International Journal of Cell & Molecular Biology (IJCMB)
Accepted on 11 November, 2010, Vol. 1 (3), pp 270-277, 1st December, 2010
Available online at http://www.ijcmb.yolasite.com/archive.php
ISSN (2220-7589) 2010

Research article

SCREENING OF PATHOGENIC MICROORGANISMS IN EGGS AND TAP WATER SAMPLES COLLECTED FROM DIFFERENT LOCALITIES OF LAHORE

Kausar Malik*, Syeda Shaista Iram and Khansa

Lahore College for Women University Lahore, Jail Road, Lahore, Pakistan.

Contamination of food and water is very common in Pakistan due to unhygienic conditions. Present work was based on the study of bacterial contamination (human pathogenic *E. coli)* of eggs and tap water. Egg and food prepared by the eggs especially raw eggs are important vehicles for *Salmonella* infection. Eggs contents may be contaminated with *Salmonella* by 2 routes transovarion or trans-shell. Drinking water contamination occurred due to mixing of sewage water. In Pakistan, older cities with aging infrastructure may have leaky sewage collection system which can cause sanitary sewage overflows. This sewage water mixed with drinking water and can cause dangerous diseases. Samples of eggs and water collected from different localities of Lahore and were tested for the presence of human pathogenic bacteria. Developed detection methods can detect more than 10,000 pathogenic *E. coli* in food and water samples while ten pathogenic *E. coli* are enough to cause human infections like diarrhea and other complicated diseases. Microbial screening of all the samples were done. Bacteria grew on the Trypticase soy broth media and MacConkey agar media after incubation period of one day. Novobiocin containing media was used for the detection of pathogenic *E. coli* in eggs and water samples. Growth were observed on petriplates. Gram staining of these bacteria confirmed the presence of pathogenic *E. coli*. Gram negative bacteria were observed under electron microscope. The present study will be helpful for creation of knowledge about these pathogenic bacteria and diseases they caused among people. So the hygienic conditions should be prevailed in order to prevent contamination of pathogenic bacteria. Clean water and pasteurized eggs should be used.

Key words: Pathogens, Microorganisms, Eggs, Tap water, *Salmonella, E. coli,* Gram staining.

Introduction

Eggs are important part of our diet, it is a rich source of protein and tap water is important source of drinking water in our daily life. But they have large amount of microbial organisms in them. As the food contamination is vary common in Pakistan so we worked on the human pathogens of eggs and water. Eggs and its products that are improperly handled can be a source of foodborne diseases, such as salmonellosis. *Salmonella* serotypes are readily found in poultry house environments (Humphrey et al., 2003). *Salmonella* is a genus of rod-shaped, Gram-negative, non-spore forming, predominantly motile enterobacteria with diameters around 0.7 to 1.5 µm, lengths from 2 to 5 µm, and flagella which project in all directions (Ryan and Ray, 2004). *Salmonella* are closely related to the *Escherichia* genus and are found worldwide in warm- and cold-blooded animals, in humans, and in nonliving habitats. They cause illnesses in humans and many animals, such as typhoid fever, paratyphoid fever, and the foodborne illness salmonellosis (Braden, 2006; CDC, 2001).

**Correspondence author Email: kausarbasit7576@yahoo.com*

Salmonella is actually a general name for a group of more than 2000 closely related bacteria that cause illness by reproducing in the digestive tract. Each Salmonella serotype shares common antigens and has its own name. *Salmonella* are spread from animal reservoirs to humans, often through foods such as eggs, meat and milk (Adak et al., 2002). Although *Salmonella* species do not generally constitute a major problem to poultry health. The propensity for *Salmonella* to persist in hostile environments, growing within a wide temperature range between 2ºC to 54ºC, though optimal growth occurs at 37ºC, is a public health concern (Gast and Beard, 1990). Once birds are exposed to the bacteria, they will shed the organisms thus contaminating other birds and the environment. Most chickens will be colonized by the second or third week of age at which point intestinal colonization is at its peak. *Salmonella* species can attach to, penetrate and invade the intestinal mucosa resulting in diarrhea from direct mucosal damage or by the action of bacterial toxins. Poultry is widely acknowledged to be a reservoir for Salmonella. Egg contents may be contaminated with salmonellae by 2 routes: trans-shell (horizontal transmission) ortransovarian (vertical transmission) The infectious dose, incubation period, symptoms and mode of transmission of salmonellosis caused by different serotypes are similar. Symptoms include diarrhoea, fever and abdominal cramps with incubation period ranges from 12 to 72 hours. The illness usually last 4 to 7 days and most people recover without treatment (Gast and Holt, 2001).

