PREVALENCE AND ANTIBIOGRAM STUDY OF PATHOGENIC BACTERIA FROM TABLE EGG

ASHRIFA AKTER MUKTA

MASTER OF SCIENCE IN MICROBIOLOGY
DEPARTMENT OF MICROBIOLOGY AND PARASITOLOGY
SHER-E-BANGLA AGRICULTURAL UNIVERSITY
DHAKA-1207

JUNE, 2019

PREVALENCE AND ANTIBIOGRAM STUDY OF PATHOGENIC BACTERIA FROM TABLE EGG

By

ASHRIFA AKTER MUKTA
REGISTRATION NO.: 12-04996

A Thesis
Submitted to the Faculty of Animal Science and Veterinary Medicine
Sher-e-Bangla Agricultural University, Dhaka-1207, In Partial Fulfillment of the Requirements for the Degree of

MASTER OF SCIENCE (MS) IN MICROBIOLOGY
DEPARTMENT OF MICROBIOLOGY AND PARASITOLOGY

SEMESTER: JAN-JUN/ 2019

Approved by

(Dr. Uday Kumar Mohanta)
Supervisor
Associate Professor
Department of Microbiology and Parasitology
Sher-e-Bangla Agricultural University
Sher-e-Bangla Nagar, Dhaka-1207

(Dr. M. A. Mannan)
Co-Supervisor
Assistant Professor
Department of Microbiology and Parasitology
Sher-e-Bangla Agricultural University
Sher-e-Bangla Nagar, Dhaka-1207

Dr. Uday Kumar Mohanta
Chairman, Examination Committee
Department of Microbiology and Parasitology
Sher-e-Bangla Agricultural University, Dhaka-1207

DEPARTMENT OF MICROBIOLOGY AND PARASITOLOGY
Sher-e-Bangla Agricultural University
Sher-eBangla Nagar, Dhaka-1207

Memo No: SAU/MIPA

CERTIFICATE

*This is to certify that the thesis entitled "**PREVALENCE AND ANTIBIOGRAM STUDY OF PATHOGENIC BACTERIA FROM TABLE EGG**" submitted to the Department of Microbiology and Parasitology, Faculty of Animal Science & Veterinary Medicine, Sher-e-Bangla Agricultural University, Dhaka-1207, as partial fulfillment for the requirements of the degree of Master of Science(MS) in Microbiology, embodies the result of a piece of bona fide research work carried out by **Ashrifa Akter Mukta**, Registration No.: **12-04996** under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma.*

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

Dated: June, 2019
Place: Dhaka, Bangladesh

Dr. Uday Kumar Mohanta
Supervisor
Associate Professor
Department of Microbiology and Parasitology
Sher-e-Bangla Agricultural University,
Dhaka-1207

ACKNOWLEDGEMENT

At the beginning, the author bows the grace and mercy of the "Almighty Allah", the omnipresent, omnipotent and omniscient, who enabled him to complete this thesis. The author express his grateful acknowledgement and indebtedness to his beloved father Md. Mokhlesar Rahman and mother Nurjahan Begum for their ending prayer, encouragement, sacrifice and dedicated efforts to educate me this level.

The author with a sense of respect, expresses his heart felt gratitude to his Supervisor Dr. Uday Kumar Mohanta, Associate Professor, Department of Microbiology and Parasitology, Sher-e-Bangla Agricultural University, Dhaka-1207 for his untiring and painstaking guidance, invaluable suggestions, continuous supervision, timely instructions, inspirations and constructive criticism throughout the tenure of research work.

Heartfelt gratitude and profound respect are due to his Co-supervisor Dr. M.A. Mannan Assistant Professor, Department of Microbiology and Parasitology, Sher-e-Bangla Agricultural University, Dhaka-1207 for his co-operation, constructive criticism, and valuable suggestions for the modification and improvement of the research work.

The author is deeply grateful to Mirza Synthia Sabrin, Assistant Professor, Department of Microbiology and Parasitology,Professor, for her kind help, advice and co-operation in the completion of the study. The author is especially grateful to Mahfuzul Islam, Assistant Professor, Department of Microbiology and Parasitology, Sher-e-Bangla Agricultural University, Dhaka- 1207 for his advice and sincere co-operation in the completion of the study.

The author is ever grateful to the Ministry of Science and Technology, Government of the People's Republic of Bangladesh for providing the NST fellowship and funding the research.

The author is also boundless grateful to all the staffs of the Department of Microbiology and Parasitology, Sher-e-Bangla Agricultural University, Dhaka-1207 for their co-operation. The author express his deep and boundless gratefulness and profound respect to his beloved parents, brother, sister, and friends Tahmina Sikder, Md. Moshiur Rahman, Tasmim Sultana, Shah Jangy Ibn Karim for their love, blessings, prayers, sacrifices, moral support and encouragement a lot in the process of building the academic career which can never repaid.

The Author

PREVALENCE AND ANTIBIOGRAM STUDY OF PATHOGENIC BACTERIA FROM TABLE EGG

ABSTRACT

The present study was carried out to isolate and identify the pathogenic bacteria from table egg with special emphasis to *E. coli*, *Salmonella* spp. and *Staphylococcus* spp. Techniques forward to isolate, identify and characterize the bacterial species by cultural, morphological, biochemical and staining. The antimicrobial susceptibility pattern of isolates were investigated by disc diffusion method. A total number of 40 eggs were collected from different markets of Dhaka, Bangladesh such as Krishi market, Bihari camp market, Agargaon market and SAU market. In Gram's staining, the organism revealed gram-negative, pink color, small rod shaped appearance, arranged in single or paired short called *E.coli*, the organism revealed gram negative, short rod shaped, singly arranged called *Salmonella* spp., the organisms were found as gram positive grapes like clusters formed by cocci. *E. coli* from 14 samples, *Salmonella* spp. from 11 samples and *Staphylococcus* spp. from 7 samples were isolated from total of 40 samples that means (35.00%) were positive for *E. coli* ,(27.50 %) were positive for *Salmonella* spp. and (17.50%) were positive for *Staphylococcus* spp. The antibiotic sensitivity test indicated that the isolated *E. coli* were highly resistant to penicillin, erythromycin and amoxicillin; and were sensitive to tetracycline, ciprofloxacin and gentamycin. The isolated *Salmonella* spp. were highly resistant to penicillin, erythromycin and amoxicillin; and were sensitive to tetracycline, ciprofloxacin and gentamycin. *Staphylococcus* spp. were resistant against Neomycin, Vancomycin and were sensitive to ciprofloxacin, erythromycin, tetracycline and gentamycin.

LIST OF CONTENTS

LIST OF TABLE

LIST OF FIGURES

LIST OF ABBREVIATIONS

MAC	MacConkey
EMB	Eosin Methylene Blue
SSA	Salmonella-Shigella Agar
MSA	Manitol Salt Agar
SAU	Sher-e-Bangla Agricultural University
CHO	Carbohydrate
BA	Blood agar
NA	Nutrient Agar
MHA	Muller Hinton Agar
µg	Micro gram
MAR	Multiple drug resistant
Spp.	Species

CHAPTER 1
INTRODUCTION

The egg is very rich in nutrients, making it one of the most valuable and most perfect foodstuffs. Eggs are considered to be an excellent source of choline and selenium, and a good source of vitamin B12, phosphorus and riboflavin. The yolk contains vitamins A, D, E and K as well as folic acid, pantothenic acid and zinc. Eggs can fully meet all the necessary nutrients for their development and life functions of organisms, including humans. At the same time, many nutrient substances present in eggs provide an excellent environment for the development of bacterial microflora, including pathogenic bacteria. Eggs can be contaminated or infected horizontally (through the shell) or vertically (transovarially) and thereby, act as a potential source of pathogens participating in the etiology of food borne diseases in humans (Stępień-Pyśniak, 2010).

Freshly laid eggs are generally sterile, however they may constitute, if contaminated, a public health hazard, leading to economic losses through spoilage. Regarding the increasing consumption of egg and its products, it is necessary to investigate different factors implicated in egg contamination. The egg shell contamination may result from deposition of fecal material on the shell, ovarium or oviduct and gut flora, debris material, egg crates, packing and storage, clothes and hands of poultry workers, dust, the environment, weather conditions, transportion and marketing (Al-Bahry *et al.*, 2012).

Food borne illness is a major public health problem and the main cause of diarrhoeal diseases affecting all developed and developing countries (Akbar and Anal, 2013b; Akbar and Anal, 2014). Table eggs are the best and easy source of food, containing quality protein, essential amino acids, essential vitamins and minerals needed for a good health (MAFF, 2009). Asia is the largest egg producing region with 65% global outputs (Ernst, 2009). A combined share of egg production from China, India and Japan are more than 46% (Peter, 2011). However, China itself is the number one of the top 10 countries that have provided 38% of the world's eggs demand in 2011 (Peter, 2011).

Eggs have natural defense system against the contaminating microbes, such as cuticle, calcium hard shell and shell membrane (Jerzy and Dagmara, 2009). The albumen contains several egg white proteins that have antimicrobial properties, especially the lysozyme. Ovomucoid is another proteinase that inhibits the ability of bacterial to use the protein in albumen. Furthermore, the pH in albumen which is about 9–10 and the viscosities of the egg white are not suitable for microbial growth (Froning, 1998). Egg can be contaminated at both egg shell and egg contents by a variety of microbes with a wide range of pathogens such as *Campylobacter jejuni*, *Escherichia coli, Yersinia enterocolitica* and especially *salmonella* (Ricke *et al*., 2001; Board and Tranter, 1995). Staphylococci are most common bacteria contaminating eggshells.

Contamination is more likely linked with cracked egg, dirty shells and storage in contaminated surroundings. It can be contaminated during formation and laying process (Abdullah, 2010). Elliott (1954) revealed that stored or aged eggs have more possibility to become infected than fresh eggs due to the degradation of natural defense mechanisms in egg over time. The eggshell contamination increasing the chances of egg contents contamination by penetration (Messens *et al.,* 2006). Bacterial contamination can happen at three main parts of egg (egg yolk, albumen and shell membrane / egg shell) (Bahrouz, 2005). *Salmonella enteritidis* is able to invade the cells of the follicles before ovulation and multiply themselves after 2 h of infection (Howard *et al.,* 2005).

Eggs are considered to be a medium to low risk food for foodborne illness which can become contaminated with bacteria, like *Salmonella* and other enteric pathogens (Chousalkar *et al*., 2010). The most common foodborne pathogens associated with food of animal origin are *Salmonella, Campylobacter, Listeria monocytogenes, Staphylococcus aureus* and *Escherichia coli* O157 (Akbar and anal, 2011; Ghasemian, 2011; Akbar and Anal, 2013a). In current study a survey was conducted for enumeration of aerobic bacterial load and the pathogens on eggshells and in egg contents. The pathogens were also examined for its antibiogram.

Objectives of the study:

i. To isolate and identify pathogenic bacteria from table egg.
ii. To know the prevalence of pathogenic bacteria in table egg.
iii. To assess antibiogram profile of isolated organisms.

CHAPTER 2
REVIEW OF LITERATURE

2.1 Isolation of pathogenic bacteria

Bufano-Nancy (2000), reported that sources of egg contamination are numerous as egg may be infected before it is laid, and also outside by the fecal matter, the lining of the nest, wash water if the eggs are washed, handling and perhaps by the material in which eggs are packed. Furthermore, according to the Centers for Disease Control and Prevention (CDC), eggs are responsible for an estimated 2,30,000 cases of food-borne illness each year.

Przybylska (2000), reported that food poisoning and food-borne infection following consumption of eggs or dishes containing eggs are usually caused by *Salmonella*, as well as *Staphylococcus aureus, Escherichia coli* and other coli bacilli.

Bufano *et al.* (2000), suggested that eggs are considered one of the most delicious and popular foods all over the world; they can be prepared in many different ways to suit everyone's taste. Eggs and egg products enter in a wide variety of foods including custard, mayonnaise, egg salad and all types of bakery products. Also there are another uses of eggs as soil fertilization, culture media, artificial insemination, cosmetics, shampoo and adhesives. Eggs are super foods which provide unique well balanced nutrients for human of all ages. They contain large amounts of protein, amino acids, vitamins and minerals, low caloric value and easily digestible make them valuable choice in many therapeutic based diets for adults.

Sim *et al.* (2000), found that most freshly laid eggs are sterile; at least from the inside due to many factors as a good management of laying flock, mechanical defense barriers as outer spongy and inner mammillary layers of the eggshell, cuticle (waxy membrane), shell membranes and biological defense barrier as the anti-microbial properties and the pH of the white.

Ricke *et al.* (2001), described that egg can be contaminated at both egg shell and egg contents by a variety of microbes with a wide range of pathogens such as

Campylobacter jejuni, *Listeria monocytogenes*, *Escherichia coli*, *Yersinia enterocolitica* and especially *Salmonella sp.*

Karem *et al.* (2001), suggested that *Salmonella* can be obtained from perfectly normal looking eggs. Eggs can be contaminated either on the outer shell surface or internally. The internal contamination can be a result of penetration through the eggshell or by direct contamination of egg contents before oviposition, originating from infection of the reproductive organs. Once inside the egg, the bacteria need to cope with antimicrobial factors in the albumen and vitelline membrane before migration to the yolk can occur. Gast *et al.,* (2002) found that *Salmonella* infections remain an important problem for humans and animals throughout the world.

Ryan and Ray (2004), found that the genus *Salmonella* is a member of the family Enterobacteriaceae, rod-shaped, gram-negative, non spore-forming bacteria related to each other phenotypically and genotypically.

Bahrouz (2005), described that bacterial contamination can happen at three main parts of egg (egg yolk, albumen and shell membrane/egg shell).Pradhan *et al.,* (2005) suggested that contamination and penetration of salmonellae into hatching eggs may comprise an important link in the transmission of these bacteria to growing birds, processed carcasses, and eventually to the consumer.

Suresh *et al.* (2006), suggested that prevalence of *Salmonella* was recorded in 6.1% of egg shells and 1.8% of egg contents in South India. De Jong and Ekdahl, (2006) reported that the disease occurs at high frequency in industrialized nations as well as developing countries and represents an important public health problem worldwide.

Holtby *et al.* (2006), suggested that *Salmonella enteritidis* can be detected inside perfectly normal-appearing eggs, and if the eggs are eaten raw or undercooked, the bacterium can cause illness. Symptoms of *Salmonella* poisoning as abdominal pain, diarrhea, vomiting, and fever may appear within 12 hours post eating the infected eggs, or may take as long as 72 hours to show up. Because the symptoms are like flu symptoms some people may not know they are sick because of what they have eaten.

WHO (2007), reported that the examination of food to detect *Salmonella* is routinely carried out for food safety and food-borne disease surveillance. Human *Salmonella* infection and food poisoning have been associated with consumption of contaminated poultry and eggs.

Aarestrup *et al.* (2007) reported that this return to its endemic nature, high morbidity and association with a wide range of foods.

Murchie *et al.* (2008), reported that *Salmonella typhimurium* and *Salmonella enteritidis* are the most frequently isolated serovars from food-borne outbreaks throughout the world.

Stepien-Pysniak and Marek (2009), suggested that *Staphylococcus aureus* dominated on the shell and in yolk compared to egg white. The degree of contamination with these bacteria regarding to the source of eggs.

Maff (2009), reported that table eggs are the best and easy source of food, containing quality protein, essential amino acids, essential vitamins and minerals needed for a good health.

Akond *et al.*(2009), conducted an experiment on isolation and identification of *Escherichia coli* from poultry sources of different poultry markets in the capital city of Bangladesh. Out of total 250 samples, 50 from each of cloacal swab, intestinal fluid, egg surface, faecal material and hand wash of chicken handlers, 145 (58%) were found to be positive for *E. coli* prevalence. 80 selected strains were thoroughly characterized by standard cultural and biochemical tests followed by final identification using latex agglutination test with several polyvalent anti-sera.

Abdullah (2010), demonstrated the degree of contamination of table egg with bacteria of genus *Staphylococcus, E. coli, Salmonella, Streptococcus* and *Clostridia* according to source of eggs. The study indicated a relatively high degree of contamination of table egg with *Staphylococcus* bacteria and Enterobacteriaceae both in yolk and on egg shell. And also found that concentration of bacterial growth is higher in winter

and lesser in summer. *Staphylococci* are most common bacteria contaminating eggshells. Contamination is more likely linked with cracked egg, dirty shells and storage in contaminated surroundings. It can be contaminated during formation and laying process.

Stępień *et al*. (2010), suggested that contamination of eggs with *Salmonella*, which is closely correlated with the spread of these bacteria among poultry is currently, one of the most important factors constituting a risk to consumer health.

Eric (2011), suggested that egg shell contamination is the main reason for *E. coli* infection. Poor hatchery sanitation can leave a residue of *E. coli* from the previous hatch leads to yolk infections which occur during hatching process.

Peter (2011), reported that a combined share of egg production from China, India and Japan are more than 46%. However, China itself is the number one of the top 10 countries that have provided 38% of the world's eggs demand in 2011.

Scallan (2011), reported that *Salmonella enterica* infections are of a significant public health concern worldwide, with an estimated 1.028 million cases, 19,000 hospitalizations, and about 400 deaths in the United States each year.

