**Detecting *Salmonella* From Egg Shell and Egg Nests**

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**Detecting *Salmonella* From Egg Shell and Egg Nests**

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LIST OF ABBREVIATION

|  |  |
| --- | --- |
| Abbreviation and Symbol | Elaboration |
| % | Percent |
| et al. | And his associate |
| CVASU | Chittagong Veterinary and Animal Sciences University |
| Spp | Species |
| US | United States |
| WHO | World Health Organization |
| Hrs | Hours |
| °C | Degree Celsius |
| CDC | Centers for Disease Control |
| Gms | Grams |
| SS | Salmonella shigella |
| BG | Brilliant Green |
| MS | Microsoft word |

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**The author**

**Chapter 1-** **Abstract**

A study was conducted to determine the prevalence of *Salmonella spp.* on quail eggshell, egg nests in Santhia upazila under Pabna district. A total number of 45 eggs and 25 egg nests were examined over a period of 2 month from two different farms. Salmonella contamination was recorded in 3 of 45 (6.6%) eggshell and 2(8%) in egg nests.. Isolated *Salmonella* was tested for resistance to eight different antimicrobial agents, using disc diffusion method. Among eight antimicrobial tested, 100% resistance were found to Ampicillin and Amoxicillin followed by Erythromycin (60-100%), Tetracycline (72-93%), Ciprofloxacin (22-66%), Colistin (27-66%), Enrofloxacin (42-54%) and Pefloxacin 23.07% across the study sites. Ciprofloxacin remained sensitive in 40.9%, Pefloxacin and Colistin appeared to be (61-72.22%) sensitive against Salmonella isolates at studied areas.

**Keywords:** *Salmonella spp*; Egg; Antimicrobial resistance

**Chapter 2-Introduction**

Eggs and egg products are nutritive food items and a vital constituent of human food in Bangladesh. Quail eggs and meats become popular day by day in Bangladesh. They are rich in protein, phosphorous, selenium, choline, riboflavin and vitamin B12. Moreover, they are also comprises with folic acid, zinc, pantothenic acid and vitamins A, D, E, and K (Anonymous, 2004). The genus *Salmonella* is divided into two species, *Salmonella enterica*, which consists of six subspecies, and *Salmonella bongori,* currently the genus includes a total of 2,500 serotypes (Popoff *et al*., 2004).*Salmonella enterica* subspecies *enterica* (subspecies I) is responsible for 99.5 % of infection in man and animal (Pignato *et al*., 1998).Most of the infections are zoonotic in origin but some serotypes like *Salmonella typhi* and *Salmonella paratyphi* infect only humans (Yan *et al*., 2003). Salmonellosis is a major problem in in Bangladesh and its prevalence ranged from 28-53.3% (Bhattacharjee *et al*., 2001; Rahman *et al*., 2004; Akter *et al*., 2007; Showkat *et al*., 2011). This disease declines e 1: Introduction