Water is generally referred to as polluted when it is impaired by anthropogenic contaminants and either does not support a human use, like serving as drinking water, and undergoes a marked shift in its ability to support its constituent biotic communities, such as fish (Brock, 1998). Waterborne microorganisms pose increasingly greater threats to public health, due to changing patterns in water use, increased water pollution, the nation's aging water treatment systems, and outmoded risk assessment protocols. Action should be taken to address microbial water quality issues through a coordinated research and policymaking effort by all agencies and institutions involved (Hurst et al., 1997b). Water contaminated with pathogenic microorganisms results not only in human suffering but also in significant economic losses (Lindsay, 1997). Coliform bacteria are a commonly-used bacterial indicator of water pollution, although not an actual cause of disease. Other microorganisms sometimes found in surface waters which have caused human health problems include: *Cryptosporidium parvum*, *Giardia lamblia*, *Salmonella*, *Novovirus* and other viruses, Parasitic worms (helminths) (Schueler and Thomas, 2000). The ability to establish the microbiological safety of water is limited by the fact that hundreds of different kinds of microorganisms cause gastroenteritis, consequently, a search for all pathogens possibly present in water supplies is impractical on a routine basis. Further, not all of the waterborne microbial pathogens causing gastroenteritis can be cultured or similarly identified. For this reason, several groups of microorganisms are used as keys, or indicator species, to establish the microbiological safety of waters (Adelman et al., 1998) Total coliforms microorganisms are aerobic, or facultatively anaerobic, gram-negative, nonspore-forming, rod-shaped bacteria able to ferment lactose with gas and acid production in 24 to 48 hr at 35 °C. This group of microorganisms has traditionally been used as an indicator of water pollution from sewage. However, these bacteria can also originate from the intestines of animals (Brenner et al., 1993).

E. coli was discovered by German pediatrician and bacteriologist Theodor Escherich in 1885, as *Bacterium coli commune*, which he isolated from the feces of newborns. The harmless strains are part of the normal flora of the gut, and can benefit their hosts by producing vitamin K_2 or by preventing the establishment of pathogenic bacteria within the intestine. A number of genera within the family are human intestinal pathogens (e.g. *Salmonella*, *Shigella*, *Yersinia*). *E. coli* is Gram-negative, facultative anaerobic and non-sporulating. Cells are typically rod-shaped and are about 2 micrometres (μm) long and 0.5 μm in diameter, with a cell volume of 0.6 - 0.7 μm^3) (Feng et al., 2002)

E. coli are not always confined to the intestine, and their ability to survive for brief periods outside the body makes them an ideal indicator organism to test environmental samples for fecal contamination (Fotadar et al., 2005; Ingledew and Poole, 1994). The regular presence of *E. coli* in the human intestine and feces has led to tracking the bacterium in nature as an indicator of fecal pollution and water contamination. As such, it is taken to mean that, wherever *E. coli* is found, there may be fecal contamination by intestinal parasites of humans (Bennett et al., 1997). *E. coli* can adhere to the mucus overlying the large intestine. Once established, an *E. coli* strain may persist for months or years. Resident strains shift over a long period (weeks to months), and more rapidly after enteric infection or antimicrobial chemotherapy that perturbs the normal flora. (Hudault et al., 2001).

