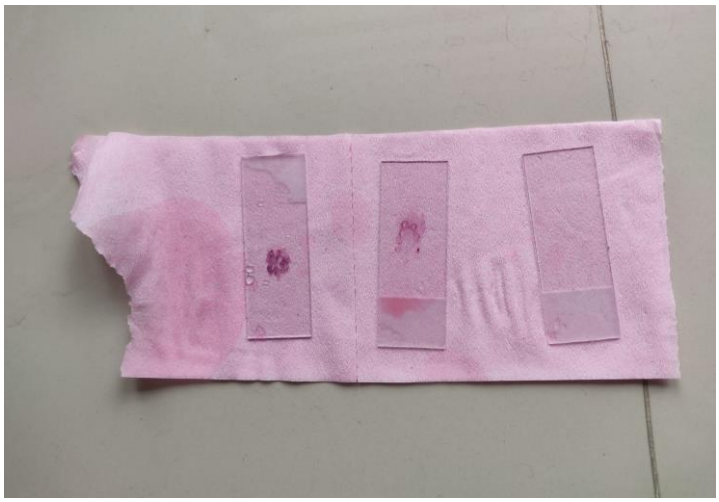

Fig 6: Slide

Fig 7: Microscopic observation

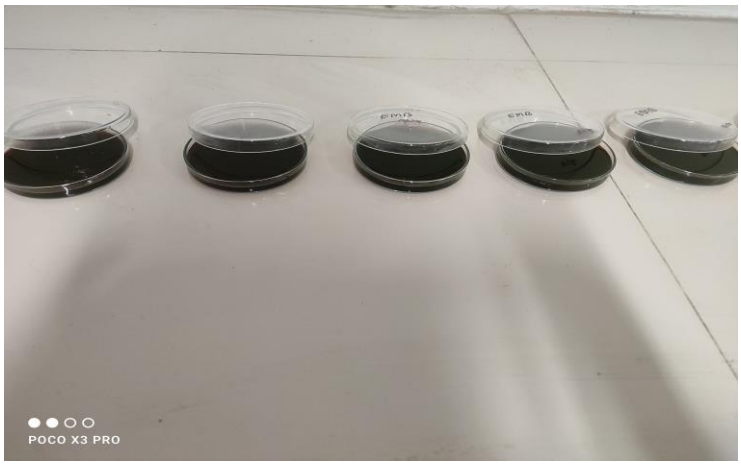

Fig 8: EMB agar media

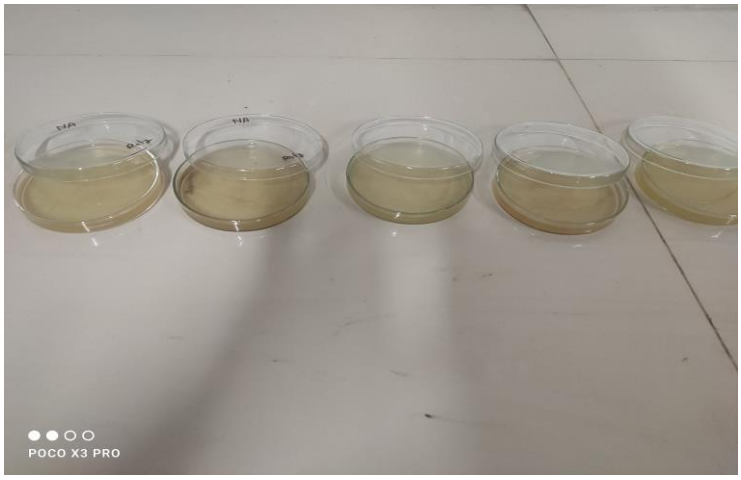

Fig 9: Nutrient agar media

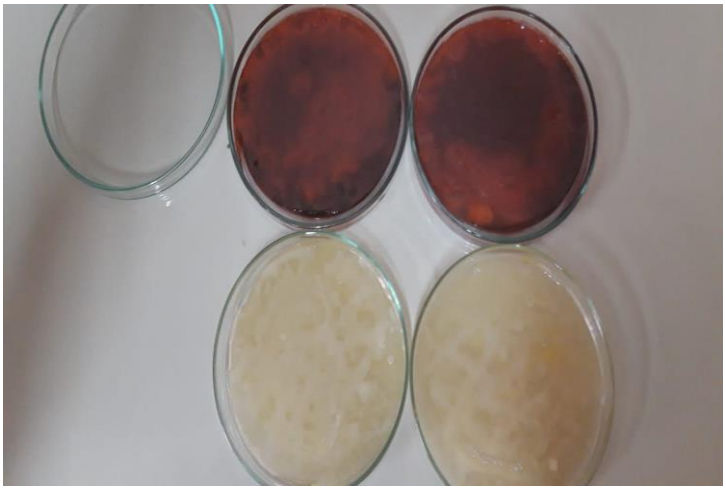

Fig 10: MacConkey agar media