Al-Bahry *et al.* (2012), described the three different mechanisms in bacterial penetration of the egg: Adherence to eggshell and penetration through the eggshell pores, survival in albumin, and multiplication and growth in yolk.

Parveen *et al.* (2012), conducted an experiment of 147 samples comprising egg shell (36), egg yolk (36), feed (45) and air (30) were collected during the period from January to May, 2012 and the collected samples were then examined for the bacteriological study by using cultural, morphological and biochemical techniques. On the basis of their cultural, morphological and biochemical properties the isolated organisms were identified as *Escherichia coli*, *Staphylococcus spp.*, *Salmonella serovars* and *Bacillus spp.* In this study it was observed that out of 147 samples a total of 51 were identified as bacterial pathogens in which egg shell containing 10 (27.78%), egg yolk 11 (30.56%), feed 20 (44.44%) and air 10 (33.33%) respectively.

In this study it was also observed that the highest prevalence of bacterial pathogens in feed samples (44.44%) in comparison with egg shell (27.78%), egg yolk (30.56%) and air samples (33.33%). In this study it was demonstrated that out of four (04) pathogens *Escherichia coli* was more abundant (39.21%) in the layer house and its environment in comparison with *Staphylococcus spp.* (25.49%), *Salmonella* (23.52%) and *Bacillus spp.* (11.76%) respectively.

Ahmed *et al.* (2012), suggested that *Salmonella* isolation by conventional culture methods are based on pre-enrichment, enrichment and plating on selective and differential media and suspected colonies are then confirmed by biochemical test.

Rather *et al.* (2012), conducted a total of 100 samples, 25 from each source, were taken for the present study. All samples were evaluated for the presence of *Salmonella* and *E. coli,* stereotyped and tested for antimicrobial susceptibility. A total of 60 isolates of *E. coli* and 12 isolates of *Salmonella* were obtained.

Akbar and Anal *et al.* (2013), reported that the most common foodborne pathogens associated with food of animal origin are *Salmonella*, *Campylobacter*, *Listeria monocytogenes*, *Staphylococcus aureus* and *Escherichia coli* O157.

Anbessa and Shiferaw *et al.* (2013), reported that Salmonella can be regarded as two types of infections. The first is primarily of importance for public health by causing food borne illness. The other type causes severe disease leads to great economic losses in poultry industry.

Sedeek *et al.* (2013), conducted an experiment on a total number of 147 egg samples (5 eggs each sample) from commercial and non-commercial layer hens, 34 duck egg samples and 15 quail egg samples were collected from different markets and localities at Alexandria governorate during spring and summer 2012. The collected egg samples were subjected to examination for the prevalence of *Salmonella* in both egg shells and contents. The results revealed number of (12&15), (09&17) and (0&2) presumptive *Salmonella* colonies recovered from chicken, duck and quail egg samples using XLD and SS agars, respectively. In addition, (3&5) out of (98) chicken egg shell samples and (1&1) out of (34) duck egg shell samples were identified as Atypical *Salmonella*

spp. Cultured on XLD agar and SS agar, respectively. Typical (1isolate) of *Salmonella* was isolated from chicken balady egg shell samples with antigenic structure Somatic "O" antigen (3,10) and Flagellar "H" antigen (Phase1 L, Z28 & Phase 2 E, n, x) which identified serologically as *Salmonella enterica serovar westpark* which represented the first record for isolation and identification of *Salmonella westpark* in Egypt. All examined egg shell and egg content samples were negative for *Salmonellae* other than *westpark.* Also, all examined quail egg samples and egg content of all samples were free from any *Salmonellae*. The public health importance of *Salmonella* organism as well as the suggested measures for improving the egg quality were discussed.

Akbar and Anal (2014), reported that food borne illness is a major public health problem and the main cause of diarrhoeal diseases affecting all developed and developing countries.

Ibrahim *et al.* (2014), conducted an experiment on a total of 304 random chicken egg samples obtained from layers of Balady and Battery systems. 144 of chicken Balady and Battery eggs (72 of each sample were divided into 18 batches) were used for bacteriological evaluation. Enterobactriacae (94.4, 33.3 and 27.7%), Salmonella (77, 29 and 22%), E.coli (44, 0 and 22%) and Staphylococcus aureus (100, 97 and 98%) were detected in shell, albumen and yolk in Balady eggs, respectively. Battery system showed lower incidence in Enterobactriacae (50, 16.6 and 22%), Salmonella (41.6, 8 and 0%), E.coli (27.7, 9 and 19%)and Staphylococcus aureus (100, 83 and 95%), respectively. Results concluded that the system in which the hens are housed contribute in rate of contamination of eggs. Staphylococcus aureus showed a higher prevalence rate compared to other pathogens in both laying systems. About 160 Baladyeggs were selected for studying the effect of different pathogen inhibitors, 80 eggs for detecting preservation after 21 days at room temperature and the other 80 fertile Balady eggs for detecting hatchability and mortality. The efficacy of application of different pathogen inhibitors as Propionic acid at different concentration 10, 30, 50, 70 and 100%, Hydrogen Peroxides (H_2O_2) 3% and Virkon S 1% on eggs were recorded. Propionic acid10%, Virkon S and H_2O_2 showed nearly similar significant inhibitory effect on pathogens on egg shells ranged from 86 to 100%, albumin from 33.4 to 100% and yolk from 34.3 to100%, while 30% Propionic

acid has highly significant inhibitory effect on pathogen load ranged from 99.8 to100%. About 30% propionic acid concentration had a preservative effect on table eggs for 21 days at room temperature and increasing hatching percent up to 90% and lowering embryonic mortality to10% in fertile eggs. The findings of this study indicate that 30% Propionic acid may be considered as a favorable disinfectant agent for the egg shell spraying.

Salihu *et al.* (2015), revealed that this study was carried out to evaluate the microbial contents of chicken eggs, sold at retail outlets in Sokoto metropolis. A total of 160 eggs were collected from 16 randomly selected retail outlets, in Sokoto metropolis, for microbial evaluation. Samples were cultured and isolated using nutrient and McConkey agar for bacteria while Sabauroud dextrose agar was employed for fungus and identified using Harvey and Green Wood method. All the 160 (100%) samples were positive for bacteria (nine different genera); while 104 (65%) egg shells were positive for fungi isolation from the genus *Aspergillus*; however, evaluation of the egg contents revealed 95(59.4%) positive for bacteria isolations from seven different genera and 86(53.8%) positive for fungi isolations from only one genus *Aspergillus*. The bacterial genera include *Escherichia coli*, *Salmonella* spp, *Shigella* spp, *Corynebacteria*, *Proteus* spp *Bacillus* spp *Staphylococcus* spp *Streptococcus* spp and *Klebsiella*. The only fungal genus was *Aspergillus*, which were identified to be *Aspergillus flavus*, *Aspergillus niger* and *Aspergillus fumigatus*. The eggs from these areas should therefore be taken with caution and the public should be educated on the dangers associated with consumption of raw and under cooked eggs and egg products, retailers should be encourage to store their eggs in refrigerators and practice good hygiene in order to prevent microbial growth on the eggs.

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

Fardows *et al.* (2015), conducted an experiment out of 150 egg shells, 120 (80%) yielded growth of different bacteria. Of them, *Staphylococcus* spp. were 80 (66.67%), *Streptococcus* spp. 8 (6.67%), *Bacillus subtilis* 20 (16.67%) and *Bacillus cereus* 12 (10%). Out of 80 *Staphylococcus* spp., 30 (25%) were *Staphylococcus aureus* and 50 (41.67%) were *Staphylococcus saprophyticus*. Most of the Gram-positive bacteria were sensitive to ciprofloxacin, ceftriaxone and imipenem. No MRSA and VRSA were found.

Ashish *et al.* (2016), said that this study aims at the isolation and identification of pathogenic enterobacteria responsible for egg contamination in poultry farms by using different biochemical tests and molecular characterization i.e. 16s rRNA gene analysis. A total of 90 eggs were collected from three different Poultry farms in Jaipur city, India. Micro-organisms were isolated by using differential medium i.e. MacConkey agar, EMB agar and characterized by using different biochemical tests like catalase test, indole test, methyl-red and voges-proskauer test (MR-VP), citrate utilization test, H2S production, Urease test, Gas production, Glucose and Lactose fermentation etc. On the basis of biochemical analysis selected isolates were subjected to 16s rRNA gene analysis, as 16s rRNA gene analysis is a powerful technique for bacterial taxonomy and identification. Both the biochemical and molecular analysis revealed that most of the isolates belong to family *Enterobacteriaceae* and were identified as *Escherichia coli* O157:H7str. EC4115, *Staphylococcus epidermidis* ATCC 14990 and *Pluralibacter gergoviae*. The study concludes that the eggs and egg products are contaminated with pathogenic microbes and may cause diseases if consumed raw or uncooked. Thus, there is a serious need to pay attention in increasing the hygienic level of commercial eggs so as to prevent the occurrence of prevalence of microbial contamination in eggs.

2.2 Antibiotic sensitivity pattern of pathogenic bacteria

Bager *et al.* (2001), reported that antimicrobial resistance is an increasingly global problem, and emerging antimicrobial resistance has become a public health issue worldwide. A variety of foods and environmental sources harbor bacteria that are resistant to one or more antimicrobial drugs used in human or veterinary medicine and in food-animal production. While comparing the antibiotic sensitivity pattern of different isolates of *E. coli* strains, it was found that the isolates were sensitive to neomycin, gentamicin, chloramphenicol, ofloxacin, ampicillin, nalidixic acid and nitrofurantoin, and resistant to tetracycline, cephalosporin, sulfisoxazole and streptomycin.

Su *et al.* (2004), reported that various *Salmonella* serovars are resistant to conventional antibiotics such as ampicillin, chloramphenicol, trimethoprim-sulfamethoxazole, and other newer antibiotics (quinolones and extended-spectrum cephalosporins) have been reported with increasing frequency in many areas of the world.

Kasper *et al.* (2005), reported that during the last decade, antibiotic resistance and multiresistance of *Salmonella* spp. have increased a great deal, especially in developing countries with an increased and indiscriminate use of antibiotics in the treatment of humans and animals. (Adesiyun *et al.*, 2005) reported higher prevalence of *E. coli* on eggshells from farms compared to eggs from markets.

Messens *et al.* (2006), reported that the eggshell contamination increasing the chances of egg contents contamination by penetration. The antibiogram study of the pathogens from eggshells showed that all the isolates were sensitive to gentamicin, enrofloxacin, tetracycline and ampicillin whereas, all the isolates showed intermediate resistance to kanamycin, isolated *Salmonella* and *E. coli* from eggs and analyzed for their antibiogram against 16 antibiotics including ampicillin, tetracycline, gentamicin, and kanamycin. (Musgrove *et al.*, 2006)

Akond *et al.* (2009), conducted an experiment on isolation and identification of *Escherichia coli* from poultry sources of different poultry markets in the capital city of Bangladesh. 50 identified strains were subjected to 13 antimicrobial agents to check their susceptibility. 88%, 82%, 80%, 76%, 70%, 68%, 64%, 58%, 52%, and 20% of the tested *Escherichia coli* strains from poultry sources were found resistant respectively to Penicillin, Ciprofloxacin, Riphampicin, Kanamycin, Streptomycin, Cefixine, Erythromycin, Ampicillin, Tetracycline, and Chloramphenicol and Neomycin. None of the strains showed resistance to Norfloxacin and Gentamicin. Sensitivity was recorded in case of 86%, 80%, 60%, 36%, 30%, and 26% of the strains to Norfloxacin, Gentamicin and Chloramphenicol, Neomycin, Tetracycline, Streptomycin and Ampicillin, respectively. Intermediate resistance/ susceptibility to various antibiotics were observed for 12-36% *Escherichia coli* strains. Both, resistance and susceptibility were exhibited against Chloramphenicol, Ampicillin, Gentamicin, Neomycin, Tetracycline, Streptomycin and Norfloxacin. Multi drug resistance was recorded in case of 6-10 antibiotics for all strains tested. More cautions are recommended for personnel hygiene in processing and handling of poultry and poultry products. Excess use or abuse of antibiotics should be reduced or stopped by judicious application of antibiotics for the safety of public health.

Kalmus *et al.* (2011), identified antimicrobial resistance in *Staphylococcus aureus*, including methicillin resistant *S. aureus (*MRSA), recovered from raw retail meat products purchased in the Washington, D.C., area. From March to August 2008, 694 samples of ground beef (n = 198), ground pork (n = 300), and ground turkey (n = 196) were collected by random sampling from stores of three grocery chains. In total, 200 *S. aureus* isolates (29%) were recovered by direct plating. When tested for susceptibility to 22 antimicrobials, 69% of the *S. aureus* isolates were resistant to tetracycline, 26% to penicillin, 17% to ampicillin, 13% to methicillin, 8% to erythromycin, 4.5% to clindamycin, 1.5% to gentamicin, and 0.5% to chloramphenicol, oxacillin, cefoxitin, or quinupristin dalfopristin. However, 27% of the isolates were susceptible to all tested antimicrobials.

Deresse *et al.* (2012), examined that all of 78 samples were contaminated with *S. aureus*. All strains were resistant to Penicillin G (PG) (10µg), Ampicillin (AP) (10µg), Amoxicillin-Clavulanic acid (AC) (30µg), Ciprofloxacin (CIP) (5µg), Erythromycin

(E) (15µg), Ceftriaxone (CRO) (30µg), Trimethoprime- Sulfamethoxazole (TMP-SMZ) (25µg) Oxacillin (Ox) (1µg) and Vancomycin (V) (30µg), 67.9%, 70.9%, 30.9%, 0%, 32.1%, 23.1%, 7.7%, 60.3% and 38.5% respectively.

Rather *et al.* (2012), conducted a total of 100 samples, 25 from each source, were taken for the present study. All samples were evaluated for the presence of *Salmonella* and *E. coli,* stereotyped and tested for antimicrobial susceptibility. A total of 60 isolates of *E. coli* and 12 isolates of *Salmonella* were obtained. The antimicrobial susceptibilities of different isolates of E. coli and Salmonella from different water sources were examined by disc diffusion method. The high in vitro sensitivity was shown by 100 % of *E. coli* isolates for ciprofloxacin, 95 % for amoxycillin/clavulanic acid, 93.33 % for each of cephotaxime and amikacin and 91.67 % for lavofloxacin and gentamicin. The isolates registered an intermediate response to rifampicin (85.00 %), lomefloxacin (83.33 %) and norfloxacin (83.33 %) and showed resistance to erythromycin (98.33 %). The isolates of *Salmonella* were 100 % sensitive for gentamicin, 91.67%for each of cefixime, ciprofloxacin, amikacin and lavofloxacin; 83.33 % for each of cephotaxime and amoxycillin/ clavulanic acid and showed resistance to erythromycin (100 %), nalidixic acid (100 %) and rifampicin (91.67%). The isolates, however, showed an intermediate resistance to lomefloxacin (83.34 %) and norfloxacin (66.67). Raza *et al.* (2012) showed that total 78 (2.0%) Salmonella serotype isolated from 3,980 blood culture samples, in which 47 (60.3%) were S. typhi and 31 (39.7%) were S. paratyphi A. Isolates were from all age group median age being the 25 years. Among the tested antibiotics S. typhi was susceptible towards Ciprofloxacin (100%) followed by Gentamicin (97.9%), Ofloxacine (95.7%), Ceftriaxone (95.7%) and Chloramphenicol (93.6%). In case of S. paratyphi A most of the tested antibiotics showed high percentage of susceptibility and least susceptible antibiotic for S. paratyphi A was Ampicillin (25.8%). Three isolates of S. typhi showed multidrug resistance.

Geidam *et al.* (2012), reported the prevalence of multi-drug resistant bacteria in apparently healthy chickens from 3 selected poultry farms in Selangor area of Malaysia. Antimicrobial sensitivity test was monitored with the disc diffusion assay against 12 antimicrobial agents. A total of 96 *Staphylococcus aureus,* 48 *E. coli,* 7 *Pasteurella sp.* and 6 *Salmonella sp.* were isolated. All *E. coli* and *Salmonella* spp.

isolates were multi-drug resistant while 77.2% of *Staphylococcus aureus* and 71.5% of *Pasteurella* spp. isolates were multi-drug resistant. The study further revealed highest resistance to tetracycline while cephalothin as the best drug of choice for treatment of infections caused by the isolates in the study area. Since not only chickens are at risk, this study recommends urgent intervention by regulatory agencies to limit the emergence and spread of these bacteria as well as prudent use of antibacterial agents among farmers in Malaysia.

Raza *et al.* (2012), showed that total 78 (2.0%) Salmonella serotype isolated from 3,980 blood culture samples, in which 47 (60.3%) were S. typhi and 31 (39.7%) were S. paratyphi A. Isolates were from all age group median age being the 25 years. Among the tested antibiotics S. typhi was susceptible towards Ciprofloxacin (100%) followed by Gentamicin (97.9%), Ofloxacine (95.7%), Ceftriaxone (95.7%) and Chloramphenicol (93.6%). In case of S. paratyphi A most of the tested antibiotics showed high percentage of susceptibility and least susceptible antibiotic for S. paratyphi A was Ampicillin (25.8%). Three isolates of S. typhi showed multidrug resistance.