Pathogens in the urban environment easily enter waters through a number of pathways, including discharge of inadequately treated sewage, stormwater runoff, combined sewer overflows and sanitary sewer overflows. Food poisoning caused by *E. coli* is usually associated with taking fecal contaminated water and eating unwashed vegetables and meat contaminated post-slaughter. O157:H7 is responsible for causing serious and even life-threatening complications like hemolytic-uremic syndrome (HUS). Symptoms usually appear within 2 to 4 days, but can take up to 8 days. Most people recover without antibiotics or other specific treatment in 5-10 days. There is no evidence that antibiotics improve the course of disease, and it is thought that treatment with some antibiotics may precipitate kidney complications. (Todar, 2007).

Present study is based on the detection of human pathogenic microorganisms in egg and tap water. This study will be helpful for the identification of pathogens who are causing the dangerous diseases by consuming contaminated egg and water. It will be helpful for the eradication of these diseases by guiding the people about these pathogens and the ways to prevent themselves from these pathogens.

Materials and Methods

A) Collection of samples

Egg samples were collected from the utility stores. Fresh egg, rotten egg and boiled eggs for 1 and 5 minutes were used in the study. The tap water samples were collected from different localities of Lahore.

B) Preparation of Medias:

Preparation of Trypticase soy broth media

7.5 g of tryptic soy broth and 0.75 g of yeast extract were dissolved in 200ml of distilled water with the help of stirrer. pH was adjusted up to 7-7.2 by adding NaOH [diluted solution] drop wise in the media. Added 3.75g agar in it. The final volume were made up to 250ml in the flask The media were autoclaved for 2-3 hours. All forms of bacteria either in cyst or in vegetative form were killed by heating it at high temperature and pressure in autoclave. Liquid media prepared by similar ways, the only difference between two is of agar solid contains agar but the liquid does not.

Preparation of MacConkey agar media with sorbitol:

Suspended 25.8g of the powder in 500ml of purified water. Mixed thoroughly. Heated with frequent agitation and boiled for 1 minute to completely dissolved the powder. The pH was maintained at 7.1.The media was autoclaved for 2-3 hours.liquid SMAC was obtained. The sterilized petriplates were poured and the plates were clear and red.

C) Microbial screening of samples

Pouring of plates

After autoclaving of the medias and other apparatus next step was the pouring of media in patriplates. After pouring in laminar flow placed the petriplates in incubator for one day at 37°C for solidification.

Innocultion on solid media and Inoculation of liquid media:

Samples of eggs and tap water were streaked on the plate with the help of inoculating loop. The plates were left in incubator for 24 hours or even more for bacterial growth temperature of incubator were adjusted at 37°C.Five ml of liquid broth were pipette out in each test tube. Sample cultures which were grown in solid media were introduced in the test tube with inoculating loop. The test tubes were left in shaker for overnight to obtain the bacterial growth.

D) Detection of pathogenic E.coli:

For the detection of pathogenic *E.coli* novobiocin containing media was used as it is specific for the growth of pathogenic becteria like *Escherichia coli.*

Prepration of novobiocin containing media:

About 3.3 g of novobiocin containing media was dissolved in 100ml of water in a beaker. Mixed it on magnetic stirrer. It was autoclaved for 2-3 hours. One ml of autoclaved media was poured in each eppendroff.

Inoculation of novobiocin media and streaking of pathogenic becteria on agar plate;

Poured in eppendroffs 1000µl of novobiocin media, 10µl of culture of samples (eggs and water) added in it. Single loop from liquid broth (having bacterial colony) were introduced in novobiocin. Bacterial colony grown on agar plate might be taken with the help of inoculating loop for the same purpose. Eppandroffs: containing novobiocin media shaked for overnight. The novobiocin media become turbid showing that the growth has been occurred. Poured plates containing solid agar media were streaked by the bacterial culture grown in novobiocin media. These plates were then incubated for one day at 37ºC.

E) Gram Staining of pathogenic bacteria

Staining of all the bacteria was done which was obtained at the plates for the detection of Gram negative pathogenic bacteria. Gram staining (or Gram's method) is an empirical method of differentiating bacterial species into two large groups (Gram-positive and Gram-negative) based on the chemical and physical properties of their cell walls. The Gram staining of all the samples were done and observed under microscope. The pathogenic bacillus rod shaped *E.coli* were confirmed.