Eggs and egg products are nutritive food items and a vital constituent of human food in Bangladesh.quail eggs and meats become popular day by day in Bangladesh. They are rich in protein, phosphorous, selenium, choline, riboflavin and vitamin B12. Moreover, they are also comprises with folic acid, zinc, pantothenic acid and vitamins A, D, E, and K (Anonymous, 2004). The genus *Salmonella* is divided into two species, *Salmonella enterica*, which consists of six subspecies, and *Salmonella bongori,* currently the genus includes a total of 2,500 serotypes (Popoff *et al*., 2004).*Salmonella enterica* subspecies *enterica* (subspecies I) is responsible for 99.5 % of infection in man and animal (Pignato *et al*., 1998).Most of the infections are zoonotic in origin but some serotypes like *Salmonella typhi* and *Salmonella paratyphi* infect only humans (Yan *et al*., 2003). Salmonellosis is a major problem in in Bangladesh and its prevalence ranged from 28-53.3% (Bhattacharjee *et al*., 2001; Rahman *et al*., gg production (Begum *et al*., 1992; Khan *et al*., 1998) and also causes huge mortality in layer birds (Bhattacharjee *et al*., 2001; Kamal *et al*., 1998; Islam *et al*., 2006). *Salmonella* is one of the major bacterial agents that cause foodborne infections in humans worldwide (Herikstad *et al*., 2002). The majority of salmonellosis outbreaks have been attributed to food such as eggs, chicken, beef and fish to human carriers. The outbreaks involving eggs almost all have occurred in the food service sector and have been the result of inadequate refrigeration and insufficient cooking. Since food animals are the reservoir for most domestically acquired human *Salmonella* infections and transmission from animals to human occurs through the food supply (Angulo *et al.*, 2000). Food borne *Salmonella* are estimated to cause approximately 1.4 million illness, 15000 hospitalization and 500 deaths per year in united state (Mead, 1999). Among food-borne bacterial zoonotic diseases Salmonellosis causes huge economic losses in terms of massive mortality and morbidity (Hafez, 2011). Huge ranges of poultry products have been contaminated with *Salmonella* spp. leading to outbreaks of Salmonellosis (Messens *et al*., 2007). Different species of *Salmonella* including *Salmonella* *choleraesuis*, *Salmonella* *enterica*, *Salmonella* *bongori*, *Salmonella* *typhi*, *Salmonella* *paratyphi* and *Salmonella typhimurium* causes GIT and typhoid fever.*Salmonella gallinarum* and *Salmonella pullorum* are responsible for fowl typhoid & Pullorum disease, respectively (Khan *et al*., 2000). *Salmonella typhimurium* and *Salmonella enteritidis* are prevalent both in poultry and human and categorized as zoonotic hazards (Rahman *et al*., 1997; Verma and Gupta, 1999).

*Salmonella* spp. contamination in egg farms and market outlets may arise at any production stage by horizontal or vertical transmission. Vertical transmission means contamination of egg yolk, albumin, membranes or eggshells. While in horizontal transmission disease is penetrated during or after ovipositor through the egg shell from the gut or contaminated feces (Aoust *et al*., 2000). The cause and the method of spread of *Salmonella* spp. between the egg items and the customers should be recognized to influence the illness manage. *Salmonella* spp. Contamination may be prevalent in farm environment. One possible cause of *Salmonella* contamination in developing countries is reusable egg trays (Utrarachkij *et al*., 2012). Outbreaks and sporadic cases of Salmonellosis are frequently associated with the intake of infected hen eggs with *Salmonella* spp. Therefore, awareness of the prevalence of *Salmonella* spp. is essential to commence a control programmed. The disease is endemic in many developing countries particularly the asian subcontinent, south and central america (Miller and pegues, 2000). Infections with bacteria of the genus *Salmonella* are responsible for a variety of acute and chronic disease of poultry has been reported in Bangladesh (Bhattacharjee *et al*., 1996 ; Kamal *et al*., 1998). In recent years problems related to *Salmonella* have increased significantly, both in terms of the incidence and severity of cases of human Salmonellosis. Since the beginning of the 1990s, strains of *Salmonella* which are resistant to a range of antimicrobials including the first choice agents for treatment of humans have emerged and are threatening to become a serious public health problem. Drug resistant *Salmonella* emerge in response to antimicrobial usage in humans and in food animals and selective pressure from the use of antimicrobials is a major driving force behind the emergence of resistance. Multi-drug resistance to critically important antimicrobials is compounding the problem (WHO, 2005). There are reports of high prevalence of resistance in *Salmonella* isolates from countries such as Taiwan (Lauderdale *et al.,* 2006), India (Mandal *et al.,* 2004; Mandal *et al.,* 2006), The Netherlands (Duijkeren *et al.,* 2003), resistant isolates from France (Weill *et al.,* 2006), Canada (Poppe *et al.,* 2006), and Ethiopia (Molla *et al.,* 2003). Similarly, there are various reports of multi-drug resistant *Salmonella* organisms isolated from chickens eggs in Bangladesh (Ahmed *et al*., 2011; Kohinur *et al*., 2010).

