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Isolation and characterization of *Salmonalla* serovars from meat of cattle, goat and chicken

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ABSTRACT

The study aimed at isolation and identification of *Salmonella* serovars from meat of cattle, goat and chicken and characterization of the isolated serovars using biochemical, serological and molecular techniques and also to study the antibiotic sensitivity pattern of the isolates. The research was conducted during the period from June 2009 to May 2010 in the Bacteriology Laboratory of the Department of Microbiology and Hygiene, BAU, Mymensingh and Enteric Microbiology Laboratory, ICDDRB, Mohakhali, Dhaka. A total of 76 samples were collected of which 11.84% were positive to *Salmonella* serovars. Among the positive samples, the prevalence was 7.69%, 13.33% and 15.00% for meat of cattle, goat and chicken respectively. All the isolates produced distinguished colonies on SS, McConkey and Brilliant Green agar. The culturally positive isolates fermented dextrose, maltose and mannitol with the production of acid and gas but did not fermented sucrose and lactose. The same isolates showed indole and V-P test negative but M-R test positive. In rapid agglutination test, all culturally and biochemically positive *Salmonella* serovars showed agglutination with poly 'O' and in case of poly 'H', all isolates gave positive reaction except chicken isolates. DNA fingerprinting analysis using PFGE of *Xbal* digested genomic DNA revealed that *Salmonella* isolates of the same species collected from the same areas have same genomic pattern. Moreover, similar band pattern of genomic organization was found in the meat of goat and chicken. The antibiotic sensitivity and resistance pattern showed that the isolated *Salmonella* serovars were lightly sensitive to ciprofloxacin and moderately to chloramphenicol, kanamycin, cotrimoxazole and malidixic acid. The positive isolates of chicken exhibited fully resistant to erythromycin but the cattle and goat isolates were of 80% resistance. The study concluded that the genetic changes and emergence of *Salmonella* serovars with multiple drug resistance are the major obstacles for the treatment of *Salmonella* infection in different hosts, although the genetic basis for emergence of new serovars with multiple drug resistance remains unknown.

Introduction

Salmonella is a disease condition caused by a large group of bacteria of the genus *Salmonella* that can affect all species of domestic animal like cattle, goat and poultry throughout the world. In recent years, food borne infections and intoxications have been assumed significant as a health hazard. *Salmonella* and the medical condition that it causes, is one of the most commonly and widely distributed food borne illness (Hanning *et al.*, 2009). As major food items, meat and meat products are important vehicles of *Salmonella* outbreaks among the people. Ingestion of raw or undercooked meat can easily transmit *Salmonella* in animal and human. Contaminated raw meat can spread *Salmonella* among people during handling, processing, transport, storage, distribution and preparation for consumption. Cross-contamination of carcasses with *Salmonella* can also occur during slaughtering operations. *Salmonella* is a worldwide issue in public health sector. People most at risk for serious complications due to *Salmonella* food poisoning include older adults, pregnant women, infants, children and people who have compromised immune systems. Salmonellosis is manifested clinically in all hosts by one of three major syndromes, per acute systemic infection, an acute enteritis or a chronic enteritis (Merchant and Packer, 1967). Symptoms are usually including headache, nausea, vomiting, fatigue, gastroenteritis, abdominal cramps and bloody diarrhea with mucus and sometimes reactive arthritis (Reiters syndrome) (Dworkin *et al.*, 2001). Following Salmonellosis dehydration with renal insufficiency and death may occur.

The importance of Salmonellosis in public health sector is a growing concern day by day throughout the world during the last decade. Salmonellosis in the past has caused tremendous loss to society in many countries around the world. Two to four million of cases have been reported annually and yet a significant number of cases have been unreported worldwide. Non-typhoidal *Salmonella* is the leading cause of food borne illness and its increasing antimicrobial resistance is associated with higher risks of hospitalization in Bangladesh (Salam *et al.*, 2003). Non-typhi *Salmonella* was found responsible for 66% cases of food illness in Bangladesh. The highest proportion (15%) was isolated in 1998 followed by in 1995 (13%) while it was less than 10% for other years. Thirty six percent were isolated during the summer while 28% were in the fall. *Salmonella* serovars, the leading cause of food borne illness, are important global burden in public health causing substantial morbidity and mortality among the population.