Results

Collection of samples

Samples of eggs include, rotten eggs, fresh eggs, eggs boiled for 1minute and eggs boiled for 5 minutes were collected from the Johar town market. Total 12 samples were collected. Samples of tap water were collected from the different localities of Lahore these are Johar town, Town ship, Model town, Muslim town, Iqbal town, Anarkali, Rehmaan pora, Ichra bazaar, Shadman, Green town. Total 50 samples of tap water were collected 5,5 from each locality. Eggs and water samples were collected for the detection of human pathogenic microorganisms. No dilution was used. Samples were passed through the process of microbial screening and then through the detection of pathogenic microorganisms.

Microbial screening of samples

Eggs and water samples were then microbially screened on different media. Different colonies were obtain on liquid broth, solid agar media, MacConkey agar with sorbitol media and novobiocin containing media. Medias were prepared.

Growth on Trypticase soy broth media

Growth on solid agar media

Measured 7.5g of Tryptic soy broth, 0.75g of yeast extract, 3.75g of agar. Dissolved tryptic soya broth and yeast extract in 200ml of distilled water, stirrer it on a magnetic stirrer until it dissolved, pH was adjusted by adding NaOH (dilute) solution. Then poured it in flask added agar in it, covered it with aluminium foil and autoclaved it for 3 hours. Poured media in petriplates and incubated it for one day. Streaking of all egg and water samples was done by sterilized red hot loop, than incubated at 37°C for overnight. On next day growth of microbes was observed on plates. Growth on all plates was observed.

Growth on liquid agar media

Liquid media was also prepared by dissolving 7.5g of Tryptic soya broth, 0.75g of yeast extract in 200ml of distilled water, stirrer it on a magnetic stirrer until it dissolved, pH was adjusted by adding NaOH solution, poured it in flask, covered it with aluminium foil and autoclaved it for 3 hours. Poured liquid samples 5, 5ml in test tubes and add 1ml of sample in them. Growth was observed on them after incubation of 24 hours.

Growth in MacConkey agar with sorbitol

MacConkey Sorbitol Agar and MacConkey II Agar with Sorbitol are selective and differential media for the detection of sorbitol-nonfermenting *Escherichia coli*. These media are also referred to as "Sorbitol MacConkey Agar." *E. coli* is a "mixed acid fermenter of sugars." That means that *E. coli* will convert sugars to a wide variety of organic acids in the absence of oxygen (anaerobic growth). Those acids are acetic, formic, succinic, etc., etc. Many other Gram(-) bacteria are mixed acid fermenters but they do now make formic acid (HCOOH), which is a rather strong organic acid. So Dr. MacConkey, in the early 1900's, concocted his medium so that it contained the sugar lactose, which few other Gram(-) bacteria can use, a pH indicator that turned red under acidic conditions, a lot of phosphate buffer that had to be overcome by all the "mixed acids" including the formic acid in order for the pH to drop into the "red" zone of the indicator, and a lot of nitrogenous material (amino acids, for example) that could sustain the growth of non-*E. coli*, if they happened to be present. But those non-*E. coli* forms would ferment the amino acids into cell mass and NH_3, which would make the pH go basic rather than acidic. Thus the color would not be red. One final thing the inventor thought of was that the dye, when acidic must be insoluble so that it stays right there wherever the bacteria happen to be growing.

Suspended 25.8g of the powder in 500ml of purified water. Mixed thoroughly. Heated with frequent agitation and boiled for 1 minute to completely dissolved the powder. The pH was maintained at 7.1. The media was autoclaved for 2-3 hours. The sterilized petriplates were poured and the plates were clear and red. The plates were inoculated with spreader containing samples (eggs and water). After 24 hours colorless and pink color colonies were observed on it.

Detection of human pathogenic microorganisms

Novobiocin containing media was used for the detection of human pathogenic microorganisms in eggs and water. Novobiocin suppresses the growth of the Gram-positive microbial flora and promote the growth of Gram-negative bacteria.

