INNSPUB

International Journal of Biosciences | IJB |
ISSN: 2220-6655 (Print) 2222-5234 (Online)
http://www.innspub.net
Vol. 3, No. 1, p. 109-120, 2013

RESEARCH PAPER OPEN ACCESS

Characterization, pathogenecity and antibiogram study of *Salmonella* species isolated from apparently healthy and diarrhoeic calves

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Key words: Salmonellosis, *Salmonella* spp. calves, characterization, pathogenicity, antobiogram study..

Article published on January 20, 2013

Abstract

Salmonellosis in calves appears as one of the major problems in Bangladesh and causes substantial economic loss. The present study was conducted with a view to isolate and identify *Salmonella* species from apparently healthy and diarrhoeic calves of Char Nilokkhiya, BAU dairy farm and calves those came to Veterinary clinic. During the present study, out of 126 rectal swab samples, 10 samples were identified as positive for *Salmonella*. The isolates were named as D1, D2, D3, D4, D5, D6, H1, H2, H3 and H4. Of the 10 isolates 4 were isolated from 71 apparently healthy calves and 6 were isolated from 55 diarrhoeic calves. In case of apparently healthy individual, the percentage of positive samples from those areas was 6.25%, 4% and 6.66% respectively whereas in case of diarrhoeic calves it was 11.11%, 9.52% and 12% respectively. The overall prevalence of *Salmonella* in diarrhoeic calves (10.90%) was remarkably higher than in apparently healthy calves (5.633%). Pathogenicity test was performed using 5 groups of day old white mice each consisting of 4 animals by inoculating the isolates orally and intraperitonially which caused diarrhoea and death to the mice used. The group of animals was numbered as Group-A, Group-B, Group-C, Group-D and Goup-E. Two mice from each of Group-A and Group-B were inoculated orally each with 0.5 ml. of the inoculum of the isolate No. H_1 and H_2 respectively, while the remaining two mice of the same groups were injected intraperitoneally each with 0.25 ml. of the inoculum of the same isolate. Similarly isolate no. D_1 and D_2 were inoculated into the animal group C and D respectively while Group-E was kept as control and inoculated with nutrient broth. The antimicrobial susceptibility of all the *Salmonella* isolates was analyzed by the disc diffusion method. Most of the isolates were highly sensitive to ciprofloxacin, gentamycin and spiramycin, moderately sensitive to amoxycillin, streptomycin and oxytetracycline and nearly resistant to penicillin and slulphamethoxazole.

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Introduction

Salmonellosis is a collective description of a group of diseases caused by the members of the genus *Salmonella* which consists of over 2,500 different serovars. *Salmonella* infection in calves appears as one of the major problems throughout the world and causes substantial economic loss both directly through mortality and poor growth after clinical infection, and indirectly from animal carriage leading to human *Salmonella* infection which is a serious food-borne infection with gastroenteritis in human and represents a serious problem for the food industry (Barrington *et al.,* 2002; Khan *et al.,* 2007 and Much *et al.,* 2007).

Bangladesh is one of the poorest countries of the third world in terms of material resources but livestock population is one of the most important resources that can be considered as the most effective weapon to uplift the overall condition of its economy. Among livestock population cattle are the most important animals in livestock sub-sector. *Salmonella* has been widely reported in cattle (Field, 1948; Hughes *et al*., 1971; Wray *et al*., 1977; Hollinger *et al*., 1998; McDonough *et al*., 1999). The infected animals may shed the organism in their feces without showing any clinical signs of disease (Gibson, 1965). Thus a rapid, specific and sensitive detection method for Salmonella is important for animal and human health and for the diagnostic industry (Gouws, 1998). *Salmonellae* are Gram negative, short plump shaped rods, nonsporeforming, noncapsulated, aerobic and facultatively. More than 2500 serovars exist based on 67 "O" antigens (for non-motile species) and the numerous "H" antigens (for motile spp.) recognized so far (Blood *et al*., 2003. The most common serovar identified in various cases of Salmonellosis in cattle is *Salmonella dublin* (McEvoy *et, al.* 2003 and Davison *et al.,* 2005).