Besides, treatment expenditure of salmonellosis seriously reduces the financial solvency of the poor and middle income families. Thus *Salmonella* makes harm to both human and financial resources of the country. So, it is of great economic concern and public health significance. But studies in this direction are scant, especially in Bangladesh. Thus the present work is an important endeavor for *Salmonella* research in Bangladesh. In view of these considerations, the present study was undertaken with objectives of isolating and identifying *Salmonella* serovars from meat of cattle, goat and chicken using cultural, biochemical, serological, molecular techniques and also to study the antibiotic sensitivity pattern of the isolates.

METHODOLOGY

The present research was conducted during the period from June 2009 to May 2010 in the Bacteriology Laboratory of the Department of Microbiology & Hygiene, BAU, Mymensingh and Enteric Microbiology Laboratory, International Centre for Diarrheal Disease Research, Bangladesh (ICDDR,B), Mohakhali. The meat samples (76) examined in this study were collected from Kamal-Ranjit Market of BAU and Natun bazar, Mesua Bazar and Municipal Slaughter House of Mymensing town. Among the samples 26 were cattle, 30 were goat and the rest 20 were chicken samples. The entire study was divided into three steps. The first step included selection of sources, collection of samples, transportation to the laboratory, isolation and identification of *Salmonella* on the basis of their colony morphology, staining property, motility and biochemical and serological characteristics. In the second step, molecular detection of *Salmonella* isolates was performed by Pulsed-Field Gel Electrophoresis (PFGE). In the third step, the current status of drugs sensitivity and resistance patterns of isolated bacteria was determined. The Salmonellae isolated were preserved in 20% buffered glycerin for further use.

Nutrient broth (NB) and Nutrient agar (NA) were used to grow the organisms from the collected samples. To have a pure isolate colonies from NA were cultured into different selective and differential media, viz- Salmonella-Shigella agar (SSA), Brilliant Green agar (BGA) and McConkey agar (MCA) as mentioned by Cheesebrough (1984). Blood agar (BA) medium was used to perform the antibiotic sensitivity study. In order to identify *Salmonella*, media used for biochemical tests were sugar media (dextrose, maltose, lactose, mannitol and sucrose), methyl red-Voges-Proskauer (MR-VP) broth, peptone broth and Triple sugar iron agar (TSIA) slant. TSIA slant was also used for preservation of *Salmonella*. *Salmonella* polyvalent antiserum (poly 'O' and poly 'H') was used for the serological identification of *Salmonella*. The isolated *Salmonella* serovars were preserved in 20% buffered glycerin. Preserved *Salmonella* were placed into ice box and transported to ICDDR,B Dhaka for performing molecular characterization by PFGF according to the procedure. Eight different antibacterial discs were selected for antibacterial sensitivity study against isolated Salmonellae.

FINDINGS AND DISCUSSION

Salmonella serovars were isolated and identified from the samples after cultivation on NA, MCA, SSA and BGA media. *Salmonella* was detected from 9 out of 76 samples (Table 1). The positive samples were collected from Kamal-Ranjit Market of BAU and Natun Bazar and Mesua Bazar of Mymensingh town. The highest recovery of *Salmonella* serovars (15.00%) was found in the meat of chicken which was followed by goat (13.33%) and cattle (7.69%). Overall 11.84% recovery of *Salmonella* was found in the samples under consideration of the present study (Table 1).