The emergence of antimicrobial-resistant *Salmonella* strains is of great concern world wide (Rowe, 2001). Antimicrobial resistance is an increasingly global problem, and emerging antimicrobial resistance has become a public health issue worldwide (Kaye *et al*., 2004). 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 (Bager and Helmuth, 2001; Anderson *et al*., 2003; Schroeder *et al*., 2004). One of the studies indicated a rise in the antibiotic resistance in *Salmonella typhi* (Gautam *et al.,* 2002). In recent years, antibiotic resistance in *Salmonella* has assumed alarming proportions (Murugkar *et al.,* 2005) and the isolates were resistant to at least one of the 15 antibiotics tested. Moreover, antibiotic treatment is considered the most important issue that promotes the emergence, selection and spreading of antibiotic-resistant microorganisms in both veterinary and human medicine (Neu, 1992; Witte, 1998). It was stated by well established evidence that antibiotics can lead to the emergence and dissemination of resistant *Salmonella* spp.which can then be passed into people via food or direct contact with infected poultry. These resistant microbes may function as a potential source in the transportation of antimicrobial resistance to human pathogens (Schroeder *et al*., 2002). Antibiotic used as a prophylactic and growth promoter in many developing countries including Bangladesh to treat and prevent bacterial and protozoal infections and diseases (Witte, 1998; Silva *et al*., 2000; Scharwz *et al*., 2001; Molla *et al*., 2003; Angulo *et al*., 2004; Enabulele *et al*., 2010). In veterinary practice, antibiotics are used in livestock production, disease prevention and as growth-promoting feed additives. Due to indiscriminate use of antimicrobials, they are randomly become resistant against different infectious diseases. The use of antibiotics in animals disrupts normal flora of intestine, resulting in to emergence of antibiotic-resistant *Salmonella* and their prolonged faecal shedding into the environment. Such practice has led to misuse of antibiotics with the associated high prevalence of antibiotic resistance among isolates from animal and food sources (Enabulele *et al*., 2010). The fatality rate in people infected with antibiotic-resistant *Salmonella* is 21 times greater than that infected with non-antibiotic resistant *Salmonella* strains. Several lines of evidence indicate that antimicrobial resistance among human *Salmonella* isolates results from the use of antimicrobials in food producing animals and resistance of antimicrobials against *Salmonella* spp. in poultry (Grob *et al*., 1998; Ahmed *et al*., 2000; Angulo *et al*., 2004; Zhao *et al*., 2007; Showkat *et al*., 2011; Adil *et al*., 2012).

This present study was designated to isolate *Salmonella* and from egg shell surface of quail which are reared in the background rural area of Santhia upazilla in Pabna district. The main objective of this study was to estimate prevalence of *Salmonella* in eggs and nests of quail and to determine antimicrobial resistance pattern of isolated *Salmonella.*

**Chapter 3- Methods & Materials**

**3.1 Sample collection**

**Sampling of Eggshell surfaces**

Experiments were conducted over a two different farms of Santhia in Pabna district. The selected population was those quails which are reared in backyard farming system of these two farms. Seventy swab samples are taken from the freshly laid quail eggshell surface with the help of sterile swabs from different flocks of those two farms and which are suspended in 1ml buffer peptone water in an eppendorf tube. At the mean time a developed questionnaire was filled up for each and every sample regarding with information of housing system, feeding system, rearing system, production state, clinical state, vaccination history etc. Those samples were brought to the laboratory for isolation of *Salmonella*.

**Sampling from nests**

Cotton swab was used to wet in sterilized peptone solution to swab on egg nests and then put the stick immediately into a sterile vial containing buffered peptone water. Individual sample in each vial was given unique identification number then immediately transferred to laboratory, Chittagong Veterinary and Animal Sciences University (CVASU) through ice box. Samples with transport media were stored temporarily in refrigerator before laboratory evaluation.