In cattle, diverse clinical signs occur in Salmonellosis; they range from unthriftiness to explosive, necrotizing diarrhoeas with high mortality. Secondary complications of pneumonia, bone and joint infections, and meningoencephalitis can result from calfhood infections (Rings, 1985).

Since infection with *Salmonella* causes abortion in cows, poor growth and considerable loss in calves through mortality, it can be treated as a threat to our developing livestock sector as the cattle population of Bangladesh is supposed to be suffering from Salmonellosis. The members of the genus *Salmonella* vary widely from each other in terms of their pathogenicity and drug sensitivity, so to suggest an appropriate line of treatment of Salmonellosis in Bangladesh there are no other alternatives to characterize the local isolates which is yet to be done. Keeping in mind the above mentioned ideas the present study had been undertaken with the following specific objectives: to isolate and identify the *Salmonella* species from apparently healthy and diarrhoeic calves and to study the pathogenicity of the isolated *Salmonella* species as well as their antibiotic sensitivity test.

Materials and methods

Study area

The study was conducted in the Bacteriology laboratory of the Department of Microbiology and Hygiene, BAU, Mymensingh, Bangladesh during the period of July 2006 to May 2007.The samples studied were collected from Bangladesh Agricultural University dairy farm, calves brought for the treatment in Veterinary clinic and Char Nilokkhiya of the sadar upazilla under Mymensingh district. The rectal swab samples were collected from apparently healthy and diarrhoeic calves and brought to the laboratory of the Department of Microbiology and Hygiene, BAU, Mymensingh for the isolation and characterization of *salmonella*.

Collection of samples

A total number of 126 field samples comprising rectal swab from apparently healthy and diarrhoeic calves of the study areas were aseptically collected and carried to the laboratory for the isolation of *Salmonella sp*. Rectal swab samples were collected from the mucosa of the rectum of the calves (Figure

1). The rectal swab was taken from the calves in aseptic way and the swabs were kept immediately in the test tube containing Bacto-selenite broth. Test tubes containing swab samples were then immediately brought to the bacteriology laboratory, Department of Microbiology and Hygiene, BAU and subjected to cultural study. One swab sample was collected from each calf. Out of 126 rectal swab samples 71 were collected from apparently healthy and 55 from diarrhoeic calves (Table 1 and 2).

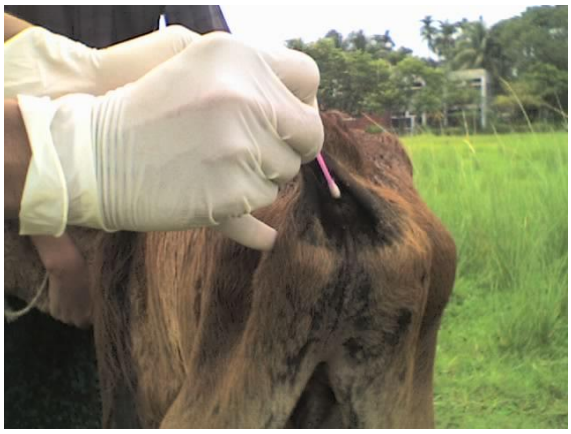

Fig. 1. Collection of rectal swab sample from calf.

Table 1. Rectal swab samples collected from apparently healthy calves of Char Nillokhiya, BAU Dairy Farm and BAU Veterinary Clinic under Mymensingh district.

Sl. No.	Source	Location	Number of samples collected
1	Rectal swab	BAU dairy farm	16
2		BAU Vet clinic	30
3		Char Nilokkhiya	25
Total			71

BAU=Bangladesh Agricultural University

Sample preparation and isolation of Salmonella species

Rectal swabs from apparently healthy and diarrhoeic calves were used in the analysis. Each swab sample was inoculated into freshly prepared selenite broth and incubated at 37° C for 24 hours aerobically. At the end of the incubation period, a loopful from each of the selective enrichment broths was streaked onto MacConkey (MC) agar, Salmonella-Shigella (ss) agar and Brilliant green agar (BG) separately. The plates were then incubated at 37° C for 24 hours and the plates containing characteristic colonies of *Salmonella* were selected. Subculturing in SS agar was performed from the suspected plates containing *Salmonella* to obtain a pure culture. The selected isolates were used for further study.