NB inoculated separately with the collected samples revealed the growth of bacteria after 24 hours of incubation at 37^0C aerobically and was indicated by the presence of turbidity (Table 1). Under the same condition, NA plates streaked separately with the samples revealed the growth of bacteria after 24 hours of incubation at 37^0C aerobically and was indicated by the growth of circular, smooth, opaque, translucent colonies. On SSA plates, the organisms were produced pinhead or lentil sized, raised, round or circular smooth, glistening, opaque, colorless, transparent or translucent colonies. These results were found to be pale pink color colonies against a pinkish background in the case of BGA plates. On MCA plates, the organisms were produced colorless and smooth colonies while they were produced black color colonies in the case of TSIA slant. The thin smears prepared with the colony from SSA, MCA and BGA for Gram's staining revealed Gram-negative, pink colored, small rod shaped appearance, arranged in single or paired under the microscopic examination. All the isolates were found to be motile having swinging movement when examined using hanging drop slide under microscope except chicken isolates (Table 2).

All of the isolates fermented dextrose, maltose and mannitol with the production of acid and gas but did not ferment lactose and sucrose. Acid production was indicated by the color change from reddish to yellow and gas production was noted by the presence of gas bubbles in the inverted Durham's tubes (Table 3). All of the isolates were found to be indole negative, MR test positive and VP test negative.

Table 1. Prevalence of *Salmonella* serovars obtained from various sources

Source of sample	No. of sample	Media used	Change in broth	Positive/negative to *Salmonella*					Prevalence (%)
				Cultural examination		Biochemical examination		Total positive sample	
				Positive	Negative	Positive	Negative		
Cattle	26	NB, NA, MCA, SSA, BGA	Turbi-dity	2	24	2	24	2	7.69
Goat	30	+	+	4	26	4	26	4	13.33
Chicken	20	+	+	3	17	3	17	3	15.00
Total	76			9	67	9	67	9	11.84

Table 2. Cultural, staining and morphological characteristics of the isolated *Salmonella* serovars

Isolate	Colony characteristics (include TSIA cultural characters)			Staining characters	Motility
	SSA	MCA	BGA		
CaKR2	Opaque, translucent, colorless, smooth, round colonies	Pale, colorless, smooth, transparent raised colonies	Pale pink color colonies against a pinkish background	Gram negative, short rod shaped	+
CaNB2	Do	Do	Do	Do	+
GKR8	Do	Do	Do	Do	+
GKR9	Do	Do	Do	Do	+
GNB10	Do	Do	Do	Do	+
GMB11	Do	Do	Do	Do	+
ChKR7	Do	Do	Do	Do	-
ChKR8	Do	Do	Do	Do	-
ChNB11	Do	Do	Do	Do	-

Legends:
CaKR2 and CANB2= isolates of cattle from KR Market and Natun Bazar respectively
GKR8 and GKR9= isolates of goat from KR Market
GNB10 and GMB11= isolates of goat from Natun Bazar and Mesua Bazar
ChKR7, ChKR8 and ChNB11= isolates of chicken from KR Market and Natun Bazar respectively

Table 3. Results of biochemical tests of the isolated *Salmonella* serovars

Isolates	Carbohydrate fermentation tests					Indole	MR	V-P
	Dextrose	Maltose	Lactose	Sucrose	Mannitol			
CaKR2	+	+	-	-	+	-	+	-
CaNB2	+	+	-	-	+	-	+	-
GKR8	+	+	-	-	+	-	+	-
GKR9	+	+	-	-	+	-	+	-
GNB10	+	+	-	-	+	-	+	-
GMB11	+	+	-	-	+	-	+	-
ChKR7	+	+	-	-	+	-	+	-
ChKR8	+	+	-	-	+	-	+	-
ChNB11	+	+	-	-	+	-	+	-

Table 4. Serotyping of *Salmonella* isolated from different sources

Isolated Salmonellae	Poly O	Poly H
CaKR2	+	+
CaNB2	+	+
GKR8	+	+
GKR9	+	+
GNB10	+	+
GMB11	+	+
ChKR7	+	-
ChKR8	+	-
ChNB11	+	-

The rapid slide agglutination test with poly 'O' and poly 'H' antisera was conducted with all the isolated *Salmonella* serovars. In this test, all culturally and biochemically positive *Salmonella* serovars showed agglutination with poly 'O' but in case of poly 'H', all isolates gave positive reaction except chicken isolates (Table 4).