**3.2 Bacteriological method:**

Isolation of *Salmonella*

The collected sample were than subculture at nutrient broth which are prepared by dissolving 25gms powder in one litter distilled water and dissolve the medium completely by heating then sterilize it by autoclaving at 10 lbs pressure (1150C) or alternatively at 15 lbs pressure (1210C) for 15 minutes or as per validated cycle. After sterilization ph of the medium should be 7.3±0.1. Normally each litter of nutrient broth medium contains 10gms peptone, 10gms beef extract and 5gms sodium chloride. Beef extract and peptone provide the necessary nitrogen compounds, carbon, vitamins and also some trace ingredients to the non-fastidious organism and sodium chloride maintain osmotic equilibrium of the medium. After giving subculture inoculation in nutrient broth, it was incubated at 35-37°C for 24 hours

For the isolation of *Salmonella* at first the subculture inoculums from nutrient broth are streaked in the *Salmonella* *Shigella* agar which is composed of lactose 10gm, bile salts 8.5gm, sodium citrate 8.5gm, sodium thiosulfate 8.5gm, beef extract 5gm, ferric citrate 1gm, brilliant green 0.33gm, neutral red 0.025gm, agar 13.5gm per liter of deionized water. The basis for differentiation on SS Agar depends on the fermentation of lactose and the absorption of neutral red as the bile salts precipitate in the acidic condition. Neutral red turns red in the presence of an acidic pH, thus showing fermentation has occurred. The inclusion of bile salts, sodium citrate, and brilliant green serve to inhibit gram-positive and coliform organisms. *Salmonella*, *Shigell*, and other non-lactose-fermenting organisms appear as transparent or translucent colorless colonies on SS Agar. Sodium thiosulfate is added to the medium as a hydrogen sulfide source, and ferric citrate is added as an indicator for hydrogen sulfide production. If lactose fermentation occurs, the medium will turn red due to the acidic pH. *Salmonella*, *Shigella*, and other non-lactose fermenters appear as transparent or translucent colorless colonies on SS Agar. Colonies of *Salmonella* spp. may appear with or without black centers. The suspected *Salmonella* colony then transferred to nutrient broth medium and incubated at 35-37°C for 24 hours. In the next step inoculums from nutrient broth again streaked in the Brilliant Green Agar (BGA) which is used for selective isolation of *Salmonella* *spp*. The medium also contains yeast extract, enzymatic digest of casein, enzymatic digest of animal tissue, NaCl, lactose, sucrose, brilliant green, phenol red and agar. The enzymatic digests provide sources of nitrogen, amino acids and carbon. The yeast extract supplies vitamins required for growth of bacteria. NaCl maintains the osmotic balance of the medium. Lactose and sucrose are the carbohydrates in the medium. Brilliant green (BG) inhibits gram-positive bacteria and most gram-negative rods other than *Salmonella* spp. The pH is 6.9. Phenol red is the pH indicator, which turns the yellow upon acidification due to fermentation of lactose and/or sucrose. Agar is the solidifying agent. *Salmonella* colonies can vary in color from red to pink-white, depending upon incubation time and strain. The agar around the colonies must be red. Finally the presence of *Salmonella* organisms in sample was confirmed by biochemical test in Triple Sugar Iron media. In *Salmonella* positive case the slants of the media become red, butt become yellow and gas was formed, blackish appearance may indicate the production of hydrogen sulfhide.