Table 2. Samples collected from diarrhoeic calves of Char Nillokhiya, BAU Dairy Farm and BAU Veterinary Clinic.

Sl. No.	Source	Location	Number of samples collected
1	Rectal swab	BAU dairy farm	9
2		BAU Vet. clinic	25
3		Char Nilokkhiya	21
Total			55

BAU=Bangladesh Agricultural University

Characterization of the selected isolates

The selected isolates were characterized by morphological and biochemical methods. Morphological characteristics such as colony size, shape, colour etc. were observed.

Morphological characterization by Gram's staining method

Smears of suspected colonies were stained with Gram's stain and examined morphologically for staining characters. Motility was tested under light microscope of 100 magnifications by using slide with a drop of young bacteria.

Biochemical characterization

Isolated organisms with supporting growth characteristics of *Salmonella* on various media were maintained on SS and BG agar media and were subjected to various biochemical tests named lactose

fermentation, sucrose fermentation, maltose fermentation, dextrose fermentation, mannitol fermentation, triple sugar iron agar slant reaction(TSI), MR-VP reaction, hydrogen sulphide production(H_2S) and indole reaction.

Table 3. Inoculation of white mice for pathogenicity test.

Group of mice	Sample from	Experimental Mice No.	Materials inoculated	Route of Inoculation	Amount of Inoculation
A	Healthy calf of BAU dairy farm	1	Isolate No. H_1	Oral	1 ml.
		2	Isolate No. H_1	Oral	1 ml.
		3	Isolate No. H_1	I/P	0.5 ml.
		4	Isolate No. H_1	I/P	0.5 ml.
B	Healthy calf of Char Nillokhiya	5	Isolate No. H_2	Oral	1 ml.
		6	Isolate No. H_2	Oral	1 ml.
		7	Isolate No. H_2	I/P	0.5 ml.
		8	Isolate No. H_2	I/P	0.5 ml.
C	Diarrhoeic calf of BAU dairy farm	9	Isolate No. D_1	Oral	1 ml.
		10	Isolate No. D_1	Oral	1 ml.
		11	Isolate No. D_1	I/P	0.5 ml.
		12	Isolate No. D_1	I/P	0.5 ml.
D	Diarrhoeic calf from BAU Veterinary clinic	13	Isolate No. D_2	Oral	1 ml.
		14	Isolate No. D_2	Oral	1 ml.
		15	Isolate No. D_2	I/P	0.5 ml.
		16	Isolate No. D_2	I/P	0.5 ml.
E		17	NB	Oral	1 ml
		18	NB	Oral	1 ml
		19	NB	I/P	0.5 ml
		20	NB	I/P	0.5 ml

Legends

H1 = Isolate from healthy calf of BAU dairy farm; H2 = Isolate from healthy calf of char Nillokhiya; D1 = Isolate from diarrhoeic calf of BAU dairy farm; D2 = Isolate from diarrhoeic calf of veterinary clinic; BAU=Bangladesh Agricultural University; I/P = Intraperitonial; NB = Nutrient broth.

Serotyping through slide agglutination test

Salmonella agglutinating antiserum "Poly "O" and "Poly-H" of S&E Reagents Lab, Bangkok, Thailand was used to do the serotyping of the isolated *Salmonellae*. The macroscopic slide agglutination tests were performed.

Pathogenicity test

Pathogenicity test was performed using day old white mice by inoculating the isolates orally and intraperitonially which caused diarrhoea and death to the mice used.

Table 4. Antimicrobial agents and their disc concentration.

Antibacterial agent	Disc concentration (µg) /disc)
Streptomycin	10
Spiramycin	100
Penicillin	10
Sulphamethoxazole+ Trimethoprim	25
Oxytetracycline	30
Gentamycin	120
Ciprofloxacin	5
Amoxycillin	25

Two isolates from apparently healthy and 2 from diarrhoeic calves were selected for pathogenicity test. To study the pathogenicity of the isolated organisms, 5 groups of day old white mice each consisting of 4 animals were selected, the group of animals were numbered as Group-A, Group-B, Group-C, Group-D and Goup-E. Two mice from each of Group-A and Group-B were inoculated orally each with 0.5 ml. of the inoculum of the isolate No. H_1 and H_2 respectively, while the remaining two mice of the same groups were injected intraperitoneally each with 0.25 ml. of the inoculum of the same isolate. Similarly isolate no. D_1 and D_2 were inoculated into the animal group C and D respectively while Group-E was kept as control and inoculated with Nutrient broth (Table 3).