PFGE analysis of the *Xba1* digested chromosomal DNA of the *Salmonella* strains yielded 12 to 17 reproducible DNA fragments ranging in size approximately from <20 to <668.9 Kbp (Table 5). PFGE analysis revealed that of the 9 *Salmonella* strains, the strains isolated from the same species of the same region displayed very similar restriction fingerprint pattern while the strains of different species of different places yielded diverse and heterogeneous banding pattern. So, major differences in band patterns were observed among the strains of different species.

Table 5. Approximate number of band of *Salmonella* serovars formed by restriction enzyme during PFGE

Isolate and source	Lab ID	Restriction Enzyme	Approximate no. of restriction fragment
Goat meat, KR Market	Gt8	Xba1	15-16
Do	Gt9	Xba1	13-15
Goat meat, Notun Bazar	Gt10	Xba1	12-13
Do	Gt10(2)	Xba1	13-15
Goat meat, Mesua Bazar	Gt11	Xba1	13-15
Do	GT11(2)	Xba1	12-13
Chicken meat, KR Market	Ch7	Xba1	15-17
Do	Ch8	Xba1	15-16
Chicken meat, Notun Bazar	Ch11	Xba1	15-16

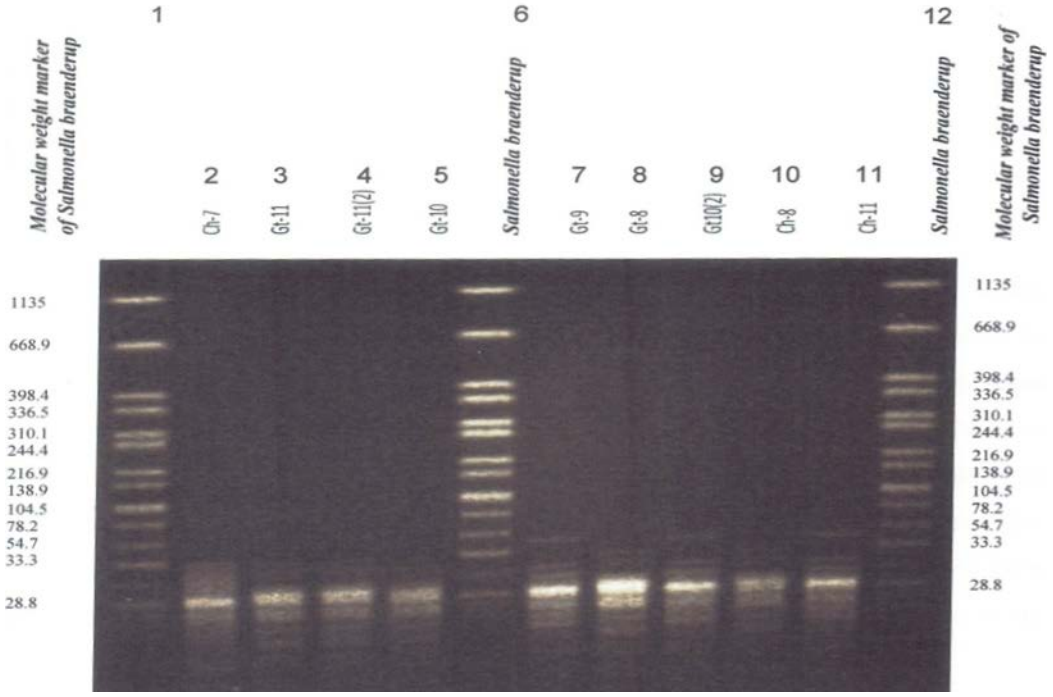

Figure 1. PFGE gel image of *Salmonella* serovars
Legends
Lane 1, 6, 12: Genomic organization of *Salmonella* braenderup (marker)
Lane 2, 10, 11: Genomic organization of isolate of chicken meat from KR Market, KR Market and Natun Bazar
Lane 3, 4, 5, 7, 8, 9: Genomic organization of isolate of goat meat from Masua Bazar, Masua Bazar, Natun Bazar, KR Market, KR Market and Natun Bazar

The fingerprint pattern in the gel was analyzed using computer software package Quantity One Version 3.0 (Applied Math BVBA, Belgium). After background subtraction and gel normalization, the fingerprint patterns were subjected to cluster analysis using Unweighted Pair Group Method with Arithmetic Mean (UPGMA) (Figure 1).