2.3Antimicrobial sensitivity test:

Antimicrobial susceptibility testing against *Salmonella* isolates was performed by using antimicrobial disc (Oxoid) according to Kirby- Bauer antimicrobial disc diffusion techniques. Mueller- Hinton agar was prepared in petri-dishes as per the instructions of the manufacturer. Pure colonies of the *Salmonella spp.* isolates were inoculated in nutrient broth and incubated at 37°C for overnight. The isolates were streaked thoroughly on the Mueller Hinton agar by using sterile glass rod (60° cone shaped) and antimicrobial discs (OXOID®) like Ampicillin (30mcg/disc), Amoxicillin (30mcg/disc), Ciprofloxacin (5mcg/disc), Colistin (25mcg/disc), Erythromycin (15mcg/disc), Enrofloxalin (30mcg/disc), Pefloxacin (5mcg/disc) and Tetracycline (30mcg/disc) were chosen and placed centrally using antimicrobial disc dispenser (Oxoid). The petri-dish and its contents were incubated in an incubator at 37°C for 24 hrs. The plates were observed for antimicrobial susceptibility pattern by measuring the zone of inhibition developed against the *Salmonella* isolates on the plate.The isolates were considered as sensitive, intermediately sensitive or resistence.

Data analysis:

Field and Laboratory data were stored and then cleaned in the MS excel-2007 programme before exporting to STATA/IC-11.0 for analysis. Descriptive analysis was performed to know the frequency & distribution of *Salmonella* and antibiotic resistance pattern.

**Chapter 4- Results**

*Salmonella* spp. isolated from egg storing trays, egg shell surface and egg content samples of six (6) wet markets in Chittagong City Corporation, Bangladesh were evaluated for antimicrobial susceptibility to estimate the prevalence and pattern of antimicrobial resistance and sensitivity among *Salmonella* spp. isolates.

**Table1:** Prevalence of *Salmonella* in different samples and sampling sites

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Variable | Categories | Positive(%) | **Chi2- value** | **P-value** |
| Sample | Egg shell surface | 3(6.6%) | **0.0431** | **0.836** |
| Egg nests | 2(8%) |
| Sampling site | Al-amin farm | 3(7.1%) | 0.0000 | 1.000 |
| Ujjal farm | 2(7.1%) |

Table shows the prevalence of *salmonella* spp. in different samples like egg shell surface & egg nests. The highest prevalence was found in egg nests (8%) and lowest (6.6%) in egg shell surface. Among the category of samples the variation in prevalence differs not significantly (p=0.836). On the other hand, prevalence was same in case of sampling site variation.

**Table 2:** Prevalence of antimicrobial resistance pattern of *Salmonella* isolates from egg shell surface

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Antibiotics** | ***Salmonella* positive isolates** | **Pattern** | | |
| **Resistance (%)** | **Intermediate (%)** | **Sensitive (%)** |
| Enrofloxacin | 3 | 47.27 | 43.63 | 20 |
| Amoxicillin | 3 | 98.18 | 1.81 | 0 |
| Colistin | 3 | 50.9 | 0 | 49.1 |
| Erythromycin | 3 | 83.63 | 9.09 | 7.27 |
| Tetracycline | 3 | 94.54 | 1.82 | 3.64 |
| Ampicillin | 3 | 100 | 0 | 0 |
| Pefloxacin | 3 | 9.09 | 40 | 50.9 |
| Ciprofloxacin | 3 | 49.1 | 23.64 | 27.27 |

The results of antimicrobial resistance pattern against *Salmonella* isolated from egg shell surface are given in Table-2. The results revealed that the isolate from eggs shell surface were highest resistance to Ampicillin (100%) followed by Amoxicillin (98.18%), Tetracycline (94.54%), Erythromycin (83.63%) and others (0-50%). Pefloxacin showed highest level of sensitivity (50.9%) followed by Colistin (49.1%) and Ciprofloxacin (27.27%). The increasing rates of resistance to Ampicillin, Amoxicillin, Tetracycline, and Erythromycin among the isolates might attributed to the emergence of multi resistance *Salmonella* spp. Figure-1 shows pattern of resistance of *salmonella* isolates from egg shell surface.