Antibiotic sensitivity test

In vitro susceptibility of the organisms to various antimicrobial agents was determined by the disc diffusion technique. The antibacterial discs used were streptomycin, amoxycillin, spiramycin, oxytetracyclin, penicillin, gentamycin, ciprofloxacin and sulphamethoxazole (Table4).

Results and discussion

The study was aimed to isolate and characterize the *Salmonella* spp. from apparently healthy and diarrhoeic calves of Char Nilokkhiya, BAU dairy farm and calves those came to Veterinary clinic of the same university for treatment purpose, under the sadar upazilla of Mymensingh district. In the present study, out of 126 rectal swab samples, 10 samples were identified as positive for *Salmonella* (Table 5 and Table 6).The isolates were named as D1, D2, D3, D4, D5, D6, H1, H2, H3 and H4.

Out of 10 isolates 4 were isolated from 71 apparently healthy calves (Table 5) and 6 were isolated from 55 diarrhoeic calves (Table 6). Among the positive samples collected from apparently healthy calves, one was from BAU dairy farm, one was from Char Nilokkhiya and two were from calves brought for the treatment in BAU Veterinary clinic. In case of apparently healthy individual, the percentage of positive samples from those areas was 6.25%, 4% and 6.66% respectively (Table 5). The overall prevalence of *Salmonella* in apparently healthy calves of those areas was 5.633% (Table 5). The prevalence of *Salmonella* was remarkably higher among the diarrhoeic calves. In case of diarrhoeic calves, the percentage of positive samples obtained from BAU dairy farm, Char Nilokkhiya and BAU Veterinary clinic was 11.11%, 9.52% and 12% respectively (Table 6). The overall percentage of salmonellosis among the diarrhoeic calves was 10.90% (Table 6). The present study reveals that the prevalence of *Salmonella* organisms in the calves of this country is not insignificant and immediate attention must be given as it has zoonotic importance and readily transmissible to human, animals and birds. The public health importance of this organism calls for strict hygienic measures in farms and slaughterhouse personnel. (Jones *et al.*; 2006, Dallal *et al.*; 2006).

In the present study, several different selective culture media were used simultaneously to culture the organism because all of them are not equally suitable for all the serovars of *Salmonell* .The media

used in this study were selected considering the experience of the past researcher worked in various fields relevant to the present study (Amin, 1969; Rahman, 1977; Buxton and Frazer, 1977). In this study, colony characteristics of *Salmonella* observed in SS, MC (Fig. 2) and BG (Fig. 3**)** agar (Table 7) were similar to the findings of other authors (Buxton and Fraser, 1977; Rahman, 1977 and Hossain, 2002).

Table 5. Isolation of *Salmonella* from rectal swab samples collected from apparently healthy calves of BAU dairy farm, Char Nillokhyia and BAU veterinary Clinic.

SL. No.	Name of the place	No. of sample tested	No. of samples positive for *Salmonella*	Percent positive samples (%)
1	BAU dairy farm, Mymensingh	16	1	6.25
2	Char Nilokkhiya, Mymensingh	25	1	4.00
3	Veterinary Clinic, BAU, Mymensingh	30	2	6.66
Total		71	4	5.633

BAU = Bangladesh Agricultural University

Table 6. Isolation of *Salmonella* from rectal swab samples collected from diarrhoeic calves of BAU dairy farm, Char Nillokhyia and BAU veterinary Clinic.

SL. No.	Name of the place	No. of sample tested	No. of samples positive for *Salmonella*	Percent positive samples (%)
1	BAU dairy farm, Mymensingh	9	1	11.11
2	Char Nilokkhiya, Mymensingh	21	2	9.52
3	Veterinary Clinic, BAU, Mymensingh	25	3	12
Total		55	6	10.90

BAU = Bangladesh Agricultural university

Table 7. Cultural, staining and morphological characteristics of isolated *Salmonellae*.