Fardows *et al.* (2012), conducted an experiment of 150 eggs collected from poultry were tested. Of 150 egg shells, 130 (86.67%) yielded growth of bacteria and 60 (40%) *E.coli*, 25 (16.67%) *Providencia rettgeri*, 5 (3.33%) *Providencia alkalifaciens*, 20 (13.33%) *Citrobacter freundii*, 10 (6.67%) *Salmonella spp*, 10 (6.67%) *Enterobacter aerogenes* were isolated. No bacteria were isolated from 150 egg contents. Total 14 (9.33%) *Salmonella spp.* from egg shells and 7 (4.67%) *Salmonella spp.* from egg contents were identified by PCR. Most of the identified serotypes were *Salmonella enteritidis* (42.86% from egg shells and 71.43% from egg contents). All (100%) *Salmonella typhi* and *Salmonella paratyphi* A were sensitive to ciprofloxacin and ceftriaxone.

Sabir *et al.* (2014), revealed that 402 samples with highest prevalence of *E. coli* (321, 80%) followed by *Staphylococcus aureus* (9.4%), *Proteus species (*5.4%) and *Pseudomonas species*(5.2%). The *E. coli* were highly resistant to penicillin (100%), amoxicillin (100%) and cefotaxime (89.7%), followed by intermediate level of resistance to ceftazidime (73.8%), cephradine (73.8%), tetracycline (69.4%),

doxycycline (66.6%), augmentin (62.6%), gentamycin (59.8%), cefuroxime (58.2%), ciprofloxacin (54.2%), cefaclor (50%), aztreonam (44.8%), ceftriaxone (43.3%), imipenem (43.3%), and low level of resistance to streptomycin (30%), kanamycin (19.9%), tazocin (14%), amikacin (12.7%) and lowest to norfloxacin (11.2%). Out of 321 *E. coli* isolates, 261 (81%) were declared as multiple drug resistant and 5 (1.5%) were extensive drug resistant.

Ejo *et al.* (2016), showed that the overall prevalence rate of 5.5% was recorded from the total analyzed food items of animal origin. *Salmonella* isolates were detected from 12% of raw meat, 8% of minced meat, 2.9% of burger samples, 18% of raw eggs, and 6% of raw milk. Furthermore, antimicrobial susceptibility test identified 47.6% resistant *Salmonella* isolates, 28.6% intermediately sensitive isolates, and 23.8% susceptible isolates. Among *Salmonella* isolates tested, 42.6%, 28.6%, and 14.3% were found to be relatively resistant to tetracycline, sulfamethoxazole-trimethoprim, and ampicillin, respectively, while 9.5%–19% were intermediately resistant to tetracycline, amoxicillin, ampicillin, cephalothin, and nitrofurantoin. Therefore, our findings provide the prevalence and drug resistance of *Salmonella* from foods of animal origin and contribute information to scientists as well as public health researchers to minimize the prevalent and resistant foodborne *Salmonella* species in Ethiopia.

Ashish *et al.* (2016), conducted an experiment on a total of 132 egg samples were collected from different areas of Jaipur, India. Gram negative enterobacteria were isolated initially on Nutrient agar and then on selective-differential media and further characterized by different biochemical tests. Commonly prescribed antibiotics in patients for gastrointestinal infection were used for antibiotic susceptibility test. Against different antibiotics different resistance pattern were found ($p<0.01$). The highest resistance rate were detected against Cefixime (86.66%) whereas highest sensitivity rate (100%) were recorded against Gentamicin, Levofloxacin and Ciprofloxacin. Also, most of the isolates (73.3%) were found to be multi drug resistant as these showed resistance against three or more antibiotics tested. Multiple antibiotic resistance index of isolated microbes from table egg ranged from 0.10 to 0.70. It can be concluded that commercial eggs which are consumed as food may

harbour multi-drug resistant bacterial pathogens and if consumed raw may cause severe ailments in consumers. Antibiotic resistance of bacterial species will make the clinical treatment of diseases more difficult. Thus aim of the present study is to assess the risk of antibiotic resistance in table eggs and egg products.

Kulkarni *et al.* (2017), conducted an experiment of total 1000 samples, 395 cases were culture-positive for *E. coli.* These isolates were tested for antibiotic susceptibility by disk diffusion method. Of the total 395 *E. coli* isolates, 170 (43%) were multi drug resistant (MDR). The isolates showed high level of resistance to Ampicillin (82.53%), Cefuroxime (72.41%), Amoxycillin-clavulinic acid (71.90%), Ceftriaxone (66.58%), Ciprofloxacin (65.82%) and Cefepime (57.47%). The isolates were sensitive to Imipenem (96.71%), Nitrfurantion (92.41%), Amikacin (90.89%), Chloramphenicol (85.82%), Piperacillin-tazobactum (80.76%), Gentamicin (59.24%), Azetreonam (54.43%) and Norfloxacin (53.67%).

Gupta *et al.* (2017**),** revealed that fecal materials originated from recto anal junction (RAJ) of 100 cattle used for primary screening on MacConkey agar. The diversities among the pink color colony producing isolates on MacConkey agar were verified by conventional cultural methods and biochemical tests. Phenotypically positive *E. coli*isolates were further investigated for the variations in the antimicrobial resistance profiles to 10 selected antibiotics, by the disk-diffusion method. This study revealed that the overall prevalence of *E. coli* was 70% of in the rectal swab sample of cattle. However, the prevalence of *E. coli* was found significantly higher (p= 0.002) in cattle under intensive farming (84%) than cattle on Bathan (56%). Antibiotic susceptibility pattern shows that among the tested isolates 83%, 73%, 68% and 64% were sensitive to chloramphenicol, gentamicin, ciprofloxacin and ampicillin, respectively. On the other hand, all the 70 (100%) *E. coli* isolates were found resistant to tetracycline and sulfamethoxazole. A high antibiotic resistance profile was also found against amoxicillin (90%), ampicillin (87%), nalidixic acid (86%) and erythromycin (83%). In total, 24 (34%) isolates were resistant against 2 antimicrobials. The result clearly shows that antibiotic resistant *E. coli* isolates are commonly present in cattle of different management systems (intensive and Bathan). Therefore, careful selection of appropriate antibiotics with optimal doses might be ensured to prevent the emergence of antibiotic resistance bacteria.

Ema *et al.* (2018), revealed that the present study was 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. A total of 40 samples were collected randomly from different grocery shops of Bangladesh Agricultural University (BAU) Campus and Kaowatkhali, Mymensingh. Following necessary preparation, the samples were streaked onto various selective media like Salmonella-Shigella (SS) agar (for *Salmonella* spp.), Eosin Methylene Blue (EMB) (for *E. coli*), and Mannitol Salt (MS) agar (for *Staphylococcus* spp.) respectively for isolation of bacteria. The bacteria were confirmed based on cultural and biochemical characteristics. Antibiotic sensitivity test of the bacterial isolates was performed using seven antibiotics (Ampicillin, Chloramphenicol, Ciprofloxacin, Gentamicin, Vancomycin, Kanamycin and Cephalexin) by following disc diffusion method. *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%, whereas 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.

CHAPTER 3
MATERIALS AND METHODS

3.1 Study period and working place

The research work was conducted at the Department of Microbiology & Parasitology, Faculty of Animal Science and Veterinary Medicine, Sher-e-Bangla Agricultural University, Dhaka-1207, during the period of January 2018 to July 2018.

3.2 Materials

3.2.1 Area of the study and collection of sample

A cross-sectional study design was used to investigate the bacteriological analysis of table egg in Agargaon Market, Bihari kamp Market, Krishi market and SAU Market from January to May 2018. A total of 40 table egg samples were collected.

3.2.2 Transportation of the samples

Samples of approximately 10 table egg were collected from different local market by using ice box. Collected samples were immediately transported on ice to the Microbiology Laboratory of the Sher-e-Bangla Agricultural University for analysis.

Table 1. No. of egg samples collected from selected areas of Dhaka city

Serial No.	Place	No. of Collected Samples
1.	Agargaon Market	10
2.	Bihari Kamp Market	10
3.	Krishi Market	10
4.	SAU Market	10
Total		40

3.3 Brief description of the experimental design

Egg samples were collected from selected areas of Dhaka city. The samples swab were first inoculated onto Nutrient Broth. The isolates were characterized by cultural characteristics on selective media eg. MacConkey (MAC) Agar Media and Eosin Methylene Blue (EMB) Agar Media for *E. coli*, Salmonella- Shigella (SS) Agar for *Salmonella* spp., Manitol Salt Agar (MSA) Media for *Staphylococcus* spp. Then Gram's staining, biochemical test was performed. Finally the isolated organisms were subjected to antibiotic sensitivity test to observe the resistant characteristics of organism on some specific antibiotic disk by spreading method.

Experimental Design

The following is a flow chart of representing design of the experiment

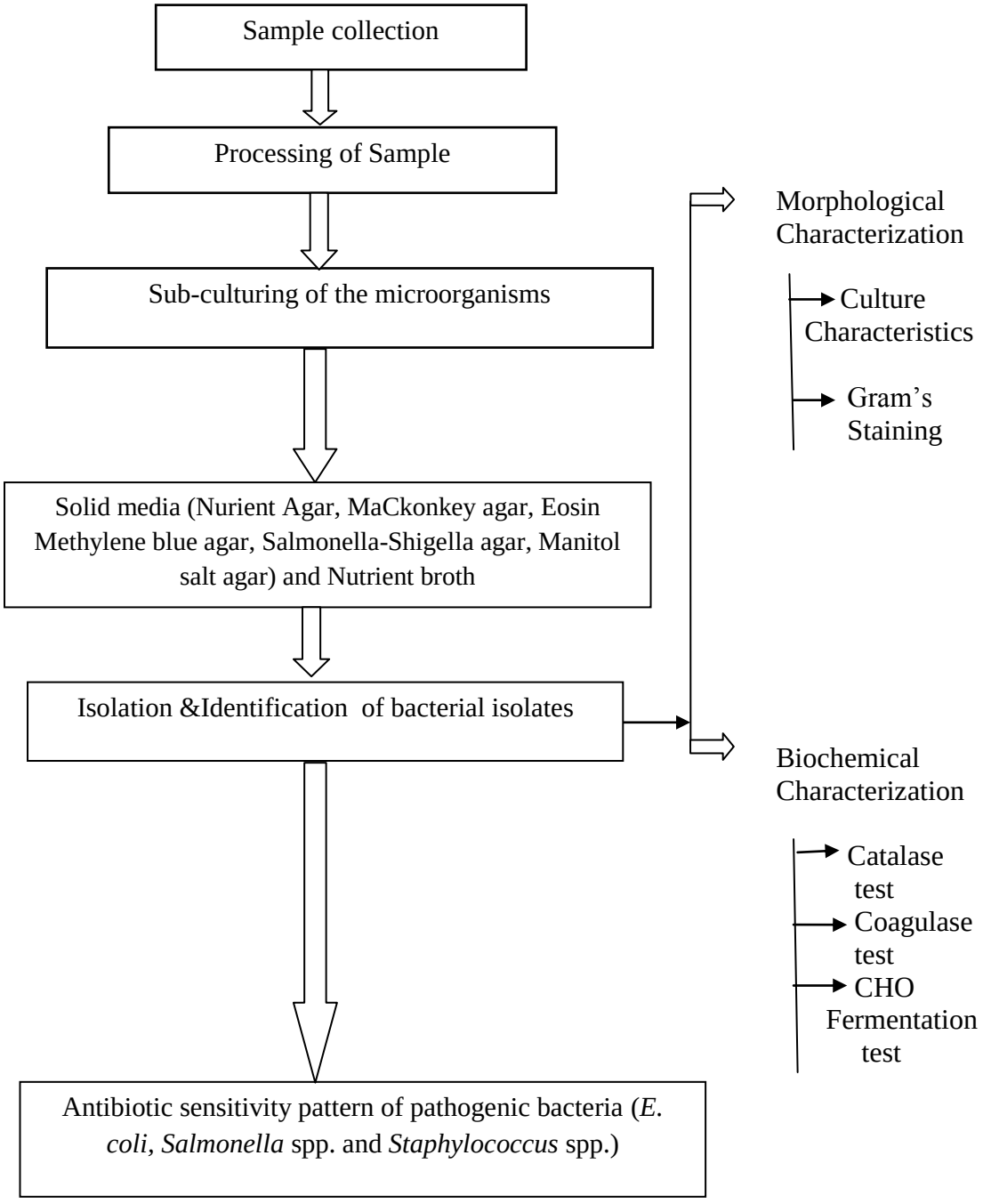

Figure 1: Schematic illustration of the experimental design

3.4 Media for Bacteriological study

3.4.1 MacConkey agar (MAC)

MAC was used as a selective medium for the identification of *E. coli* organisms. MAC agar media was prepared according to the instruction of (Hi media, India) manufacturer company.

3.4.2 Eosin Methylene Blue (EMB) Agar

EMB agar was used as a selective medium for the identification of *E. coli* organisms. EMB agar media was prepared according to the instruction of (Hi media, India) manufacturer company.

3.4.3 Salmonella Shigella Agar (SSA)

SS agar was used as a selective medium for the identification of *Salmonella* spp. organisms. SS agar media was prepared according to the instruction of (Hi media, India) manufacturer company.

3.4.4 Mannitol Salt Agar (MSA)

MSA agar was used as a selective medium for the identification of *Staphylococcus* spp. organisms. MSA agar media was prepared according to the instruction of (Hi media, India) manufacturer company.

3.4.5 Blood agar (BA)

Blood agar (Hi media, India) enriched with 5% sheep blood was used to observe the hemolytic property of *Staphylococcus* spp.

3.4.6 Muller Hinton Agar (MHA)

Muller Hinton Agar plates were used for the antibiotic sensitivity test which was prepared according to the instruction of Manufacturer Company (Hi media, India).

3.4.7 Nutrient Broth and Nutrient Agar

Nutrient broth and Nutrient Agar were used for the primary growth of *E.coli, Salmonella* spp. *and Staphylococcus* spp. which were prepared according to the instruction of Manufacturer (Merck Specialities Private Limited) company.

3.4.8 Glass ware and other necessary instruments

During the study period, sterile test tubes, petri-culture dishes, conical flasks, pipette, slides, hand gloves, glass spreader, bacteriological loop, electric balance, spirit lamp, bacteriological incubator, electronic light microscope, sterilized cotton, immersion oil were used.

3.4.9 Chemicals and reagents

Phosphate buffered saline (PBS) solution, reagents for Gram's staining (Crystal violet, Gram's iodine, Acetone alcohol and Safranin), 3% Hydrogen peroxide, Normal saline solution, Sugar solutions, Rabbit plasma, 50% buffered glycerin, Alcohol and other common laboratory chemicals and reagents and distilled water were used for this research.

3.4.10 Antibiotic disc

Commercially available antibiotic disc (Oxoid, England) was used for the test to determine the drug sensitivity pattern. The test was performed using disc diffusion method. This method allowed for the rapid determination of the efficacy of the drugs by measuring the diameter of the zone of inhibition that resulted from different diffusion of the agent into the medium surrounding the disc. The following antibiotic discs were used:

Table 2. Lists of antibiotics and their dosage

Name of the antibiotics	Dose (µg/disc)
Penicillin (P)	10
Erythromycin (E)	15
Tetracyclin (TE)	30
Amoxicillin (AML)	10
Gentamycin(GM)	10
Ciprofloxacin (CIP)	5
Neomycin (N)	30
Vancomycin (V)	30

3.5 Methods

3.5.1 Isolation of *E.coli* from egg sample

All the samples were cultured primarily in nutrient broth at 37ºC for 18-24 h, then subcultured onto the Nutrient agar, MacConkey and EMB agar by streak plate method (Cheesbrough, 1985) to observe the colony morphology (shape, size, surface texture, edge and elevation, colour, opacity etc). The organisms showing characteristic colony morphology of *E. coli* was repeatedly subcultured onto EMB agar until the pure culture with homogenous colonies were obtained.

3.5.2 Gram's staining method

According to methodology all suspected cultures of *E.coli* species were subjected to Gram's staining and observed under a electronic light microscope. Gram's staining was performed as per procedures described by Merchant and Packer (1969) to determine the size, shape and arrangement of bacteria. Briefly, a small colony was picked up from MacConkey agar with a bacteriological loop, smeared on separate glass slide with a drop of distilled water and fixed by gentle heating. Crystal violate (Hi-media, India) was then applied on each smear to stain for two minutes followed by washing with running water. Few drops of Gram's Iodine (Hi-media, India) was then added, which acted as mordant for one minute and then washed with running water. Acetone alcohol (Hi-media, India) was then added (acts as decolorizer) for few seconds. After washing with water, safranin was added as counter stain and allowed to stain for two minutes. The slides were then washed with water, dried in air and then examined under light microscope with high power objective (100X) using immersion oil. The organisms revealed gram negative, pink colored with small rod shaped appearance and arranged in single or in pair were suspected as *E. coli.*

3.5.3 Biochemical test for *E.coli*

For Carbohydrate fermentation test, the durhams tube were inversely placed in the test tube. Then 5ml sugar solutions were taken in the test tube after tyndalization transfer in incubator for sterility test. The isolates were placed in the solutions. Incubation at 37ºC for 24-48 hours. If the organisms are positive, produced acid (development of yellow colour) and gas in the test tube.