Media was prepared by dissolving about 3.3 g of novobiocin containing media in 100ml of distilled water in a beaker, it was autoclaved for 3 hours. Poured in eppendroffs 1000µl of novobiocin media, 10µl of culture of samples (eggs and water) added in it. Shaked the eppendrofs in rotary shaker at 37°C temperature and 150 rpm (revolution per minutes) for overnight. Check the growth of pathogenic microorganisms in eppendroffs next day, eppendroffs with growth were turbid. Streaking of these pathogens were done by red hot loop, and incubated it for one day. On next day growth was observed on plates.

Table: 1 Microbial screening of tap water samples on TSB solid media, TSB liquid media and MacConkey agar with sorbitol media collected from different localities of Lahore. (+ve sign= Presence of growth, -ve sign= Absence of growth

Sr. No.	Samples of Tap water	Bacterial growth on solid TSB media	Bacterial growth on liquid TSB medis	Bacterial growth on MacConkey agar with sorbitol media
1.	Johar Town-1	+ve	+ve	+ve
2.	Johar Town -2	+ve	+ve	+ve
3.	Johar Town -3	+ve	+ve	+ve
4.	Johar Town -4	+ve	+ve	+ve
5.	Johar Town -5	+ve	+ve	+ve
6.	Model town -1	+ve	+ve	+ve
7.	Model town -2	+ve	+ve	+ve
8.	Model town -3	+ve	+ve	+ve
9.	Model town -4	+ve	+ve	+ve
10.	Model town -5	+ve	+ve	+ve
11.	Iqbal town-1	+ve	+ve	+ve
12.	Iqbal town-2	+ve	+ve	+ve
13.	Iqbal town-3	+ve	+ve	+ve
14.	Iqbal town-4	+ve	+ve	+ve
15.	Iqbal town-5	+ve	+ve	+ve
16.	Muslim town-1	+ve	+ve	+ve
17.	Muslim town-2	+ve	+ve	+ve
18.	Muslim town-3	+ve	+ve	+ve
19.	Muslim town-4	+ve	+ve	+ve
20.	Muslim town-5	+ve	+ve	+ve
21.	Green town-1	+ve	+ve	+ve
22.	Green town-2	+ve	+ve	+ve
23.	Green town-3	-ve	-ve	-ve
24.	Green town-4	-ve	-ve	-ve
25.	Green town-5	-ve	-ve	-ve
26.	Anarkali-1	+ve	+ve	+ve
27.	Anarkali-2	+ve	+ve	+ve
28.	Anarkali-3	+ve	+ve	+ve
29.	Anarkali-4	+ve	+ve	+ve
30.	Anarkali-5	+ve	+ve	+ve
31.	Ichra bazaar-1	+ve	+ve	+ve
32.	Ichra bazaar-2	+ve	+ve	+ve
33.	Ichra bazaar-3	+ve	+ve	+ve
34.	Ichra bazaar-4	+ve	+ve	+ve
35.	Ichra bazaar-5	+ve	+ve	+ve
36.	Shadman-1	+ve	+ve	+ve
37.	Shadman-2	+ve	+ve	+ve
38.	Shadman-3	+ve	+ve	+ve
39.	Shadman-4	-ve	-ve	-ve
40.	Shadman-5	-ve	-ve	-ve
41.	Town ship-1	+ve	+ve	+ve
42.	Town ship-2	+ve	+ve	+ve
43.	Town ship-3	+ve	+ve	+ve
44.	Town ship-4	+ve	+ve	+ve
45.	Town ship-5	+ve	+ve	+ve
46.	Rehmaan pora-1	+ve	+ve	+ve
47.	Rehmaan pora-2	+ve	+ve	+ve
48.	Rehmaan pora-3	-ve	-ve	-ve
49.	Rehmaan pora-4	-ve	-ve	-ve
50.	Rehmaan pora-5	-ve	-ve	-ve

Table: 2 Microbial screening of eggs samples on TSB solid media, TSB liquid media and MacConkey agar with sorbitol media collected from different localities of Lahore.