From the antibiogram study, it was revealed that among the isolates from cattle 100%, 80%, 40%, 20% and 20% were found to be highly sensitive to ciprofloxacin (CI), cotrimoxazole (CT), nalidixic acid

(NA), kanamycin (KA) and chloramphenicol (CK) respectively. 60%, 40%, 40%, 20% and 20% were moderately sensitive to NA, KA, CK, CT and cephalexin (CP) respectively. 80%, 40%, 40% and 20% were less sensitive to CP, amoxicillin (AX), erythromycin (ER) and CK respectively. 80%, 60% and 20% were resistant to ER, AX and CK respectively (Table 6). Among the isolates from goat 100%, 60%, 60%, 25% and 20% were found to be highly sensitive to CI, CT, NA, KA and CK respectively. 80%, 60%, 40%, 20% and 20% were found to be moderately sensitive to KA, NA, CT, CK and CP respectively. 60%, 20% and 20% were found to be less sensitive to CP, AX and ER respectively. 100%, 80% and 20% were resistant to ER, AX and CP respectively. In the case of *Salmonella* isolates from chicken 100% were highly sensitive to CI, 60% to CK and KA but 20% were to CP and CT. On the other hand 60% were moderately sensitive to NA, 20% to CP and KA but 40% to CK and CT. 60%were less sensitive to CP, 40% were less sensitive to CT and NA acid, 20% were less sensitive to ER and KA; but 100% were highly resistant to AX while 100% were resistant to ER.

Table 6. Antibiotic sensitivity pattern in percent

Isolate from	Sensitivity pattern	% of isolated strain sensitive to various antibiotics							
		ER	AX	CP	CK	CT	KA	CI	NA
Cattle	Resistance	80	60	0	20	0	0	0	0
	Less sensitive	20	40	80	20	0	0	0	0
	Moderately sensitive	0	0	20	40	20	40	0	60
	Highly sensitive	0	0	0	20	80	60	100	40
Goat	Resistance	80	80	20	0	0	0	0	0
	Less sensitive	20	20	60	0	0	0	0	0
	Moderately sensitive	0	0	20	20	40	80	0	60
	Highly sensitive	0	0	0	80	60	20	100	40
Chicken	Resistance	100	100	0	0	0	0	0	0
	Less sensitive	0	0	60	0	40	20	0	40
	Moderately sensitive	0	0	20	40	40	20	0	60
	Highly sensitive	0	0	20	60	20	60	100	0

In this study the colony characteristics of *Salmonella* serovars observed on MCA, SSA and BGA were similar to the findings of other authors (Hossain, 2002 and Cherry *et al.*, 2004). In Gram's staining, the morphological characteristics of the isolated *Salmonella* exhibited Gram negative, small rod shaped, single or paired in arrangement under microscope which was supported by other studies (Sogard *et al.*, 2007; Gene, 2002). In present study the isolated *Salmonella* serovars were recorded as both motile and non motile. The fundamental basis for the detection of motile and non-motile *Salmonella* was the motility test in which all isolates of cattle and goat were found to be motile but isolates of chicken were non-motile. This result is correlated with the results of Buxton and Fraser (1977) and Merchant and Packer (1967). *Salmonella* isolates were able to ferment the five basic sugars by producing both acid and gas. However, differentiation of *Salmonella* into species level was difficult based on their sugar fermentation pattern. All the isolates of this study fermented dextrose, maltose and mannitol and produced acid and gas but did not ferment sucrose and lactose which satisfied the statement of Buxton and Fraser (1977) and Hossain (2002). Some strains produced hydrogen sulfide when the isolated organisms produced acid and gas in dextrose and mannitol and variable in maltose, it was primarily considered that the isolates were *S. pullorum* (Shivprasad, 1997). It was created difficulties when organisms produced only acid in dextrose, maltose and mannitol because *S. pullorum* sometimes did not produce gas as produced by *S. gallinarum* (Williams, 1992). Robinson *et al.* (2000) and Rahman (2003) used this method for serogrouping of *Salmonella* as a diagnostic tool. All the isolates were found to be negative to indole tests positive to MR and negative to VP.