3.5.4 Isolation of *Salmonella* spp. from egg sample

All the samples were cultured primarily in nutrient broth at 37ºC for 18-24 h, then subcultured onto the Nutrient agar, Salmonella-Shigella agar by streak plate method (Cheesbrough, 1985) to observe the colony morphology (shape, size, surface texture, edge and elevation, colour, opacity etc). The organisms showing characteristic colony morphology of *Salmonella* spp. was repeatedly subcultured onto SS agar until the pure culture with homogenous colonies were obtained.

3.5.5 Gram's staining method

According to methodology all suspected cultures of *Salmonella* spp. species were subjected to Gram's staining and observed under a electronic light microscope. Gram's staining was performed as per procedures described by Merchant and Packer (1969) to determine the size, shape and arrangement of bacteria. Briefly, a small colony was picked up from Salmonella-Shigella agar with a bacteriological loop, smeared on separate glass slide with a drop of distilled water and fixed by gentle heating. Crystal violate (Hi-media, India) was then applied on each smear to stain for two minutes followed by washing with running water. Few drops of Gram's Iodine (Hi-media, India) was then added, which acted as mordant for one minute and then washed with running water. Acetone alcohol (Hi-media, India) was then added (acts as decolorizer) for few seconds. After washing with water, safranin was added as counter stain and allowed to stain for two minutes. The slides were then washed with water, dried in air and then examined under light microscope with high power objective (100X) using immersion oil. The organisms revealed gram negative, pink colored with small rod shaped appearance and arranged in single or in pair were suspected as *Salmonella* spp.

3.5.6 Biochemical test for *Salmonella* spp.

For Carbohydrate fermentation test, the durhams tube were inversely placed in the test tube. Then 5ml sugar solutions were taken in the test tube after tyndalization transfer in incubator for sterility test. The isolates were placed in the solutions. Incubation at 37ºC for 24-48 hours. If the organisms are positive, produced acid (development of yellow colour) and gas in the test tube.

3.5.7 Isolation of *Staphylococcus* spp. from egg sample

Swabs from egg samples were in inoculated in nutrient broth and incubated at 37^0 for 24 hours. Then spread in nutrient agar by streak plate method. Then spread plated 5% sheep blood agar (Supplied by Hi media company, India). The plates were incubated aerobically at 37°C for over night. Consequently, the characteristic *Staphylococcus* spp. colonies that produced β hemolysis further purified by sub-culturing onto Blood agar plates and the plates were incubated aerobically at 37°C for over night. Other than *Staphylococcus* spp. could not produced β hemolysis were discarded. These isolates were retained for further bacterial identification and inoculate on to MSA plate for further characterization.

3.5.8 Gram's staining method

According to methodology all suspected cultures of *Staphylococcus* species were subjected to Gram's staining and observed under a electronic light microscope for Gram's reaction, size, shape and cell arrangements. The Staphylococci colonies were characterized morphologically using Gram's stain according to the method described by Deresse *et al.* (2012). Briefly, a small colony was picked up from mannitol salt agar with a bacteriological loop, smeared on separate glass slide with a drop of distilled water and fixed by gentle heating. Crystal violate (Hi-media, India) was then applied on each smear to stain for two minutes followed by washing with running water. Few drops of Gram's Iodine (Hi-media, India) was then added, which acted as mordant for one minute and then washed with running water. Acetone alcohol (Hi-media, India) was then added (acts as decolorizer) for few seconds. After washing with water, safranin was added as counter stain and allowed to stain for two minutes. The slides were then washed with water, dried in air and then examined under light microscope with high power objective (100X) using immersion oil. The gram-stained smears from typical colonies that showed gram-positive cocci occurring in bunched, grapelike irregular clusters and stained as violet color were taken as presumptive *Staphylococcus* species.

3.5.9 Biochemical studies for the identification of *Staphylococcus* spp. isolates

Catalase test:

Pure culture of the isolates were picked up using a sterile loop from the agar plate and mixed with a drop of 3% H_2O_2 on a clean glass slide. If the organism was positive, bubbles of oxygen were liberated and formed bubble within a few seconds and the catalase negative isolates did not produce bubbles. The catalase positive cocci were considered as staphylococci.

Coagulase test:

For the coagulase test, 0.5 ml of rabbit plasma was diluted with sterile physiological saline (1:5) separately in two different test tubes containing an equal volume of 24 hours old Staphylococcal cultured broth and incubated at 37°C for 4 hours. The tubes were examined after 2-4 hours for detecting the presence of clots of plasma. The negative tubes were left at room temperature for over night and then re-examined. A simple slide coagulase test was also performed by mixing an equal volume of freshly cultured broth with rabbit plasma on a glass slide. A positive result was indicated by macroscopically clumping of the bacterial cells within five seconds because fibrinolysin enzyme lysis the rabbit plasma. Pathogenic Staphylococci showed coagulase-positive reaction whereas nonpathogenic Staphylococci showed negative result. In coagulase test, *Staphylococcus* spp. convert fibrinogen to fibrin and formation of clotting occur.

3.6 Antibiotic Sensitivity test

Antibiotic sensitivity tests were performed against all isolates (*E.coli*, *Salmonella* spp. and *Staphylococcus* spp.) to determine their antibiotic-resistance profiles. Fresh overnight cultures were prepared and used for antibiotic sensitivity tests. An aliquot (100µL) from each 25 isolate suspension was spread on mueller hinton agar by sterile cotton swab. Susceptibilities of the isolates to a panel of eight different antibiotic discs (penicillin, erythromycin, amoxicillin, tetracycline, gentamicin, ciprofloxacin Neomycin, Vancomycin) were determined. Antibiotic discs were gently pressed onto the inoculated mueller hinton agar to ensure intimate contact with the surface and the plates were incubated aerobically at 37°C for over night. Inhibition of zone diameters were measured and values obtained from the National Committee on Clinical Laboratory Standards were used to interpret the results obtained. *S. aureus* isolates were then classified as resistant, intermediate resistant or susceptible to a particular

antibiotic based on the standard interpretation table (Table 2) updated according to the Clinical and Laboratory Standards Institute (CLSI) guidelines (CLSI, 2007). Multiple drug resistant (MAR) phenotypes were recorded for isolates showing resistance to three and more antibiotics.

Table 3. Zone diameter interpretive standards for *E.coli* (according to the CLSI, 2007).

Name of Antibiotics	Resistant (mm in diameter)	Intermediate (mm in diameter)	Sensitive (mm in diameter)
Penicillin	13	14-16	17
Erythromycin	≤ 13	14-22	≥23
Tetracyclin	≤11	12-14	≥15
Amoxicillin	≤13	14-17	≥18
Gentamycin	≤12	13-14	≥15
Ciprofloxacin	≤ 15	16-20	≥ 21

Table 4. Zone diameter interpretive standards for *Salmonella* spp. (according to the CLSI, 2007).

Name of Antibiotics	Resistant (mm in diameter)	Intermediate (mm in diameter)	Sensitive (mm in diameter)
Penicillin	13	14-16	17
Erythromycin	≤ 13	14-22	≥23
Tetracyclin	≤11	12-14	≥15
Amoxicillin	≤13	14-17	≥18
Gentamycin	≤12	13-14	≥15
Ciprofloxacin	≤ 15	16-20	≥ 21

Table 5. Zone diameter interpretive standards for *Staphylococcus* spp. (according to the CLSI, 2007).

Name of Antibiotics	Resistant (mm in diameter)	Intermediate (mm in diameter)	Sensitive (mm in diameter)
Erythromycin	≤ 13	14-22	≥23
Tetracyclin	≤18	19-22	≥23
Gentamycin	≤12	13-14	≥15
Ciprofloxacin	≤ 15	16-20	≥ 21
Neomycin	≤12	13-14	≥17
Vancomycin	-	-	≥15

3.7.1 Inoculation of test plates

i. Optimally, within 15 minutes after adjusting the turbidity of the inoculums suspension, a sterile cotton swab was dipped into the adjusted suspension. The swab was rotated several times and pressed firmly on the inside wall of the tube above the fluid level. This removed excess inoculums from the swab.

ii. The dried surface of a Muller-Hinton agar plate was inoculated by streaking the swab over the entire sterile agar surface. This procedure was repeated by streaking two more times, As a final step, the rim of the agar is swabbed.

3.7.2 Application of discs to inoculated agar plates

i. The predetermined battery of antimicrobial discs was dispensed onto the surface of the inoculated agar plate using sterile forceps. Each disc was pressed down to ensure complete contact with the agar surface. Whether the discs ware placed individually or with a dispensing apparatus, they were distributed evenly so that they are no closer than 24 mm from center to center. Ordinarily, no more than 12 discs should be placed on one 150 mm plate or more than 5 discs on a 100 mm plate. Because some of the drug diffuses almost instantaneously, a disc should not be relocated once it has come into

contact with the agar surface. Instead, place a new disc in another location on the agar. In this case 7 discs were placed on media containing isolated organisms.

ii. The plates were inverted and placed in an incubator set to 35°C within 15 minutes after the discs were applied kept for over night incubation.

3.7.3 Reading plates and interpretation of results

i. After overnight incubation, each plate was examined. If the plate was satisfactorily streaked, and the inoculums were correct, the resulting zones of inhibition will be uniformly circular and there will be a confluent lawn of growth. If individual colonies are apparent, the inoculums were too light and the test must be repeated. The diameters of the zones of complete inhibition (as judged by the unaided eye) are measured, including the diameter of the disc. Zones were measured to the nearest whole millimeter, using a ruler, which is held on the back of the inverted petridish.

ii. The zone margin should be taken as the area showing no obvious, visible growth that can be detected with the unaided eye. Faint growth of tiny colonies, which can be detected only with a magnifying lens at the edge of the zone of inhibited growth, is ignored. However, discrete colonies growing within a clear zone of inhibition should be sub cultured, re identified, and retested.

iii. The sizes of the zones of inhibition were interpreted by referring to Table 4 (Zone Diameter Interpretative Standards) of the CLSI (2007). Performance Standards for Antimicrobial Susceptibility Testing: Seventeenth Informational Supplement and the organisms are reported as susceptible, intermediate or resistant to the agents that have been tested.

3.8 Maintenance of stock culture

i. Stock culture requires for the further analysis. For the preparation of stock culture, the organisms (*E. Coli, Salmonella* spp. and *Staphlococcus* spp.) that were isolated and identified were inoculated onto specific Eosin Methylene blue agar, Salmonella-Shigella agar, Mannitol salt agar respectively and incubated at 37° C temperatures for overnight and examined for the growth.

ii. Then some bacterial colony was taken by the sterile inoculating loop and was mixed with 50% buffered glycerol in an eppendorf tube.

iii. This mixture was subjected to proper mixing by the vortex machine and finally stored at -20° C.

iv. By this method bacteria can be preserved with no deviation of their original characters for several years.

CHAPTER 4

RESULTS AND DISCUSSION

All egg samples from selected market were bacteriologically tested and found positive for *E. coli*, *Salmonella* spp. and *Staphylococcus* spp. The results of isolation and identification of *E. coli*, *Salmonella* spp. and *Staphylococcus* spp. are presented under the following heading:

4.1 Results of cultural examination for *E. coli*

4.1.1 Culture in Nutrient broth

Rapid turbidity in Nutrient broth was characterized by the growth of *E. coli*

4.1.2 Culture on Nutrient agar

On nutrient agar media large, circular, low convex, smooth and greyish white colonies formed by *E. coli* were observed.

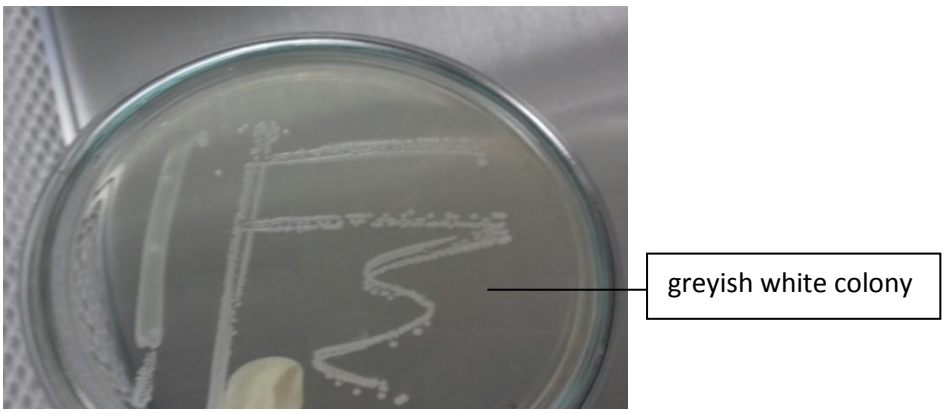

Figure 2: Growth of *E. coli* in Nutrient Agar Media

4.1.3 Culture in MacConkey Agar Media

On MacConkey Agar Media, large, pink, lactose fermenter colonies are characterized by *E. coli*.

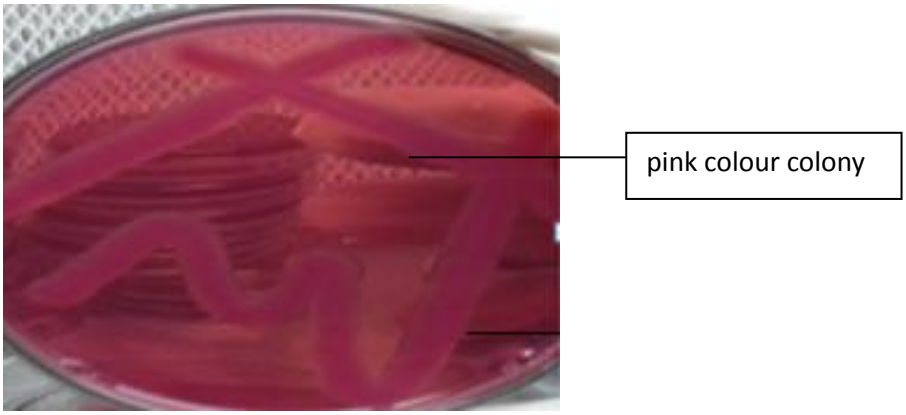

Figure 3: Growth of *E. coli* in MacConkey Agar Media

4.1.4 Culture in Eosin Methylene Blue Agar Media

On Eosin Methylene Blue Agar Media, *E. coli* produced yellow green metallic sheen colonies.

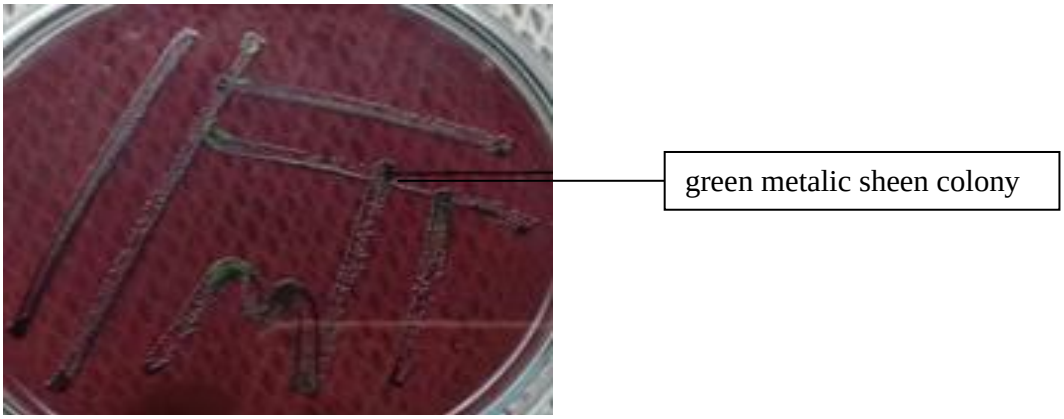

Figure 4: Growth of *E. coli* in Eosin Methylene Blue Agar Media

4.1.5 Microscopic examination

In Gram's staining, the organism revealed gram-negative, pink color, small rod shaped appearance, arranged in single or paired short

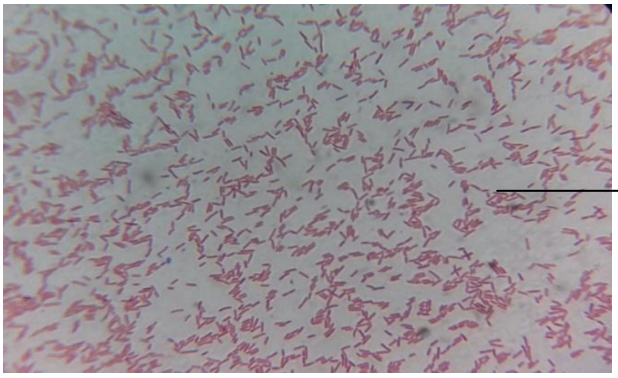

Figure 5: Staining Characteristics of *E. coli* (100X)

4.1.6 Results of biochemical tests of *E. coli*

Table 6: Carbohydrate fermentation test for the identification of *E. coli* isolates

Name of Biochemical (CHO Fermentation test)	*E. coli*
Glucose fermentation	+ve*
Sucrose fermentation	+ve*
Maltose fermentation	+ve*
Lactose fermentation	+ve*
Mannitol fermentation	+ve*

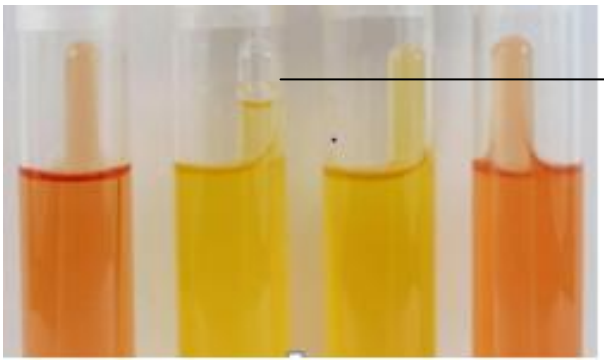

Figure 6: Carbohydrate Fermentation test of *E. coli*

4.2 Results of cultural examination for *Salmonella* spp.

4.2.1 Culture in Nutrient broth

Uniform turbidity with formation of granular sediment in Nutrient broth was characterized by the growth of *Salmonella* spp.