Sr No.	Samples of eggs	Bacterial growth on solid TSB media	Bacterial growth on liquid TSB medis	Bacterial growth on MacConkey agar with sorbitol media
1.	Rotten egg-1	+ve	+ve	+ve
2.	Rotten egg-2	+ve	+ve	+ve
3.	Rotten egg-3	+ve	+ve	+ve
4.	Fresh egg-1	+ve	+ve	+ve
5.	Fresh egg-2	+ve	+ve	+ve
6.	Fresh egg-3	+ve	+ve	+ve
7.	Boiled egg for 1 minute-1	+ve	+ve	+ve
8.	Boiled egg for 1 minute-2	+ve	+ve	+ve
9.	Boiled egg for 1 minute-3	-ve	-ve	-ve
10.	Boiled egg for 5 minutes-1	+ve	+ve	+ve
11.	Boiled egg for 5 minutes-2	+ve	+ve	+ve
12.	Boiled egg for 5 minutes-3	-ve	-ve	-ve

+ve sign= Presence of growth, -ve sign= Absence of growth

Table:3 Detection of pathogenic microorganisms in tap water samples collected from different localities of Lahore, by growth on Novobiocin containing media and by Gram staining.

Samples	Growth on novobiocin media	Gram staining	Identified as
Johar town-1	+ve	-ve, Pink bacteria	Pathogenic
Johar town-2	+ve	-ve, Pink bacteria	Pathogenic
Johar town-3	+ve	-ve, Pink bacteria	Pathogenic
Johar town-4	+ve	-ve, Pink bacteria	Pathogenic
Johar town-5	+ve	ve, Pink bacteria	Pathogenic
Model town-1	+ve	-ve, Pink bacteria	Pathogenic
Model town-2	+ve	-ve, Pink bacteria	Pathogenic
Model town-3	+ve	-ve, Pink bacteria	Pathogenic
Model town-4	+ve	-ve, Pink bacteria	Pathogenic
Model town-5	+ve	-ve, Pink bacteria	Pathogenic
Iqbal town-1	+ve	-ve, Pink bacteria	Pathogenic
Iqbal town-2	+ve	-ve, Pink bacteria	Pathogenic
Iqbal town-3	+ve	-ve, Pink bacteria	Pathogenic
Iqbal town-4	+ve	-ve, Pink bacteria	Pathogenic
Iqbal town-5	+ve	-ve, Pink bacteria	Pathogenic
Green Town-1	+ve	-ve, Pink bacteria	Pathogenic
Green Town-2	+ve	-ve, Pink bacteria	Pathogenic
Anarkali-1	+ve	-ve, Pink bacteria	Pathogenic
Anarkali-2	+ve	-ve, Pink bacteria	Pathogenic
Anarkali-3	+ve	-ve, Pink bacteria	Pathogenic
Anarkali-4	+ve	-ve, Pink bacteria	Pathogenic
Anarkali-5	+ve	-ve, Pink bacteria	Pathogenic
Ichra bazaar-1	+ve	-ve, Pink bacteria	Pathogenic
Ichra bazaar-2	+ve	-ve, Pink bacteria	Pathogenic
Ichra bazaar-3	+ve	-ve, Pink bacteria	Pathogenic
Ichra bazaar-4	+ve	-ve, Pink bacteria	Pathogenic

Ichra bazaar-5	+ve	-ve, Pink bacteria	Pathogenic
Town ship -1	+ve	-ve, Pink bacteria	Pathogenic
Town ship-2	+ve	-ve, Pink bacteria	Pathogenic
Town ship-3	+ve	-ve, Pink bacteria	Pathogenic
Town ship-4	+ve	-ve, Pink bacteria	Pathogenic
Town ship-5	+ve	-ve, Pink bacteria	Pathogenic
Shadman-1	+ve	-ve, Pink bacteria	Pathogenic
Shadman-2	+ve	-ve, Pink bacteria	Pathogenic
Shadman-3	+ve	-ve, Pink bacteria	Pathogenic
Rehmaanpora-1	+ve	-ve, Pink bacteria	Pathogenic
Rehmaanpora-2	+ve	-ve, Pink bacteria	Pathogenic

Table:4 Detection of pathogenic microorganisms in eggs samples collected from different localities of Lahore, by growth on Novobiocin containing media and by Gram staining.