**Figure 1 :** Resistance pattern of *Salmonella* isolates from egg shell surface

**Table 3:** Antimicrobial resistance pattern of *Salmonella* isolates from egg nests

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Antibiotics** | ***Salmonella* positive isolates** | **Pattern** | | |
| **Resistance (%)** | **Intermediate (%)** | **Sensitive (%)** |
| Enrofloxacin | 2 | 60 | 40 | 15 |
| Amoxicillin | 2 | 100 | 0 | 0 |
| Colistin | 2 | 52.5 | 0 | 47.5 |
| Erythromycin | 2 | 90 | 10 | 0 |
| Tetracycline | 2 | 82.5 | 12.5 | 5 |
| Ampicillin | 2 | 97.5 | 2.5 | 0 |
| Pefloxacin | 2 | 5 | 32.5 | 62.5 |
| Ciprofloxacin | 2 | 27.5 | 42.5 | 30 |

The prevalence and pattern of antimicrobial resistance of *Salmonella* isolates from egg nests has been outlined in Table-3

# Figure 2: Resistance pattern of *Salmonella* isolates from egg nests.

Resistance patterns of *Salmonella* were highest in Amoxicillin (100%) followed by Ampicillin (97.5%), Erythromycin (90%), Tetracycline (82.5%), Enrofloxacin (60%), Colistin (52.5%), Ciprofloxacin (27.5%) and Pefloxacin (5%). It was revealed that no isolates were found sensitive to Ampicillin, Amoxicillin and Erythromycin. Pefloxacin showed highest level of sensitivity (62.5%) followed by Colistin (47.5%) and Ciprofloxacin (30%). In current research, all the isolates of *Salmonella* showed multiple antimicrobial resistances.

**Chapter 5- Discussion**

In our study the prevalence of detecting Salmonella were 6.6% on shell surface and 8% on egg nests. E coli was not detected in both shell surface and egg nests. Salmonella is a vertically transmissible disease and E coli generally contaminates egg shell during egg laying. In our study it reflects there was no fecal contamination of E coli on egg shell. It indicates the birds were reared under good hygienic management. However the birds are infected with Salmonella. The present investigation revealed the prevalence of diverse serotypes of Salmonella in commercial layer hen eggs when compared to other types of eggs such as eggs of Japanese quail. Another popular food item among the locals is Japanese Quail eggs, which is cheaper and considered highly nutritious. While none of the quail eggs had Salmonella contamination in their contents, 3 different serotypes such as S.typhimurium, S.worthington and S.bareilly were isolated from the shell surface.( Sander J et al) reported dynamics of Salmonella contamination in commercial quail operations. However, reports from India are not available for comparison.

Overall, five antimicrobial resistance were observed among *Salmonella* isolated from egg, egg shell surface and egg storing trays. *Salmonella* spp. was resistant to five of the eight antimicrobials tested with simultaneous multi-drug resistance to antimicrobials. A similar study in Turkey showed higher multi-drug resistance in *Salmonella* spp. isolates from chicken as compared to human and egg isolates (Icgen *et al*, 2002). *Salmonella* spp. was also found to be comparatively resistant to as many as 5 drugs tested in United States (Berrang *et al.,* 2006). The increasing rates of resistance to Ampicillin, Amoxicillin, Tetracycline and Erythromycin among the isolates might be attributed to the emergence of multi resistance *Salmonella* spp.