4.2.2 Culture on Nutrient agar

On nutrient agar media small, blue, moist, circular and entire by *Salmonella* spp. were observed.

4.2.3 Culture in Salmonella-Shigella (SS) Agar

On SS agar *Salmonella* spp. produced non-lactose fermenting with black centres.

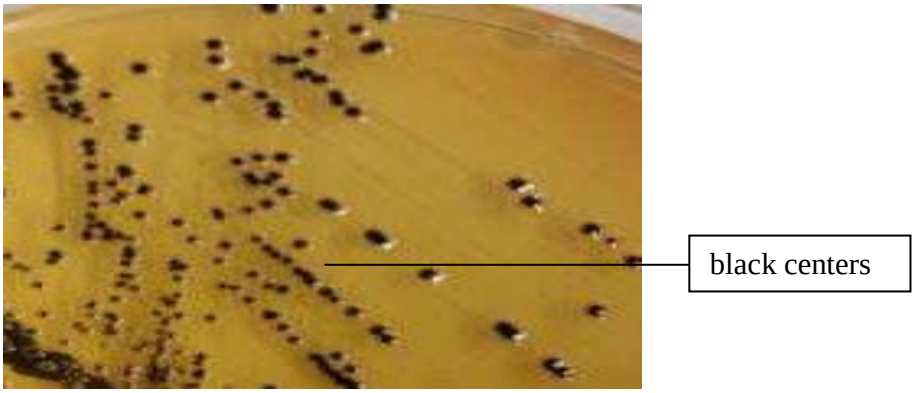

Figure 7 : *Salmonella* spp. in SS agar

4.2.4 Microscopic examination

In Gram's staining, the organism revealed gram negative, short rod shaped, singly arranged.

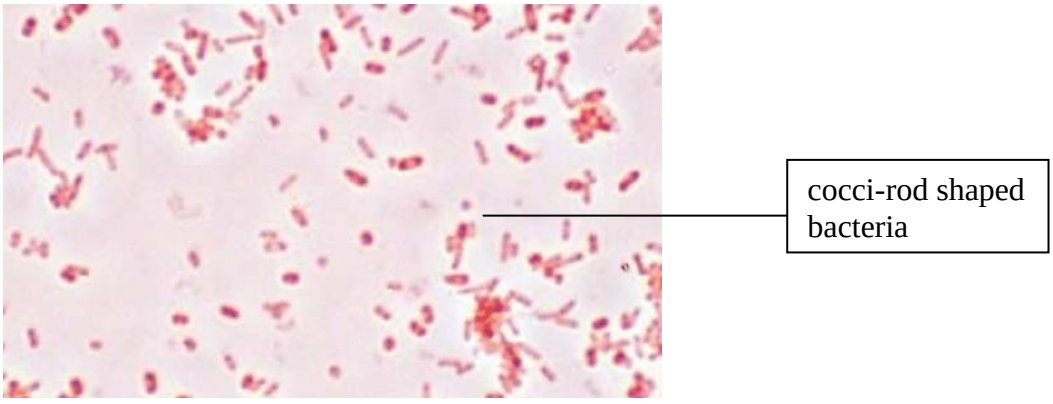

Figure 8: Staining Characteristics of *Salmonella* spp. (100X)

4.2.5 Biochemical tests of *Salmonella* spp.

Table 7. Carbohydrate fermentation test for the identification of *Salmonella* spp. isolates

Name of Biochemical Test	*Salmonella* spp.
Glucose fermentation	+ve*
Sucrose fermentation	-ve
Maltose fermentation	+ve*
Lactose fermentation	+ve*
Mannitol fermentation	+ve*

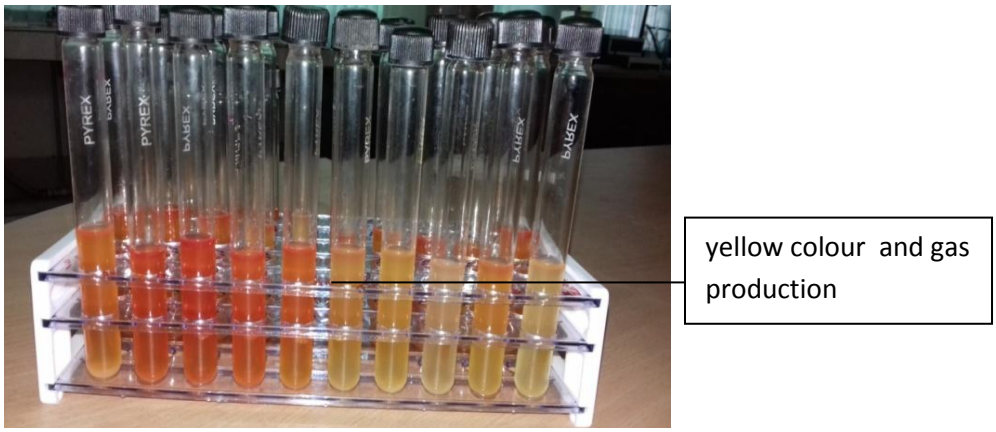

Figure 9: Carbohydrate Fermentation test of *Salmonella* spp.

4.3 Results of cultural examination for *Staphylococcus* spp.

4.3.1 Culture on Mannitol Salt Agar Media

After overnight incubation on MSA media, some plates showed yellow colony and some plates showed whitish colony. All the suspected *Staphylococcus* spp**.** which produced β hemolysis on 5% blood agar were able to ferment mannitol salt agar characterized by the formation of yellow colony and white/transparent colony indicated other *Staphylococcus* spp.

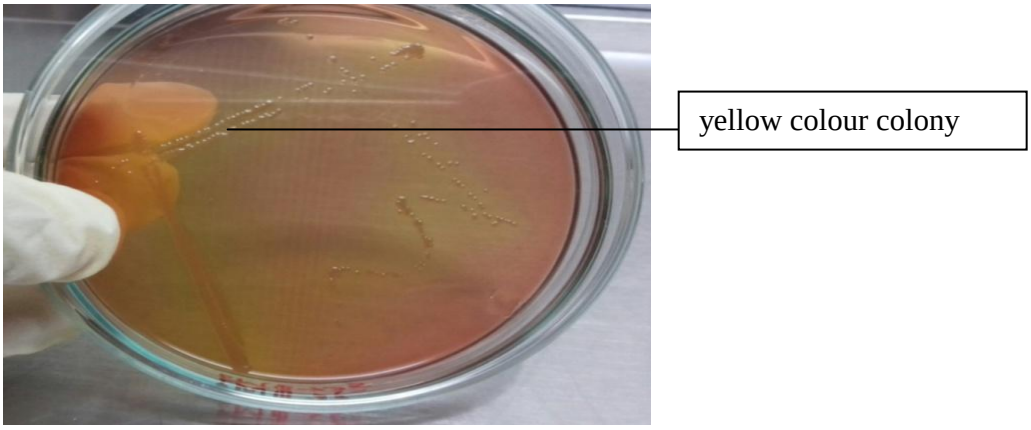

Figure 10: Growth of *Staphylococcus* spp. in MSA media

4.3.2 Culture on Blood agar

β hemolytic colonies of *Staphylococcus* spp. on blood agar media enriched with 5% sheep blood agarwere circular, small, smooth raised with yellowish in color.

4.3.3 Culture in Nutrient broth

Diffused turbidity in Nutrient broth was characterized by the growth of *Staphylococcus* spp.

4.3.4 Culture on Nutrient agar (NA)

On nutrient agar media small, circular and smooth, raised, yellowish white colonies formed by *Staphylococcus* spp. were observed.

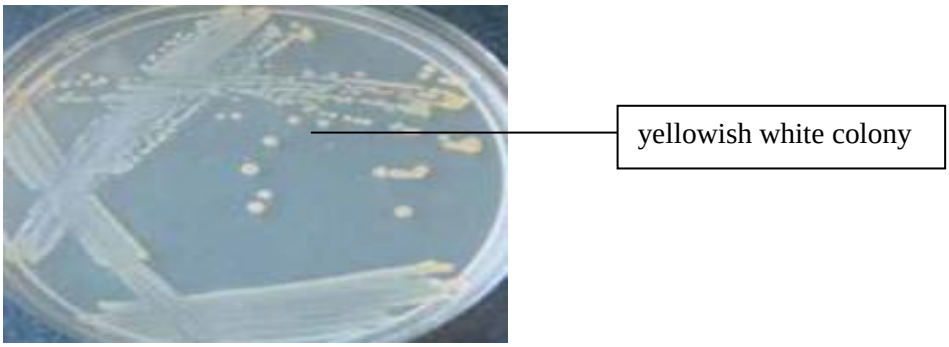

Figure 11: Growth of *Staphylococcus* spp. on NA media

4.3.5 Microscopic examination

In Gram's staining, the organism revealed Gram positive, violet colored, cocci shaped and arranged in grapes like cluster under light microscope.

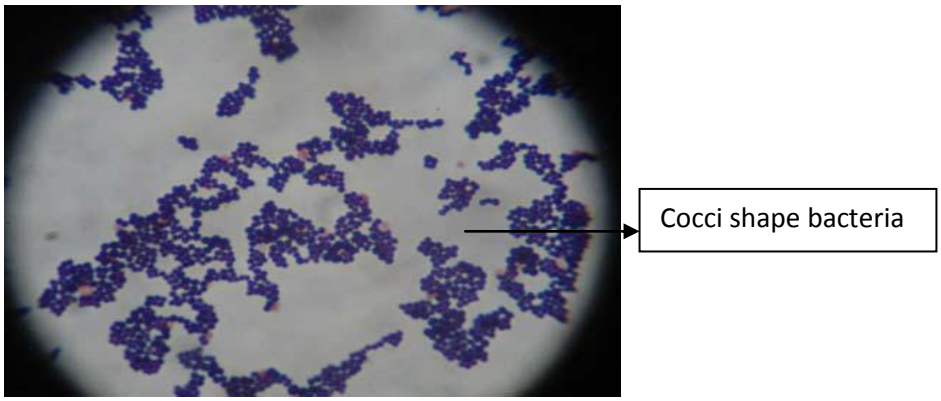

Figure 12: Staining Characteristics of *Staphylococcus* spp.(100X)

4.3.6 Biochemical tests

4.3.6 Catalase test

Catalase test was performed to differentiate Staphylococci (catalase producer) from Streptococci (non-catalase producer). Hydrogen peroxide was breakdown into water and oxygen. Production of oxygen was indicated by bubble formation. All *Staphylococcus* spp. isolates were catalase positive. 7 samples were found catalase positive, whereas the negative control did not produce any bubble. The catalase test was done by slide method.

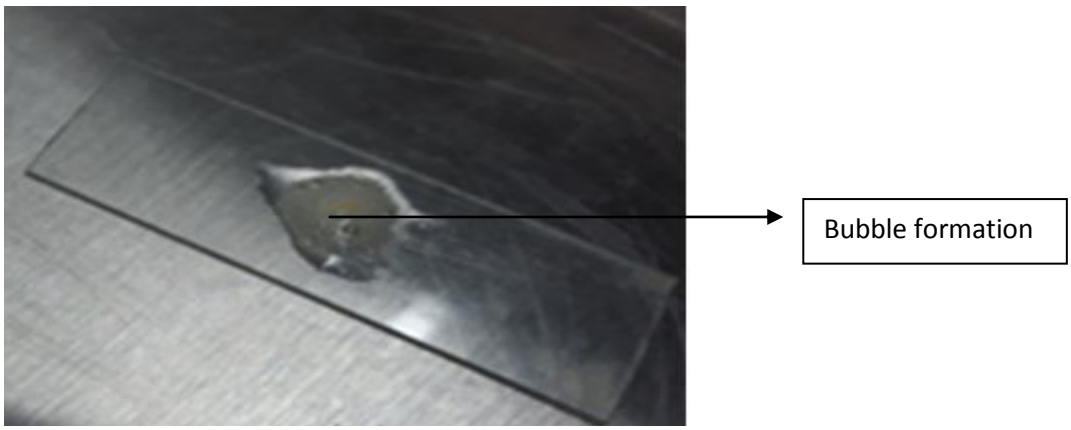

Figure 13 : Catalase test of *Staphylococcus* spp.

Table 8. The summary of the results of laboratory examination of *E. coli* in different cultural media

No. of Samples	Properties of *E. coli*			
	MacConkey Agar Media	**Eosin Methylene Blue Agar Media**	**CHO fermentation test**	**Gram's staining**
1	Large, pink, lactose fermenter colonies are characterized by *E. coli*	*E. coli* produced yellow green metallic sheen colonies	Produced both acid and gas	Gram-negative, pink color, small rod shaped appearance, arranged in single or paired short rods
3	Do	Do	Do	Do
8	Do	Do	Do	Do
10	Do	Do	Do	Do
12	Do	Do	Do	Do
15	Do	Do	Do	Do
20	Do	Do	Do	Do
22	Do	Do	Do	Do
26	Do	Do	Do	Do
29	Do	Do	Do	Do
30	Do	Do	Do	Do
32	Do	Do	Do	Do
35	Do	Do	Do	Do
39	Do	Do	Do	Do

Table 9. The summary of the results of laboratory examination of *Salmonella* spp. in different cultural media

No. of samples	Properties of *Salmonella* spp.		
	Salmonella-Shigella Agar	**CHO fermentation test**	**Gram's staining**
5	*Salmonella* spp. produced non-lactose fermenting colony with black centres.	Produced acid	Gram negative, short rod shaped, singly arranged.
6	Do	Do	Do
9	Do	Do	Do
14	Do	Do	Do
21	Do	Do	Do
25	Do	Do	Do
28	Do	Do	Do
31	Do	Do	Do
36	Do	Do	Do
37	Do	Do	Do
40	Do	Do	Do

Table 10. The summary of the results of laboratory examination of *Staphylococcus* spp. in different cultural media

No. of samples	Properties of *Staphylococcus* spp.			
	Manitol Salt Agar Media	**Blood Agar Media**	**Catalase Test**	**Gram's staining**
4	Small, circular, yellowish colonies with fermentation	Small, circular, smooth raised yellowish colonies with β hemolysis.	Bubble formation	Gram positive, blue colored, cocci shaped and arranged in grape like cluster
13	Do	Do	Do	Do
18	Do	Do	Do	Do
27	Do	Do	Do	Do
33	Do	Do	Do	Do
34	Do	Do	Do	Do
38	Do	Do	Do	Do

4.4 Prevalence of bacterial infection (*E. coli, Salmonella* spp. *and Staphylococcus* spp.)

A total of 40 egg samples were collected from different local market of Dhaka city. Out of 40 samples 20 samples (50.00%) were found to be positive for *E,coli* ,11 samples (27.50 %) were found to be positive for *Salmonella* spp. and 7 samples (17.50 %) were found to be positive for *Staphylococcus* spp. 10% mixed contamination were found.

Table 11. Prevalence of *E. coli, Salmonella* spp. *and Staphylococcus* spp. in different egg sample

Sl No.	Source and Location	Total no. of egg sample collected	No.of samples positive for *E. coli*	No.of samples positive for *Salmonella* spp.	No.of samples positive for *Staphylococcus* spp.	Prevalence %
1	Agargaon Market	10	2	3	3	80%
2	Bihari Kamp Market	10	5	3	1	90%
3	Krishi Market	10	4	4	1	90%
4	SAU Market	10	3	1	2	60%
	Total	40	14	11	7	

4.5 Antibiotic sensitivity test

The antibiotic susceptibility test was performed using agar disc diffusion assay as described by Clinical and Laboratory Standards Institute (CLSI, 2000). Antibiotics used were penicillin (10µg) amoxicillin (10µg), Erythromycin (15µg), Ciprofloxacin (5µg), Gentamycin (10µg) and Tetracycline (30µg).

Pure colonies of isolated *E.coli, Salmonella* spp. *and Staphylococcus* spp. were emulsified in normal saline and turbidity was matched with 0.5 McFarland turbidity standards. Selected antibiotic discs were placed on Mueller Hinton Agar plates seeded with bacteria. These plates were incubated at 37°C for 24 hours. The organisms were observed for antibiotic sensitivity based on diameters of zones of inhibition on petridishes. Susceptible and resistant isolates were defined according to the criteria suggested by the CLSI.