Samples	**Growth on novobiocin media**	**Gram staining**	**Identified as**
Rotten eggs 1	+ve	-ve, Pink bacteria	Pathogenic
Rotten eggs 2	+ve	-ve, Pink bacteria	Pathogenic
Rotten eggs 3	+ve	-ve, Pink bacteria	Pathogenic
Fresh eggs-1	+ve	-ve, Pink bacteria	Pathogenic
Fresh eggs-2	+ve	-ve, Pink bacteria	Pathogenic
Fresh eggs-3	+ve	-ve, Pink bacteria	Pathogenic
Boiled eggs for 1 minute -1	+ve	-ve, Pink bacteria	Pathogenic
Boiled eggs for 1 minute-2	+ve	-ve, Pink bacteria	Pathogenic
Boiled eggs for 5 minutes-1	+ve	-ve, Pink bacteria	Pathogenic
Boiled eggs for 5 minutes-2	+ve	-ve, Pink bacteria	Pathogenic

Discussion

The main objective of the study was the screening of pathogenic microorganisms in eggs and tap water, and to examine the pathogenic *E.coli* in eggs and water samples in our city. The main aim of the study was to create the awareness among people about the pathogenic bacteria and the diseases they cause. In the present research it was noted that out of 62 samples of water and eggs 42 showed the presence of pathogenic *E.coli.* Samples of eggs and water were inoculated on the TSB. It was placed in incubator for 24 hours. Solid, agar-based media can be used to identify colonial characteristics (shape, size, elevation, margin type), but can also serve to select for particular bacterial groups and differentiate between two or more different species. *Salmonella* are Gram negative rod like formed shiny convex colonies with entire margin. *Escherichia coli* are also rod like Gram negative formed shiny mucoid colonies with entire margin and are slightly raised older colonies often have a darker center. In MacConkey agar with sorbitol *Salmonella* formed circular, smooth, translucent and colourless colonies due to absence of sorbitol fermentation while *E.coli* colonies were circular and flat pink due to sorbitol fermentation with central umbonation. The result were clear on the petriplates. After that grown colonies were inoculated in liquid broth, by placing it in a shaker for overnight at 37ºC. Turbidity confirmed the bacterial growth. For the growth of pathogenic *E.coli.* Novobiocin containing media was streaked with TSB liquid broth On the next day, the growth of pathogenic bacteria were observed. The bacterial colonies were mucoid colonies, which have entire margins and were slightly rose. In Novobiocin the grown colonies of pathogenic *E.coli* were off-white. Novobiocin suppress the growth of Gram-positive bacteria and promotes the growth of Gram- negative bacteria. So the purpose of the study was to established a screening procedure for the detection of pathogenic *E.coli* from eggs and water samples to investigate its prevalence in Pakistan.

Gram staining of pathogenic bacteria confirmed the presence of pathogenic *E.coli*. After all the procedure the smear was of pink color. Gram negative, rod shaped

bacteria were observed under electron microscope in a single or cluster arrangement. These eggs and water samples showed the large amount of pathogenic *E.coli* which are the cause of many human and animals diseases. Pathogenic *E.coli* is vary dangerous bacteria which spreading large amount of gastrointestinal diseases among humans and large number of deaths occurring due to theses diseases. Large number of childrens are suffering from the diarrheal disease in our city due to drinking of contaminated water.