In the present study, out of 76 different samples 11.84% were identified as positive for *Salmonella*. The prevalence rate is 7.67% for cattle meat, 13.33% for goat meat and 15.00% for chicken meat. So, the results are more or less in agree with the findings of the previous workers who conducted research investigations on *Salmonella* from meat source (Duffy *et al.*, 2008; Molla *et al.*, 2006). The slight differences among the prevalence percentages might be due to the species differentiation, hygienic, environmental and geographic variation and technical limitation of the laboratory of the study. In rapid slide agglutination test, all culturally and biochemically positive *Salmonella* serovars showed agglutination with polyvalent poly 'O' but in the case of poly 'H', all isolates gave positive reaction except chicken. In PFGE method, chromosomal DNA was digested with a restriction endonuclease that generates large fragments. The restriction fragments were resolved in a pattern of discrete bands. The DNA restriction patterns of the isolates were compared with one another to determine their relatedness. Choice of restriction enzyme is an important factor to obtain reproducible and well discriminatory banding pattern in PFGE. A number of previous studies (Xia *et al.*, 2009; Bolton *et al.*, 2007) suggested that Xbal gave the best discriminatory banding pattern of *Salmonella* serovars. From the result of PFGE it can be concluded that same species from the same

region have the similarity in genomic organization whereas different species have different genomic organization. Moreover, similar band pattern of genomic organization was found in the meat of goat and chicken. In antibiotic sensitivity study, it was exhibited that all the isolates from cattle were highly sensitive to ciprofloxacin, cotrimoxable and kanamycin; moderate sensitive to nalidixic acid and chloramphenicol; less sensitive to cephalexin and amoxicillin; and resistant to erythromycin. All isolates from goat were highly sensitive to kanamycin, cotrimoxable and ciprofloxacin; moderate sensitive to nalidixic acid; less sensitive to cephalexin and chloramphenicol; and resistant to amoxicillin and erythromycin. Chicken isolates showed fully resistant to erythromycin and amoxicillin while the isolates were fully sensitive to kanamycin, ciprofloxacin and chloramphenicol but moderately sensitive to cotrimoxazole and nalidixic acid. These findings are in support of Sato *et al.* (1997), Banani *et al.* (2003) and Kobayashi *et al.* (2007). The antibacterial resistance observed here in the isolated Salmonellae might be due to routine indiscriminate use of those antibacterial agents in field condition in study areas and/or rapid chromosomal mutation and presence of specific plasmid DNA. This will provide a guideline to the veterinarians and physicians to select appropriate antibiotics to reduce economic loss through selecting the sensitive antibiotics.

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REFERENCES

Banani, M., Pourbakhsh, S.A., Khaki, P. and Nikookhesal, G.H. (2003). Serotyping and drug sensitivity of Salmonella isolates from buffaloes submitted to RaziInstitute.Pajouhesh-va-sadandegi in Animal-Sciences. 59:92-96.

Bolton, D.J., Meally, A., McDowell, D. and Blair, I.S. (2007). A survey for serotyping, antibiotic resistance profiling and PFGE characterization of and the potential multiplication of restaurant Salmonella isolates. J. Appl. Microbiol. 103(5): 681-690.

Buxton, A. and Fraser, G. (1977). Animal microbiology.Vol.1. Blackwell scientific publications, Oxford. London, Edinburg, Melbourne. 85-110.

Cheesbrough, M. (1984). Medical laboratory manual for tropical countries. First edition. Vol-ii Microbiology, Chapter 35, London English Lnguage Book Society, pp 4057.

Cherry, B., Burns, A., Johnson, G.S. and Dumas, N. (2004). Salmonella typhimuriumout break associated with veterinary clinic. EmwrgingIq/ec. Dis. 10(12):2249-2252.