Table 12. Results of antimicrobial sensitivity test of *E. coli*

Sample no.	List of Antibiotics					
	P	E	TE	AML	CIP	GM
St-1	R	R	S	R	S	S
St-3	R	R	S	R	S	S
St-8	R	R	S	R	S	S
St-10	R	R	S	R	S	S
St-12	R	R	S	R	I.S	S
St-15	R	R	S	R	S	S
St-20	R	R	S	R	S	S
St-22	R	R	S	R	S	S
St-26	R	R	S	R	S	S
St-29	R	R	S	R	S	S
St-30	R	R	S	R	I.S	S
St-32	R	R	S	R	S	S
St-35	R	R	S	R	S	S
St-39	R	R	S	R	S	S

P= Penicillin, E= Erythromycin, AML= Amoxicillin, TE= Tetracyclin, GM=Gentamicin, CIP= Ciprofloxacin, and R= Resistant, I.S= Intermediate Sensitive, S= Sensitive.

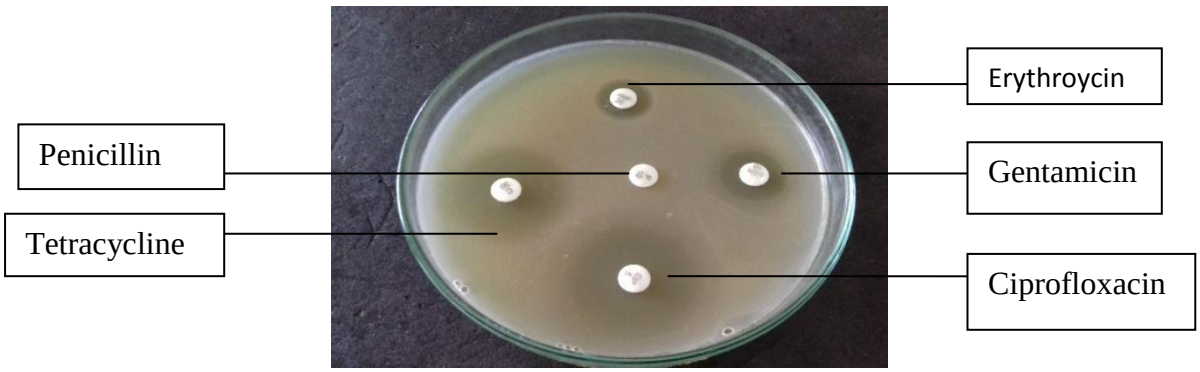

Figure14: Antibiotic sensitivity test of *E. coli*

Table 13. Results of antimicrobial sensitivity test of *Salmonella* spp.

Sample no.	List of Antibiotics					
	P	E	AML	TE	CIP	GM
St-5	R	R	R	S	S	S
St-6	R	R	I.S	S	S	S
St-9	R	R	R	S	S	S
St-14	R	R	R	S	S	S
St-21	R	R	R	S	S	S
St-25	R	R	I.S	S	S	S
St-28	R	R	R	S	S	S
St-31	R	R	R	S	S	S
St-36	R	R	R	S	S	S
St-37	R	R	I.S	S	S	S
St-40	R	R	R	S	S	S

P= Penicillin, E= Erythromycin, AML= Amoxicillin, TE= Tetracyclin, GM=Gentamicin, CIP= Ciprofloxacin, and R= Resistant, I.S= Intermediate Sensitive, S= Sensitive.

Table 14. Results of antimicrobial sensitivity test of *Staphylococcus* spp.

Sample no.	List of Antibiotics					
	N	V	E	TE	CIP	GM
St-4	R	I.S	S	S	S	S
St-13	R	R	S	S	S	S
St-18	R	R	S	I.S	S	S
St-27	R	R	I.S	S	S	S
St-33	R	R	S	I.S	S	S
St-34	R	R	S	S	S	S
St-38	R	R	S	I.S	S	S

N=Neomycin, E= Erythromycin, V=Vancomycin, TE=Tetracycline, GM=Gentamicin, CIP= Ciprofloxacin, and R= Resistant, I.S= Intermediate Sensitive, S= Sensitive.

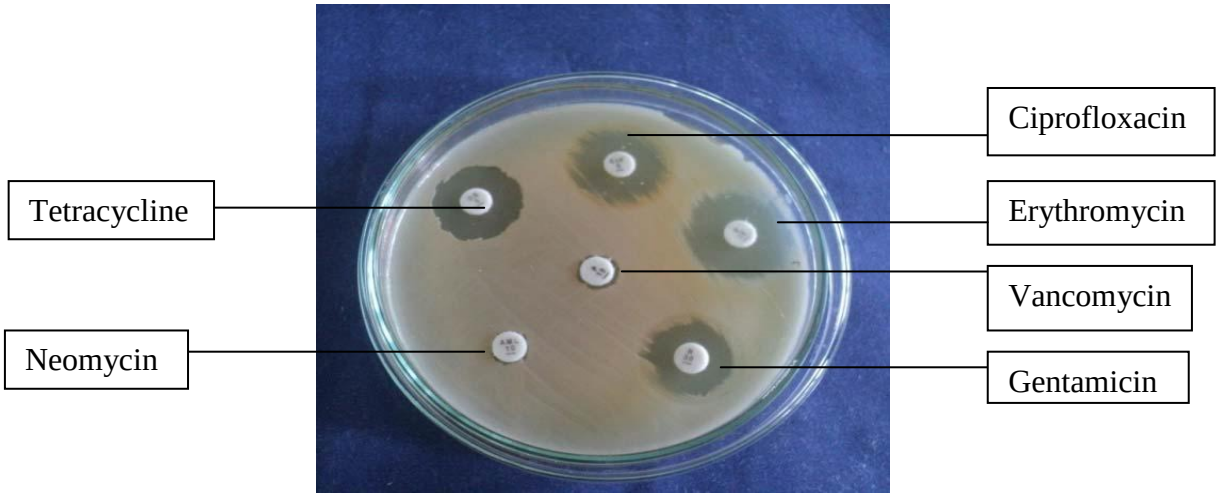

Figure15: Antibiotic sensitivity test of *Staphylococcus* spp.

DISCUSSION

The requirements for the table eggs include high nutritional value and digestibility as well as safety of use in the everyday diet of millions of people worldwide. Taking these criteria into account, the presence of pathogenic bacteria in food, including table chicken eggs, may pose a serious health problem like food poisoning and food borne infections (Pyzik *et al.* 2012). The purpose of the present study was to determine the presence of pathogenic bacteria (*E. coli, Salmonella* spp. and *Staphylococcus* spp.) in the table eggs.

Escherichia coli is known to contaminate the surface of eggs while mechanical process can spread further the bacteria. Present study has demonstrated an overall prevalence (35.0%) of *E. coli* contamination in table eggs where the highest prevalence was recorded 50.00% at Bihari kamp market and lowest (20.0%) in Agargaon market.The highest prevalence in bihari kamp market was probably due to the poor hygienic practice in the market. This finding is in agreement with the previous study where the prevalence of *E. coli* was reported as 37.0% in table eggs (AurAngzeb *et al.* 2015).

In present study, the prevalence of *Salmonella* spp. in table egg from selective market was 27.50% where the highest prevalence was 40.00% at Krishi market, and lowest 10% in SAU market. Similar prevalence of *Salmonella* spp.(23.52) was also reported previously (Parveen *et al.* 2012). Contamination of egg by *Salmonella* spp. may occur at any stage of production like collection, transportation or marketing either through vertical or horizontal transmission. Importantly, reusable egg tray is a potential source for contaminating egg shell by *Salmonella* spp. in developing country like Bangladesh.

Staphylococcus spp. isolation rate in table egg from different market was 17.50% where the highest prevalence was 30.00% at Agargaon market, and lowest 10% in Bihari kamp market. The above result is lower than the results reported by Parveen *et al.* (2012) referred prevalence of *Staphylococcus* spp. 25.49% in table egg.

Antibiogram profile studies of bacterial isolates from various poultry sources have recently drawn considerable attention due to the probable dissemination of multi-drug resistant (MDR) bacteria from birds to humans.

The antibiotic resistance of *E. coli* isolated from table egg were studied and were highly resistant to penicillin, erythromycin and amoxicillin, whereas they showed sensitivity to tetracycline ciprofloxacin and gentamycin. Gentamycin and ciprofloxacin is the best choice for treatment of *E. coli* infection. This findings were correlated with Akond *et al.*(2009) where they analysed 88%, 80%, 76%, 70%, 68%, 64%, 58%, 52%, and 20% of the tested *Escherichia coli* from poultry sources were found resistant to penicillin, riphampicin, kanamycin, streptomycin, cefixine, erythromycin, ampicillin, and chloramphenicol and neomycin respectively. Sensitivity was recorded in case of norfloxacin, gentamicin and chloramphenicol, neomycin, tetracycline, streptomycin and ampicillin as 86%, 80%, 60%, 36%, 30%, and 26%. *E. coli was* resistant to penicillin , amoxicillin and erythromycin due to their structural difference. Theis antibiotic cell wall contain peptidoglycan layer which make the antibiotic resistant to *E.coli.*

The isolated *Salmonella* spp. were highly resistant to penicillin, erythromycin, amoxicillin but were sensitive to gentamycin, tetracycline and ciprofloxacin. The observation was similar to Rather *et al.*(2012) who found that the high sensitivity of *Salmonella* spp. was 100 % sensitive for gentamicin, 91.67% for each of cefixime, ciprofloxacin, amikacin and lavofloxacin; 83.33 % for each of cephotaxime and amoxicillin. Therefore, *Salmonella* spp. showed resistance to erythromycin (100%), nalidixic acid (100%) and rifampicin (91.67%).

The isolated *Staphylococcus* spp. were resistant against neomycin, vancomycin and were sensitive to ciprofloxacin, erythromycin, tetracycline and gentamicin. These findings are almost in line with Brintya *et al*. (2014). Who found that *S. aureus* were highly susceptible to gentamycin (78.6-91.7%), streptomycin (86.1%) and ciprofloxacin (94.4%). *Staphylococcus* spp. were resistant against neomycin, vancomycin due to enzymatic modification of drug binding site, alteration of the drug target that prevents the inhibitor from binding.

CHAPTER 5

SUMMARY AND CONCLUSION

It could be concluded from present investigation that table eggs sold in selective retail market of Dhaka district contained *E. coli*, *Salmonella* spp. and *Staphylococcus* spp. hence may pose a health hazard to human beings if consumed improperly cooked or raw eggs. The pathogenic bacteria isolated from this study are of public health importance and their high levels of resistance to commonly used antibiotics in human and vet medicine make them a great risk to human and animals Antibiotic sensitivity pattern of the isolates were performed against the positive samples. The antibiotic sensitivity test indicated that the isolated *E. coli* were highly resistant to penicillin, erythromycin and amoxicillin; and were sensitive to tetracycline, ciprofloxacin and gentamycin. The isolated *Salmonella* spp. were highly resistant to penicillin, erythromycin and amoxicillin; and were sensitive to tetracycline, ciprofloxacin and gentamycin. *Staphylococcus* spp. were resistant against neomycin, vancomycin and were sensitive to ciprofloxacin, erythromycin, tetracycline and gentamycin. There is need to educate the people to adopt signifcant hygienic measures in handling of table eggs and should not be consumed inadequately cooked eggs or egg products.

From the present study, it may included that

i. *E. coli* was present in 35.00% in egg samples
ii. *Salmonella* spp. was present in 27.50% in egg samples
iii. *Staphylococcus* spp. was present in 17.50% in egg samples
iv. *E. coli*, *Salmonella* spp., *Staphylococcus* spp. successfully isolated and confirmed by different cultural media, biochemical tests.
v. *E. coli*, *Salmonella* spp. and *Staphylococcus* spp. is more prevalent in Krishi market and bihari kamp market compare to other sources.
vi. Most of the isolates showed multi-drug resistance

Recommendations

i. Prevention of undue contamination of table egg prior to and after processing
ii. Thorough washing of table egg
iii. The hand washing practices of farmer need to be improved
iv. Education of hygiene and sanitation to egg handlers

REFERENCES

Aarestrup, F.M., Rene, S., Hendriksen, J.L., Katie, G., Kathryn, T., Patrick, F., McDermott, F.J. and Peter, G.S. (2007). International spread of multidrug-resistant *Salmonella Schwarzengrund* in food products. *Emerg. Infect.. Dis.* **13**(5):726-731.

Abdullah, I.N. (2010). Isolation and identification of some bacterial isolates from table egg. *Al-Anbar J. Vet. Science***. 3** (2):59-67.

Abdel Karem, H. and Z., Mattar (2001). Heat resistance and growth of *Salmonella enteritidis, Listeria monocytogenes* and *Aeromonas hydrophila* in whole liquid egg. *Acta Microbiol. Pol.* **50**(1):27-35.

Adesiyum, A., Offiah, N., Seepersadsingh, N., Rodrigo, S., Lashley, V., Musai, L. and Georges, K. (2005). Microbial health risk posed by table eggs in Trinidad. *Epidemiol. Infect.* **133**(6):1049-56.

Adeyanju, T.G. and Ishola, O. (2014). *Salmonella* and *Escherichia coli* contamination of poultry meat from a processing plant and retail markets in Ibadan, Oyo State, Nigeria. *Springer Plus*. **3**:139.

Abdul Aziz, S., Bahobail Sabry, A. and Hassan Bahig (2012). A Microbial quality and content aflatoxins of commercially available eggs in Taif, Saudi Arabia. *African J. Microbiol. Res*. **6**(13): 3337-3342.

Ahmed, M.M., Hossain, M.S., Mahbub, K.R., Khaleque, H.N., Hossain, Z., Fakruddin, M., Chowdhury, A., Hossain, M.N. and Alam, M.Z. (2012). Performance analysis of multiplex PCR based detection of *Salmonella spp.* and *Salmonella* Typhimurium in chicken egg sample. *J. Sci. Res.* **2**(1):25-32.

Ahmed, M.M., Rhaman, M.M., Mahbub, K.R. and Wahiduzzaman, M. (2011). Characterization of antibiotic resistant *Salmonella spp*. isolated from chicken eggs of Dhaka city. *J. Sci. Res.* **3**(1):191- 96.

Akond, M.A, Alam, S., Hassan, S.M.R., Shirin, M. (2009). Antibiotic resistance of *Escherichia coli* isolated from poultry and poultry environment of Bangladesh. Int. J. Food Safety. **11**(3): 19-23.

Ahmed, A.M. and Shimamoto, T. (2014). Isolation and molecular characterization of *Salmonella enterica, Escherichia coli* O157:H7 and *Shigella* spp. from meat and dairy products in Egypt. *Int. J. Food Microbiol.* **168-169**: 57-62.

Akbar, A. and Anal, K.A. (2014). Zinc oxide nanoparticles loaded active packaging, a challenge study against *Salmonella* Typhimurium and *Staphylococcus aureus* in ready- to-eat poultry meat. *Food Control*. **38**: 88–95.

Akbar, A. and Anal, A.K. (2011). Food safety concerns and food-borne pathogens, *Salmonella*, *Escherichia coli* and *Campylobacter*.*FUUAST J. BIOL.* **1**(1):5–17.

Akbar, A. and Anal, A.K. (2013a). Prevalence and antibiogram study of *Salmonella* and *Staphylococcus aureus* in poultry meat. *Asian Pac. J. Trop. Biomed.* **3**(2):163–168.

Akbar, A. and Anal, A.K. (2013b). Occurrence of *Staphylococcus aureus* and evaluation of anti- staphylococcal activity of *Lactococcus lactis* subsp. *lactis* in ready-to-eat poultry meat. Annals of Microbiology (online first). DOI 10.1007/s13213-013-0641-x.

Al-Bahry, S. N., Mahmoud, I. Y., Al-Musharafi, S. K. and Al-Ali, M. A. (2012). Penetration of spoilage and food poisoning bacteria into fresh chicken egg: A public health concern. *G.J.B.B.* **1**(1):33-39.

Al-Jiffri, O., El-Sayed, Z. and Al-Sharif F. (2011). Urinary tract infection with Esherichia coli and antibacterial activity of some plants extracts. *Int. J. Microbiol. Res*. **2**:1–7.

Alizadeh, A.H., Behrouz, N., Salmanzadeh, S., Ranjbar, M., Azimian, M.H., Habibi, E., Jaafari, F., Zolfagharian, K. and Zali, M.R. (2007). *Escherichia coli*, *Shigella* and *Salmonella* species in acute diarrhea in Hamedan, Islamic Republic of Iran. *East. Mediter. Hlth. J.* **13**(2):243-249.

Anbessa, D. and Shiferaw, D. (2013). Correlation of traditional knowledge with laboratory bases salmonella detection on egg shell and content of market sold egg. *Asian J. Biological Sci.* **6**:168-174.

Ansah, T., Dzoagbe, G. S. K., Teye, G. A., Adday, S. and Danquah, J. K. (2009). Microbial quality of table eggs sold on selected markets in the Tamale municipality in the Northern Region of Ghana. *Livestock Research for Rural Development* **21**(9):4-7.

Andreoletti, O. H., Budka, S., Buncic, P., Colin and Collins, J. (2009). Special measures to reduce the risk for consumers through *Salmonella* in table eggs-e.g. cooling of table eggs. *EFSA J.* **957**:1-29.

Bahrouz, M.A. and Al-Jaff. (2005). The risk of bacterial contamination in hen eggs of Sulaimani poultries. Journal of Zankoy Sulaimani. **8A** (1): 63–71

Bager, F. and Helmuth, R. (2001). Epidemiology of Resistance to Quinolones in Salmonella. *Vet. Res*. **32**:285-290.