Conclusion

The present study based on screening of pathogenic bacteria from eggs and water samples. Pathogenic *Escherichia coli* were present in 43 tap water and 10 eggs samples. The procedure used for the isolation of bacterial colonies was very effective. The present project will give indications for the most effective sampling plan for determination of eggs and tap water contamination with pathogenic *Escherichia coli.* The samples of eggs and water were collected from different localities of Lahore, shows that due to improperly handling and storage of eggs and mixing of sewage water in drinking water due to leaky sewage pipe lines as a result of poor infrastructure, leads to growth of pathogenic bacteria in them. The consumption of these contaminated eggs and water can cause dangerous disease in human like typhoid fever and diarrhea. The primary information provided by the study will form the basis for the further development of a decent combative programme at grass route level and helping the people in living a life in a healthy manner.

References

Adak, GK., Long, SM. and O'Brien, SJ. (2002). Trends in indigenous foodborne disease and deaths: England and Wales 1992 to 2000. *Gu.t* 51: 832-841.

Adelman, DD., Stansbury, J. and Tabidian, MA. (1998). "A risk/cost analysis to manage viral contamination of groundwater," Water Research. 38:1-6

Bennett, JV., Holmberg, SD., Rogers, MF. and Solomon, SL. 1997. Infectious and parasitic diseases. Am. J. Preventive Med. 3: 102–114

Braden, CR. (2006). *Salmonella enterica* serotype Enteritidis and eggs: a national epidemic in the United States. Clin Infect Dis. 43:512–517

Brenner, KP., Rankin, YR., Roybal, YR., Stelma, G.N., Scarpino, PV. and Dufour, AP. (1993). "New medium for the simultaneous detection of total coliforms and Escherichia coli in water," Applied and Environmental Microbiology. 59: 3534-3544.

Brock, TD. (1998). "Coliform bacteria in streambed sediments in a subtropical rainforest conservation reserve," Water Research. 32:1852-1856

CDC (Centers for Disease Control and Prevention). 2001. *Salmonella*. Surveillance: Annual Summary, (2000). Atlanta: US Department of Health and Human Services.

Fotadar, U., Zaveloff, P. and Terracio, L. (2005). "Growth of *Escherichia coli* at elevated temperatures". J. Basic Microbiol. 45(5): 403–4

Gast, RK. and Beard, CW. (1990). Isolation of *Salmonella enteritidis* from internal organs of experimentally infected hens. Avian Dis. 34: 991-993

Gast, RK. and Holt, PS. (2001). Multiplication in egg yolk and survival in egg albumen of *Salmonella enterica* serotype Enteritidis strains of phage types 4, 8, 13a, and 14b. J. Food Prot. 64: 865–868

Hudault, S., Guignot, J. and Servin, AL. (2001). "*Escherichia coli* strains colonising the gastrointestinal tract protect germfree mice against *Salmonella typhimurium* infection". Gut 49(1): 47–55

Humphrey, TJ., Baskerville, A., Mawer, S., Rowe, B.and Hopper, S. (2003). Numbers of *Salmonella* Enteritidis in the contents of naturally contaminated hens' eggs. Epidemiology and Infection, 106: 489-496

Hurst, CJ., Knudsen, GR., McInerney, MJ., Stetzenbach, LD and Walter, MV. 1997b. "Modeling the fate of microorganisms in water, wastewater, and soil,". Manual of Environmental Microbiology. ASM Press, Washington, DC**.** 31: Pp: 213-221

Ingledew, WJ. and Poole, RK. (1994). "The respiratory chains of Escherichia coli". Microbiol. Rev. 48(3): 222–71

Lindsay, JA. 1997. Chronic sequelae of foodborne disease. *Emerg. Infect. Dis.* 3:443–452

Ryan, KJ. and Ray, CG . (2004). Sherris Medical Microbiology . McGraw Hill. 4: 362–8

Schueler, and Thomas, R. (2000). "Microbes and Urban Watersheds: Concentrations, Sources, & Pathways." Reprinted in *The Practice of Watershed Protection*. Center for Watershed Protection. Ellicott City, MD. **(**2nd ed.): 134-190.

Todar, K. (2007). "Pathogenic *E. coli*". Online Textbook of Bacteriology. University of Wisconsin-Madison Department of Bacteriology. Pp: 34-67.