Duffy, L., Barlow, R., Fegan, N. and Vanderline, P. (2008). Prevalence and serotypes of Salmonella associated with goat at two Australianabattoirs. LettApplMicrobiol. 48(2): 93-97.

Dworkrin, M.S., Shoemaker, P.C., goldoft, M.J. and Kobayashi, J.M. (2001). "Reactive arthritis and Reiter's syndrome following an outbreak of gastroenteritis caused by Salmonella enteritidis''.Clin. Infect. Dis. 33(7): 10-14.

Gene, O. (2002). The isolation, identification and serotyping of Salmonella isolated from domestic poultry in Kars district. KafkasUniversixesiVeterinerFakultesi, Dergisi. 8(1): 23-30.

Hanning, I.B., Nutt, J.D. and Ricke, S.C. (2009). Salmonellosis outbreaks in the United States due to fresh produce: sources and potential intervention measures. Foodborne Pathog. Dis. 6(6): 35-48.

Hossain, K.M. (2002). Characterization of bacteria isolated from diarrhoeic calves. M.S. Thesis, Department of Microbiology and Hygiene.Faculty of Veterinary Science, BAU, Mymensingh.

Kobayashi, H., Kanazaki, M., Shimizu, Y., Nakajima, H., Khatun, M.M., Hata, E. and Kubo, M. (2007). Salmonella isolates from rectal swabs and feaces of animals in the immediate environment of "Tokyo Bay. J. Vet. Med. Sci., 69(3):309-IL

Merchant, I.A. and Packer, R.A. (1967).Veterinary bacteriology and virology, 7thedn.The Iowa University Press, Ames, Iowa, USA, pp. 286-306.

Molla, W., Molla, B., Alcmychu, D., Mucklc, A., Cole, L., and Wilkie, E. (2006). Occurrence and antimicrobial resistance of Salmonella serovars in apparently healthy slaughtered sheep, buffalo and goats of central Ethiopia. Trop. Ani. Heal. And Produc. 38(6): 455-462.

Robinson, H., Mdegela, G.S., Yongolo, U., Minga, M., and Johin. E. (2000). Molecular epidemiology of *Salmonella gallinarum* in chickens in Tanzania. Avian Path. 29: 457-463.

Salam, M.A, Saha, D., Khan, W.A. and Bennish, M.L. (2003). Non-Typhoidal Salmonella Infection and their Antimicrobial Resistance inBangladesh.Interscience Conference on Antimicrobial Agents Chemotherapy (43rd: 2003: Chicago, 3). Agents Chemother. 2003 Sep 14_17; 43: abstract no. C2-864. ICDDR,B, Dhaka, Bangladesh.

Sato, Y., Sato, G., Tuchili, I., Pandey, G.S., Nakjima, A., Chimana, H., and Sinsungwe, H. (1997). Status of Salmonella gallinarumpullorum Infections in poultry in Zambia. Avian Dis. 41: 490-495.

Shivaprasad, H.L. (1997). Pullorum disease and fowl typhoid, In: Calnek, B.W., Jarnes, H.J., Beard, C. W., McDougald, L. R. and Saif, Y. M. (Eds.). Disease of poultry.10thedn.Ames, Iowa State University Press, USA. Pp. 82-96.

Sogard, M., Norgaard, M. and Schonheyder, H.C. (2007). First notification of positive blood cultures and the high accuracy of the gram stain report. J. Clin. Microbial. 45(4):1113-1117.

Williams, J. E. (1992). In Hofstad, M. S., John Barnes, H., Calnek, B. W., Reid, W. M. and Yoder, Jr. H. W. (eds). Diseases of poultry, 8thedn., Panima Educational Bool Agency, New Delhi, India. P.65.

Xia, S., Hendriksen, R.S., Xie, Z., Huang, J., Guo, W., Xu, B., Ran, L. and Aarestrup, E.M. (2009). Molecular characterization and antimicrobiansusceptibility of Salmonella isolates from infections in Henan Province, China. J. Clin, Microbiol. 47(2):401-409.