Bailey, W.R., S.M., Finegold, W.J., Martin and E.G., Scott. (1978). Bailey and Scott's Diagnostic Microbiology. 5th Edn., C.V. Mosby, St. Louis. MO., ISBN: 9780801604218, Pages: 514.

Bauer, A.W., Kirby, W.M.M, Sherris, J.C. and Turck, M. (1996). Antibiotic susceptibility testing by a standardized single disk method. *Am. J. Clin. Pathol.* **45**:493–496.

Begum, K., Reza, T.A., Haque, M., Hossain, A., Hassan, F.M.K., Hasan, N., Akter, N., Ahmed, A., Barua, U. (2010). Isolation, identification and antibiotic resistance pattern of *Salmonella spp.* from chicken eggs, intestines and environmental sample. *Bangladesh J. Pharmacol.* **13**(1): 245-50.

Begum, F. (1993). Studies on the immune response in chickens with experimentally prepared *Salmonella gallinarum* vaccine. M.S. thesis submitted to the Department of Microbiology and Hygiene, Bangladesh Agricultural University, Mymensingh.

Bhowmick, B.K. and Rashid, H. (2004). Prevalence and antibiotic susceptibility of *E. coli* isolated from Urinary Tract Infection (UTI) in Bangladesh. *Pak. J. Biol. Sci.* **7**:717-720.

Bichler, L. A., Nagaraja, K. V. and Halvorson, D. A. (1996). *Salmonella enteritidis* in eggs, cloacal swab specimens and internal organs of experimentally infected White Leghorn chickens. *Am. J. Vet. Res*. **57**:479-495.

Blumenthal, D. (1990). From the chicken to the egg. FDA Consumer (April, 1999). Pp. 7-10.

Board, R.G. and Tranter, H.S. (1995). The microbiology of eggs. In: W. J. Stadelman and Coterill O. J. (eds). Egg Science and technology. 4th ed. Haworth Press Inc. New York.

Bradshaw, J. G., Shah, D. B., Forney, E. and Madden, J. M. (1990). Growth of *Salmonella enteritidis* in yolk of shell eggs from normal and seropositive hens. *J. Food. Prot.* **53**:1033-1036.

Breed, R.S., Murray, E.G.D. and Smith, N.R. (1957). Bergey's Manual of Determinative Bacteriology. 7th Edn., Williams and Wilkins Co., Baltimore.

Brown, S.J. and Brown, W.L. (1978). Fate of *Salmonella* inoculated into Beef for Cooking. *J. Food Prot.* **41**(8):598–605.

Brooks, J.E., Bulet, J.S., Morse, S.A. (2002). Medical Microbiology (Jawets M and Aldelberged), 22nd Edn., MacGraw Hill, New Delhi, India. p. 197–202.

Bruce, J. and Drysdale, E.M. (1994). Trans-shell transmission. Microbiology of the avian egg. Chapman and Hall, London. Pp 63-91.

Bufano-Nancy, S. (2000). Keeping eggs safe from farm to table. *Food Technol.* **54**(8):192.

Carter, G.R. and Cole, R.J. (1990). Diagnostic Procedures in Veterinary Bacteriology and Mycology. 5th Edn., Academic Press, San Diego, California.

Casey, B.B., Ian, T.W. and Robert, V.T. (2012). Emerging food borne pathogens and problems; expanding prevention efforts before slaughter or harvest. Improving food safety through one health approach. Work shop summary. National academies press (US), 500 Fifth Street, NW. Washington DC. Pp 1-418.

Center for Disease Control and Prevention (CDC) (2013). *Salmonella* in egg: An Unwelcome summer visitor. Atlanta: Center for Disease Control and Prevention.

Cheesbrough, M. (1985). Medical laboratory manual for tropical countries.1stedition, Microbiology, English Language Book Society, London. p. 400-480.

Chousalkar, K.K., Flynn, P., Sutherland, M., Roberts, J.R. and Cheetham, B.F. (2010). Recovery of *Salmonella* and *Escherichia coli* from commercial egg shells and effect of translucency on bacterial penetration in eggs. *Int J Food Microbiol.* **142**: 207–213.

Chousalkar, K. and Gole, V.C. (2016). Salmonellosis acquired from poultry. *Curr. Opin. Infect. Dis.* **29**:514–519.

CLSI (formerly NCCLS) (2007). Performance standards for antimicrobial susceptibility testing. 17th Informational Supplement document M100-S17: 1, Wayne, Pennsylvania. 2007; p. 32–50.

Costa, P.M., Oliveira, M., Bica, A.P., Vaz-Pires and Bernardo, F. (2007). Antimicrobial resistance in *Enterococcus* spp. and *Escherichia coli* isolated from poultry feed and feed ingredients. *Vet. Microbiol.***120**:122-131.

Cortes, C.R., Isaias, G.T., Cuello, C.L., Villaseca-Flores, J.M., Anderson, R.C. and Campos, C.E. (2004). Bacterial isolation rate from fertile eggs, hatching eggs and neonatal broilers with yolk sac infection. *Rev. Latinoam Microbiol.* **46**:12-16.

Cox, N.A., Musgrove, M.T., Jones, D.R., Northcutt, J.K., Harrison, M.A., Ladely, S.R. (2006). Antimicrobial resistance in *Salmonella* and *Escherichia coli* isolated from commercial shell eggs. *Poult. Sci.* **851**:59–66.

Day, J.B., Basavanna, U., Sharma, S.K, (2009). Development of a cell culture method to isolate and enrich *Salmonella* Enteritidis from shell eggs for subsequent detection by real-time PCR. *Appl. Environ. Microbiol.* **4**:612-30.

De, Jong, B. and Ekdahl, K. (2006). The comparative burden of Salmonellosis in the European Union member states, associated and candidate countries. *BMC. Public Health*. **6**: 4 -12.

De, Reu, K., Grijspeerdt, K., and Heyndrickx, M. (2005). Bacterial eggshell contamination in conventional cages, furnished cages and aviary housing systems for laying hens. *Br. Poult. Sci*. **46**:149-55.

Dewan, A.M., Corner, R., Hashizume, M. and Ongee, E.T. (2013). Typhoid Fever and Its Association with Environmental Factors in the Dhaka Metropolitan Area of Bangladesh: A Spatial and Time-Series Approach. *PLoS. Negl. Trop. Dis*. **7**(1): e1998.

Department of Livestock Services (2009). Annual report of Department of Livestock Services, Khamarbari Road, Farmgate, Dhaka.

Dey, R.K., Khatun, M.M., Islam, M.A., Hosain, M.S. (2013). Prevalence of multidrug resistant *Escherichia coli* in Pigeon in Mymensingh, Bangladesh. *Microbes and Health*. **2**:5–7.

Doosti, A., Mahmoudi, E., Mokhtari-Farsani A. and Doosti, E. (2015). Frequency of *Salmonella* typhimurium in egg shell and determination of antibiotic resistance of isolates. *Int. J. Basic Applied Biol*. **2**:186-189.

Deresse, D., Solomon, G. and Dawit, Y. (2012). Antibiotic-resistance *Staphylococcus aureus* isolated from cow's milk in the Hawassa area, South Ethiopia. *Ann. Clin. Microbio.l Antimicrob.* **11**:26.

El-Prince and Enas, M. (1988). Microbiological quality of hens' egg. *M.V. Sci. Thesis*, Fac. Vet. Med. Assiut Univ.

Elliott, R.P. (1954). Spoilage of shell eggs by pseudomonads. *Appl. Microbiol.* **2**: 158–163.

Ellis and M.E. (1998). Gram Negative Bacillary Pneumonia. In: Infectious Diseases of the Respiratory Tract, Ellis, M.E. (Ed.). Cambridge University Press, Cambridge, UK., ISBN: 9780521405546, pp: 130-148.

Ellen, H.H., Bottcher, R.W., Wachebfelt, E. and Takai, H. (2000). Dust levels and control methods in poultry houses. *J. Agric. Saf. Health*. **6**(4):275-282.

Egg Nutrition Center(ENC)(2004). Egg protein fact sheet. *Indian J. Dermatol*. **4**:667–668.

Eric, G.(2011). *E. coli* infections in poultry. Nutrition on line. Diamond V, pp: 1-4.

Ernst, C. (2009). Asia dominates in egg consumption. Sanovo Staalkat group, Sjanghai, China.

Etches, R.J., (1992). The influence of housing aspect of egg quality. Proceedings of the 19th Worlds Poultry Congress, September 19-24, 1992, Amsterdam, Netherlands, pp: 164-167.

Frazier, W.C. & Westhoff, D.C. (1987). Contamination, preservation and spoilage of eggs. *Food Microbiology*, Third edition. McGraw-Hill, Inc., New York. Pp 11-20.

Freeman, B.A. (1985). Burrows textbook of microbiology. 22nd Edn., WB Saunders Company, Philadelphia, London, Toronto, Mexico City, Rio de Janerio, Sydney, Tokyo. p. 464–472.

Friend, M. and Fransom, J. C. (1999). Salmonellosis Field Manual of Wild Life Diseases. General field procedures and diseases of birds", Edition USGS 999, 99-109.

Froning, G.W. (1998). Recent advances in egg products research and development. University of Nebraska-Lincoln.

Gautron, J. and Nys, Y. (2006). Egg shell matrix proteins and natural defenses of the egg. Proceedings of the Symposium COA/INRA Scientific Cooperation in Agriculture, November 7-10, Tainan, pp: 7-10.

Gast, R.K., Nasir, M.S., Jolley, M.E., Holt, P.S. and Stone, H.D. (2002). Serologic detection of experimental *Salmonella enteritidis* infections in laying hens by fluorescence polarization and enzyme immunoassay. *Avian Dis.* 137-142.

Gantois, I., Ducatelle, R., Pasmans F., Haesebrouck, F., Gast, R., Humphrey, T.J. and Van ,Immerseel, F. (2009). Mechanisms of egg contamination by *Salmonella enteritidis*. *FEMS .Microbiol Rev.* **33**:718–738.

Ghasemian, S. (2011). The prevalence of bacterial contamination of table eggs from retails markets by *Salmonella* spp., *Listeria monocytogenes*, *Campylobacter jejuni* and *Escherichia coli* in Shahrekord, Iran. *Iran J. Microbiol*. **4**(4):249–253.

Gupta, V, Yadav, A. and Joshi, R.M. (2002). Antibiotic resistance pattern in uropathogens. *Indian J. Med. Microbiol.* **20**:96–8.

Habib, F., Rind, R., Durani, N., Bhutto, A.L., Buriro, R.S., Tunio, A., Aijaz, N., Lakho, S.A., Bugti, A.G. and Shoaib, M. (2015). Morphological and cultural characterization of *Staphylococcus aureus* isolated from different animal species. *J. Appl. Microbiol*. **5**:15–26.

Hang'Ombe, B.M., Sharma, R.N., Tuchili, L.M. and Skjerve, O. (1999). Isolation of bacteria from table eggs in Zambia. *INDIAN J. ANIM. SCI.* **69**(6):445-447.

Harsha, H.T., Reshmi, R., Varghese, R., Divya,P.S., Rahiman K.M.M. and Hatha, A.A.M. (2011). Prevalence and antibiotic resistance of *Salmonella* from the eggs of commercial samples. *J. Microbiol. Infect. Dis*.**1**:93-100.

Hartland, E.L. and Leong, J.M. (2013). Enteropathogenic and enterohemorrhagic *E. coli*: Ecology, pathogenesis and evolution. *Front. Cell. Infect. Microbiol.* **3**:10.3389

Harry, E.G. (1963). The relationship between egg spoilage and the environment of the egg when laid. *Brt. Poult. Sci.* **4**(1):91-100.

Hasan, B., Nahar, S.G., Akter, L. and Sale, A. (2011). Antimicrobial sensitivity pattern of *Salmonella* Typhi isolated from blood culture in a referral hospital. *Bangladesh J. Med. Microbiol*. **5**(1):16-20.

Hasenson, L. B., Kaftyreva, L., Lhszló, V. G., Woitenkova, E. and Nesterova, M. (1992). Epidemiological and microbiological data on *Salmonella enteritidis*. *Acta. Microbiol. Hung.* **39**:31-39.

Hartmann, G. and Rohde, B.H. (1980). *Textbook of introductory mycology with examples.* New age international (P) publishers. New Delhi. Pp 110-111.

Hawkey, J., Edwards, D.J., Dimovski, K., Hiley, L., Billman-Jacobe, H., Hogg, G. and Holt , K.E. (2013). Evidence of microevolution of *Salmonella typhimurium* during a series of egg-associated outbreaks linked to a single chicken farm. *BMC. Genomics*. **14**:800.

Holtby, I., Tebbutt, M., Anwar, M., Aislabie, J., Bell, B., Flowers, Hedgley, W. and Kelly, P. (2006). Two separate outbreaks of *Salmonella enteritidis* phage type 14b food poisoning linked to the consumption of the same type of frozen food. *Public Health*. **120** (9):817-823.

Holt, J.G., Krieg, N.R., Sneath, P.H.A., Staley, J.T. and Williams, S.T. (1994). Facultatively Anaerobic Gram-Negative Rods. In: Bergey's Manual of Determinative Bacteriology, Holt, J.G. (Ed.). 9th Edn., Lippincott Williams and Wilkins, Baltimore, MA., USA., ISBN-**13**: 175-290.

Holt, J.H. (1994). Bergey's manual of determinative bacteriology, ninth edition. Lippincott Williams & Wilkins, Philadelphia, PA. Pp 787.

Hossain, M.T., Siddique, M.P., Hossain, F.M.A., Zinnah, M.A., Hossain, M.M., Rahman, A.M.T., Choudhury, K.A.(2004). Isolation, identification, toxin profile & antibiogram of *Escherichia coli* isolated from broiler and layer in Mymensingh district of Bangladesh. *Bangl. J. Vet. Med*. 6:1–5.

Howard, Z.R., Moore, R.W., Zabala-Diaz, I.B., Landers, K.L., Byrd, J..A., Kubena, L.F., Nisbet, D.J., Birkhold, S.G. and Ricke, S.C. (2005). Ovarian laying hen follicular maturation.

Howard, Z.R., O'Bryan, C.A., Crandall, P.G. and Ricke, S.C. (2011). *Salmonella enteritidis* in shell eggs: Current issues and prospects for control. *Int. J. Environ.. Res. Public Health.* **45**:755–764.

Hope, B.K., Baker, R., Edel, E.D., Hogue, A.T., Schlosser, W.D., Whiting, R., McDowell, R.M. and Morales, R.A. (2002). An overview of the *S. enteritidis* risk assessment for shell eggs and egg products. *Risk Analysis*. **22**(2):203-218.

Humphrey, T. J. (1994). Contamination of egg shell and contents with *Salmonella enteritidis*. *Intl. J. Food Microbiol*. **21**:31-40.

ICMSF. (1985). Microorganism in foods, samples for Microbiological Analysis: Principles and specific applications. Recommendation of the International Commission on Microbiological Specification for Foods. Association of Microbiological Societies, Toronto, University of Toronto Press. p. 4–38.

Jakociune, D., Pasquali, F., de Silva, C.S., Löfström, C., Hoorfar, J., Klein, G., Manfreda, G. and Olsen, J.E. (2014). Enumeration of salmonellae in table eggs, pasteurized egg products, and egg-containing dishes by using quantitative real-time PCR. *Appl. Environ. Microbiol.* **80**:1616–1622.

Jahan (2013). Isolation, Identification And Molecular Characterisation Of *Staphylococcus aureus* From Raw Milk Sample.M.S. thesis, Mymensingh, Dhaka.

Jerzy, R. and Dagmara, S.P. (2009). Antimicrobial defense mechanisms of chicken eggs and possibilities for their use in protecting human and animal health. Division of avian diseases, Institute of biological bases of animal diseases University of life sciences in Lublin.

Jones, D.R., Musgrove, M.T. and Northcutt, J.K. (2004). Variations in external and internal microbial populations in shell eggs during extended storage. *J. Food Protect.* **67**: 2657-2660.

Joshi, S., Singh, R. and Singh, S.P. (2012). Antibiotic resistance profile of *Escherichia coli* isolates from colibacillosis in and around Pantnagar, India. *Vet. World.* **5**:405-408.

Kabir, S.M.L., Sikder M.H., Alam, J., Neogi, S.B. and Yamasaki, S. (2017). Colibacillosis and its impact on egg production. In: Egg Innovations and Strategies for Improvements. Edited by: Patricia Hester. 523-536 Oxford: Academic Press isbn: 978-0-12-800879-9.

Khaton, R., Haider, M.G., Paul, P.K., Das, P.M. and Hossain, M.M. (2008). Colibacillosis in commercial chickens in Bangladesh. *Bangl. vet*. **25**:17–24.

Kim, A., Lee, Y.J., Kang, M.S., Kwag, S.I. and Cho, J.K. (2007). Dissemination and tracking of *Salmonella* spp. in integrated broiler operation. *J. Vet. Sci.* **8**:155-161.

Kinde, H., Shivaprasad, H.L., Daft, B.M., Read, D.H., Ardans, A. and Breitmeyer, R. (2000). Pathologic and bacteriologic findings in 27-week-old commercial laying hens experimentally infected with *Salmonella enteritidis*, phage type 4. *Avian Disease*, **44**(2):239-48.