Ciprofloxacin is extensively used against *Salmonella* infection in Bangladesh. However, this drug still remained sensitive against *Salmonella* spp. in studied sites. The results of sensitivity for ciprofloxacin in this study are more or less similar with other investigations conducted in different places in Bangladesh (Rahman *et al*., 2004; Islam *et al*., 2006; Akter *et al*., 2007; Showkat *et al*., 2011) and other parts of the world (Dahal *et al*., 2007; Enabulele *et al*., 2010; Adil *et al*., 2012; Eliana *et al*., 2012). This may be due to the reason of fact that Ciprofloxacin is naturally less resistance drug against *Salmonella* spp. (Griggs *et al*., 1994; Tassions *et al*., 1997; Gorman and Adely, 2004; Akter *et al*., 2007; Munawwar *et al*., 2010). Pefloxacin and Colistin appeared to be sensitive against *Salmonella* spp. isolated from egg samples. some variants of *Salmonella* have developed multidrug-resistance as an integral part of the genetic material of the organism, and are therefore likely to retain their drug-resistant genes even when antimicrobial drugs are no longer used, a situation where other resistant strains would typically lose their resistance (WHO, 2005). *Salmonella* isolate from chicken egg and its environment showed resistance to 10 antimicrobials in the US (NARMS, 2004), the isolates of *Salmonella* in this study were found resistant to Ampicillin, Amoxicillin, Erythromycin, and Tetracycline. A higher proportion of antibiotic resistance in *Salmonella enteritidis* has been reported from southern Brazil (Oliveira *et al.,* 2005). Ongoing infection with *Salmonella* organism and use of medication at breeder level could considerably increase the prevalence of multiple resistant *Salmonella* in poultry rearing environment in Bangladesh. Therefore, present study demonstrated that the *Salmonella* organism were present in poultry egg and its environment and showed different antibiotic resistance pattern which may cause serious health problem in our country

**In our study it showed that no E coli was found on eggshell surface of quail. However quails are harboring Salmonella. We did not study whether this salmonella has zoonotic importance or not or it is just only commensal or pathogenic importance. Further studies are needed to identify these questions.**

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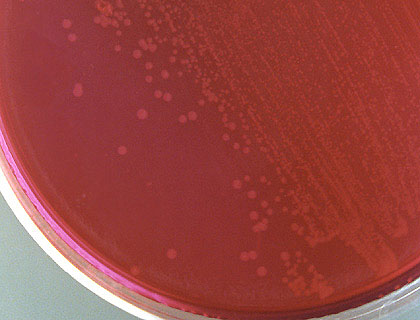
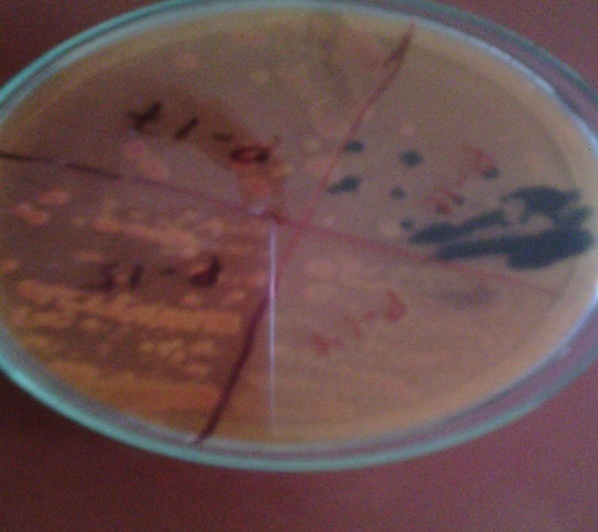
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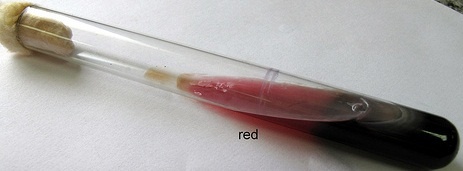
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**Photogallery**



**Fig: Salmonella on BGA agar**

**Fig: Salmonella on SS agar**

**Fig : SS & BGA agar**

**Fig: Salmonella on TSI**

**Brief Biography of the Student**

This is **MD.TARIQUL ISLAM;** son of **MD.ALHAJ UDDIN** and **MOST.FARIDA YASMIN**. He has passed the Secondary School Certificate Examination in 2007 followed by Higher Secondary Certificate Examination in 2009. Now, he is a candidate of Internship programme for the degree of Doctor of Veterinary Medicine (DVM) from Chittagong Veterinary and Animal Sciences University (CVASU), Bangladesh