Koowatananukul, C., Saitanu, K., Jerngklinchan, J. and Sasipreeyajan, J. (1994). Detection of salmonellae in hen eggs in Thailand. *Southeast Asian J. Trop. Med. Public Health*. **25**: 324-327.

Latif, E.F.A. and Saad, M.F. (2015). Microbiological profile of leaking chicken table eggs. *Int. J.of Sci. and Nature*. **6**:51–55.

Layman, D.K. and Rodriguez, N.R. (2009). Egg protein as a source of power, strength and energy. Nutrition Today. **44**:43–48.

Linares, J.A. and Wigle, W.L.(2001). *Staphylococcus aureus* pneumonia in turkey poults os Salmonella in broiler, Layer and breeder flocks in Thailand. *Trop. Anim. Health Prod.* **28**:490-495.

Lepoutre, A., Salomon, J., Charley, C. and Querrc, L. (1994). Les toxi-infections alimentaires collectives en 1993. *Bulletin Epidé -miologique Hebdomadaire*. **52**:245−248.

Mahmud, M.S., Kabir, M.L., Alam, S.M.S., Ali, M.M. and Towhid, S.T. (2015). Prevalence of *Salmonella spp.* in poultry eggsfrom different retail markets at Savar area, Bangladesh. *American Journal of Food Science and Health*. **1**:27–31.

Maha, A.M., AL-Ashmawy (2013). Prevalence of Enterobacteriaceae in table eggs with particular reference to enterovirulent *Escherichia coli* strains. *Int. J Poult. Sci.* **12**:430–435.

Madadi, M.S., Azari, M. and Yazdani, A. (2012). Evaluation of different plating medias and PCR in the detecting of *Salmonella* Enteritidis from eggs laid by Experimentally infected hens. *J. Anim. Poult. Sci*. **1**(1):13-23.

Mahdavi, M., Jalali, M., Safaei, H.G. and Shamloo, E. (2012). Microbial quality and prevalence of *Salmonella* and *Listeria* in eggs. *Int. J. Environ. Health Eng.* **1**:16-20.

Martin, H.M., Bernatdo, F.M. and Martins, M.L. (1998). Effect of *Saccharomyces cerevisiae* ATCC97631 on Aflatoxins production, 4th World Congress of Foodborne Infection and Intoxication 4. Book of abstracts. Pp 288.

Manikandan, S., Ganesapandian, S., Singh, M. and Kumaraguru, A.K. (2011). Antimicrobial susceptibility pattern of urinary tract infection causing human pathogenic bacteria. *Asian J. Med. Sci.* **3**:56–60.

Manges, A.R., Tabor, H. Tellis, P., Vincent, C. and Tellier, P.P. (2008). Endemic and epidemic lineages of *Escherichia coli*that cause urinary tract infections. *Emerg. Infect. Dis.* **14**:1575-1583.

Manikandan, S., Ganesapandian, S., Singh, M. and Kumaraguru, A.K. (2011). Emerging of multidrug resistance human pathogens from urinary tract infections. *Curr. Res. Bacteriol.* **4:** 9-15.

Moges, A.F., Genetu, A. and Mengistu, G. (2002). Antibiotic sensitivities of common bacterial pathogens in urinary tract infections at Gondar Hospital, Ethiopia. *East Afr. Med. J.* **79**:140-142.

Messens, W., Grijspeerdt, K. and Herman, L. (2006). Eggshell penetration of hen's eggs by *Salmonella enteric* serovar *enteritidis* upon various storage conditions. *Br. Poult. Sci.* **47**:554–560.

Ministry of Agriculture, Fisheries and Food (MAFF, UK). (2009). The Egg Quality Guide. Department for Environment Food and Rural Affairs (DEFRA).

Ministry of Agriculture, Fisheries & Food (MAFF) (2000). Eggs and poultry meat frequently asked question.

Mishra, A., Sharda, R., Chhabra, D. and Moghe, M.N. (2002). *Escherichia coli* isolated from domestic poultry farm. *Indian J. Animol. Sci***. 72**:727–729.

Moffatt, C.R., Musto, J., Pingault, N., Miller, M., Stafford, R., Gregory, J., Polkinghorne, B.G. and Kirk, M.D. (2016). *Salmonella typhimurium* and outbreaks of egg-associated disease in Australia, 2001 to 2011. *Foodborne Pathog. Dis.* **13**:379-385.

Musgrove, M.T., Northcutt, J.K., Jones, D.R., Cox, N.A, and Harrison, M.A. (2008). Enterobacteriaceae and related organisms isolated from shell eggs collected during commercial processing. *Poult. Sci.* **87**: 1211-1218.

Murchie, I., Bin, X., Robert, H., Madden, A., Paul, W. and Louise, K. (2008). Qualitative exposure assessment for *Salmonella* spp. In shell eggs produced on island of Ireland. *Int. J. Food Microbiol.* **125**(3):308-319.

Musgrove, M.T., Jones, D.R., Northcutt, J.K., Cox, N.A. and Harrison, M.A. (2004). Identification of *Enterobacteriaceae* from washed and unwashed commercial shell eggs. *J. Food Protect.* **67**: 2613-2616.

Nagappa, K., Shantanu, T., Brajmadhuri, Saxena, M.K. and Singh, S.P. (2007). Isolated of *Salmonella* Typhimurium from poultry eggs and meat of Tarai region of Uttaranchal. *Indian J. Biotech.* **123**:407-9.

Neamatallah, A.A., El-leboudy A., Amer, A.A. and El-shenawwy, N.M. (2009). Biosafety against fungal contamination of hen's eggs and mycotoxins producing species. *JKAU: Met., Env. & Arid. Land. Agric. Sci.* **20**(2):63-73.

Omobowale, O., Ephraim, N., John, P., Dayo, P. and Edward, K. (2009). Trends and drivers of agricultural productivity in Nigeria. International food policy research institute. Washington, DC 20006-1002 USA, series number 1. Pp 1-34.

Otto, M. (2009). *Staphylococcus epidermidis*-the accidental pathogen. *Nat. Rev. Microbiol.* **7**: 555-567.

Owen, M., Jorgensen, F., Willis, C., McLauchlin, J., Elviss, N., Aird, H., Fox, A., Kaye, M., Lane, C. and de Pinna, E. (2016). The occurrence of *Salmonella spp*. in duck eggs on sale at retail or from catering in England. *Lett. Appl. Microbiol.* **63**:335–339.

Pan, T.M. and Liang, Y.H. (2002). Identification of *Salmonella* Enteritidis isolates by polymerase chain reaction and multiplex polymerase chain reaction. *Microb. Immunol. Infec.* **35**:147- 51.

Papadopoulou, C., Dimitriou, D., Levidiotou, S., Gessouli, H., Panagiou, A., Golegou, S., Golegou, S. and Antoniades, G. (1997). Bacterial strains isolated from eggs and their resistance to currently used antibiotics: is there a health hazard for consumers? *Comp. Immunol. Microbiol. Infect. Dis.* **20**(1):35-40.

Peter, B. (2011). Majority of eggs worldwide produced by 15 countries. Executive Guide to world poultry trends. 31–35.

Pradhan, A.K., Li Y., Swem, B.L. and Mauromoustaks, A. (2005). Predictive model for the survival, death and growth of *Salmonella typhimurium* in broiler hatchery. *Poult. Sci*. **84**(12):1959-1966.

Przybylska, A. (2000). Foodborne infections and intoxications in Poland. *Przegl. Epidemiol.* **54**:103-114.

Pui, C.F., Wong, W.C., Chai, L.C., Tunung, R. and Jeyaletchumi, P. (2011). *Salmonella*: A foodborne pathogen. *Int. Food Res. J.* **18**: 465-473.

Pyzik, E. and Marek, A. (2013). Plasmid profile analysis and evaluation of antibiotic susceptibility of *Staphylococcus aureus* strains isolated from table chicken eggs. *Pol. J. Vet. Sci.* **16**:307–312.

Rahimi, E., Momtaz, H., Ameri, M., Ghasemian-Safaei, H. and Ali-Kasemi, M. (2010). Prevalence and antimicrobial resistance of *Campylobacter* species isolated from chicken carcasses during processing in Iran. *Poult Sci*. **89**:1015-20.

Reu De, K., Messens, W., Heyndrickx, M., Rodenburg, T. B., Uyttendaele, M. and Herman, L.(2008). Bacterial Contamination Of Table Eggs and The Influence of Housing Systems. *World's Poult. Sci.* **64**:5-19.

Rusul, A., Marek and Rzedzicki, J. (1996). Occurrence of bacteria of the genus *Staphylococcus* spp. in table eggs descended from different sources. *Pol. J. Vet. Sci.* **4**:56-4.

Rahman, M.T., Saha, S. and Islam, M.A. (1999). Prevalence of Salmonella in poultry feed of Mymensingh, Bangladesh. *The Bangladesh Veterinary Journal*. **16**:45-46.

Riley, P.S., Hollis, D.G., Utter, G.B., Weaver, R.E. and Baker, C.N. (1979). Characterization and identification of 95 diphtheroid (group JK) cultures isolated from clinical specimens. *J. Clin. Microbiol.* **9**(3):418-424.

Ricke, S.C., Birkhold, S.G. and Gast, R.K. (2001). Eggs and egg products. In compendium of methods for the microbiological examination of foods, 4th ed. Downes F. P. and Ito K. eds. American Public Health Association ,Washington, D.C. 473–479

Roberts-Witteveen, A.R., Campbell, B.A., Merritt, T.D., Massey, P.D., Shadbolt, C.T. and Durrheim, D.N. (2008). Egg-associated Salmonella outbreak in an aged care facility, New South Wales.Communicable Dise.

Sabarinath, A., Guillaume, V., Guillaume, B., Mathew, V., DeAllie, C. and Sharma, R. N. (2009). Bacterial contamination of commercial chicken eggs in Grenada, West Indies. *West Indian Veterinary Journal* **9**(2): 4-7. 2009

Saitou, N. and Nei, M. (1987). The neighbor-joining method: A new method for reconstructing phylogenetic trees. *Mol. Biol. Evol.* **4**:406-425.

Safaei, G., Jalali, M., Hosseini, A., Narimani, T., Sharifzadeh, A. and Raheim, E. (2011). The prevalence of bacterial contamination of table eggs from retails markets by *Salmonella spp*., *Listeria monocytogenes*, *Campylobacter jejuni* and *Esch. coli* in Shhrekord, Iran. *Jundishapur J. Microbiol*. **4** (4):249- 53.

Saif, Y.M., Calnek, B.W., Barnes, H.J., Beard, C.W. and McDougald, L.R. (2009). Diseases of Poultry.10th edition, Iowa State University Press, Iowa, USA.

Samad, M.A. (2005). Avian salmonelosis.International Poultry Science and Medicine, 1st edn. The Lyric Epic Prokasone, BAU campus, Mymensingh, Bangladesh. p. 504–514.

Samah, E.I.D., Soad, A.N. and Ahmed, M.E. (2015). Multidrug resistant bacterial pathogens in eggs collected from backyard chickens. *Assiut Vet. Med. J.* **61**:87–103.

Scallan, E. (2011). Food borne illness acquired in the United States-major pathogens. *Emerg. Infect. Dis.* **17**:7–15.

Schoeni, J.L. and Doyle, M.P. (1994). Variable colonization of chickens per orally inoculated with *Escherichia coli* O157: H7 and subsequent contamination of eggs. *Appl. Environ. Microbiol.* **60**(8):2958-2962.

Schoeni, J.L., Glass, K.A., McDermott, J.L. and Wong, A.C.L. (1995). Growth and penetration of *Salmonella enteritidis*, *Salmonella heidelberg* and *Salmonella typhimurium* in eggs. *Int. J. Food Microbiol.* **24**(3):385-396.

Schutze, G.E., Faweett, H.A., Lewno, M.J., Flick, E.L. and Kirby, L.S. (1996). Prevalence of *Salmonella enteritidis* in poultry egg shells in Arkansas. *South Med. J*. **89**: 899-891.

Schoenfelder, S.M., Lange, C., Eckart, M., Hennig, S., Kozytska, S. and Ziebuhr, W. (2010). Success through diversity-how *Staphylococcus epidermidis* establishes as a nosocomial pathogen. *Int. J. Med. Microbiol.* **300**:380-386.

Shazad, A., Mahmood, M.S., Hussain, I., Siddique, F., Abbas, R.Z. (2012). Prevalence of *Salmonella* species in hen eggs and egg storng-trays collected from poultry farms and marketing outlets of Faisalabad, Pakistan. *Pak. J. Agri. Sci*. **49**(4):565-68.

Suresh, T., Hatha, A.A.M., Sreenivasan, D., Sangeetha, N. and Lashmana P.S.P. (2006). Prevalence and antimicrobial resistance of *Salmonella enteritidis* and other salmonellas in the eggs and egg storing trays from retails markets of Coimbatore, South India. *Food Microbiol.* **23**:294-299.

Sim, J.S., Sunwoo, H.H. and Lee, E.N. (2000). Ovoglobulin IgY. [In:] Natural FoodAntimicrobial System. Naidu A.A. (ed.), CRC Press, New York, 227–252.

Singh, S., Yadav, A.S. and Singh, S.M. (2010). Prevalence of Salmonella in chicken eggs collected from poultry farms and marketing channels and their antimicrobial resistance. *Food Res. Int.* **43**(8):2027-2030.

Smith, A., Rose, S.P., Wells, R.G. and Pirgozliev, V. (2000). The effect of changing the excreta moisture of caged laying hens on the excreta and the microbial contamination of their egg shells. *Br. Poult. Sci.* **41**(2):168-173.

Stępień-Pyśniak, D., Marek, A.R. and Rzedzicki, J. (2009). Occurrence of bacteria of the genus Staphylococcusin table eggs descended from different sources. *Pol. J. Vet. Sci.* **12**:481-484.

Stępień-Pyśniak, D. (2010). Occurrence of Gram-negative bacteria in hen's eggs depending on their source and storage conditions. *Pol. J. Vet. Sci*. **13**(3):507-513.

Stephens, N., Coleman, D. and Shaw, K. (2008). Recurring outbreaks of *Salmonella typhimurium* phage type 135 associated with the consumption of products containing raw egg in Tasmania. *Commun. Dis. Intell. Q. Rep.* **32**:466–468.

Suresh, T., Hatha, A.A.M., Sreenivasan, D., Sangeetha, N. and Lashmanaperumalsamy, P. (2006). Prevalence and antimicrobial resistance of *Salmonella* enteritidis and other salmonellas in the eggs and egg-storing trays from retails markets of Coimbatore, South India. *Food Microbiol.* **23**:294-299.

Tamura, K., Stecher, G., Peterson,D., Filipski, A. and Kumar, S. (2013). MEGA6: Molecular evolutionary genetics analysis version 6.0. *Mol. Biol. Evol*. **30**:2725-2729.

Thampapillai, G., Lan, R. and Reeves, P.R. (1994). Molecular Evolution in the gnd Locus of *Salmonella enteric*. *Mol. Biol., Evol.* **11**(6):813-828.

Theron, H, Enter, P.V. and Lues, J.F.R. (2003). Bacterial growth on chicken eggs in various storage environments. *Food Res. Int.* **36**(9):969-975.

Thomas, A.R., Bruce, A.D., Stacy, A., Genagon, N.M., Warholic, U.M., Patrick, D., Pawlicki, J.M., Beannan, R.O., Burce, A.H. and Paul, R.K. (2005). *Escherichia coli* virulence factor hemolycin induces neutrophil apoptosis and necrosis/lysis in vitro and necrosis/lysis and lung injury in a rat pneumonia model. *Am. J. Physiol.* **289**:207–261.

Terzolo, H.R. and Shimizu, A. (1979). Biological characters and bacteriophage typing of *Staphylococcus aureus* isolated from chicken *staphylococcosis* and commercial balanced chicken food in Argentine. *Rev. Argent. Microbiol*. **11**:89-101.

United State Department of Agriculture, Food Safety & Inspection Services (USDA) (2011). Shell eggs from farm to table.

Van, I.l.F,. De Buck, J., Boyen, F., Pasmans, F., Bertrand, S. and Collard, J.M. (2005). *Salmonella* dans la viande de volaille et dans les oeufs, un danger pour le consommateur qui demande la mise en place d′un programme de lutte efficace. *Ann. Méd. Vét.* **149**(1):34-48.

Walsh C (2000). Molecular Mechanisms that Confer Antibacterial Drug Resistance. *Nature*, **406**:775-781.

World Health Organization (WHO) (2007). *Salmonella* epidemiology.

Woo, P.C.Y., Lau, S.K.P, Teng, J.L.L Tse, H. and Yuen, K.Y. (2008). Then and now: Use of 16S rDNA gene sequencing for bacterial identification and discovery of novel bacteria in clinical microbiology laboratories. *Clin. Microbiol. Infect.* **14**: 908-934.

Yurdakul, N.E., Erginkaya, Z. and Unal, E. (2013). Antibiotic resistance of *Enterococci*, coagulase negative *Staphylococci* and *Staphylococcus aureus* isolated from chicken meat. *Czech. J. Food Sci.* **31**:14–19.