Name of isolates	Colony characteristics			Staining characters	Motility
	SS agar	MC agar	BG agar		
D_1	Opaque, translucent, colorless, smooth, round colonies	Pale, colorless smooth, transparent, raised colonies	Pale pink color colonies against a pinkish back ground	Gram negative, short rod shaped	+
D_2	Opaque, translucent, colorless, smooth, round colonies.	Pale, colorless smooth, transparent, raised colonies	Pale pink color colonies against a pinkish back ground	Gram negative, short rod shaped	+
D_3	Opaque, translucent, colorless, smooth, round colonies.	Pale, colorless smooth, transparent, raised colonies	Pale pink color colonies against a pinkish back ground	Gram negative, short rod shaped	+
D_4	Opaque,translucent, colorless, smooth, round colonies.	Pale, colorless smooth, transparent, raised colonies	Pale pink color colonies against a pinkish back ground	Gram negative, short rod shaped	+
D_5	Opaque, translucent, colorless, smooth, round colonies.	Pale, colorless smooth, transparent, raised colonies	Pale pink color colonies against a pinkish back ground	Gram negative, short rod shaped	+

D_6	Opaque, translucent, colorless, smooth, round colonies	Pale, colorless smooth, transparent, raised colonies	Pale pink color colonies against a pinkish back ground	Gram negative, short rod shaped	+
H_1	Opaque, translucent, colorless, smooth, round colonies	Pale, colorless smooth, transparent, raised colonies	Pale pink color colonies against a pinkish back ground	Gram negative, short rod shaped	+
H_2	Opaque, translucent, colorless, smooth, round colonies.	Pale, colorless smooth, transparent, raised colonies	Pale pink color colonies against a pinkish back ground	Gram negative, short rod shaped	+
H_3	Opaque, translucent, colorless, smooth, round colonies.	Pale, colorless smooth, transparent, raised colonies	Pale pink color colonies against a pinkish back ground	Gram negative, short rod shaped	+
H_4	Opaque, translucent, colorless, smooth, round colonies.	Pale, colorless smooth, transparent, raised colonies	Pale pink color colonies against a pinkish back ground	Gram negative, short rod shaped	+

H1 = Isolate from healthy calf of dairy farm; H2 = Isolate from healthy calf of Char Nillokhiya; H3 and H4 = Isolate from healthy calf of Veterinary clinic; D1 = Isolate from diarrhoeic calf of BAU dairy farm; D2, D3 and D4 = Isolates from diarrhoeic calf of Veterinary clinic; D5 and D6 = Isolates from diarrhoeic calves of Char Nillokhiya; SS = Salmonella-Shigella agar; MC = MacKonkey agar; BG= Briliant Green; (+) = Positive.

In the present study, it was found that all the isolates were gram negative and were able to ferment dextrose, maltose and manintol but unable to ferment lactose and sucrose (Table 8) which satisfy the statement of Buxton and Fraser (1977). In Gram's staining, the morphology of the isolated bacteria exhibited short plump shaped rod, arranged in single or paired which was supported by other authors (Freeman, 1985 and Hossain, 2002). The suspected *Salmonella* isolates were motile (Table7). Motility test was fundamental basis for the detection of motile and non motile *Salmonella* organisms (Buxton and Fraser, 1977; Freeman, 1985 and Hossain, 2002). Again, all the isolates were positive for MR test, H_2S production and negative for VP test. Isolate no. D_4 and H_3 were found to be positive for indole test. This variation implies that the isolates belong to different serovars of the genus *Salmonella*. All of the representative isolates were agglutinated with polyvalent O (Poly "O") and polyvalent H (Poly "H") antisera. The results confirmed that all the isolates were *Salmonella* spp. (Fig. 4).

Inoculation of overnight culture of the isolates in NB into day old mice revealed the pathogenic nature of the organisms. Weakness, diarrhoea and depression were common symptoms in the inoculated mice before death (Fig. 5). No death was observed within the control group.

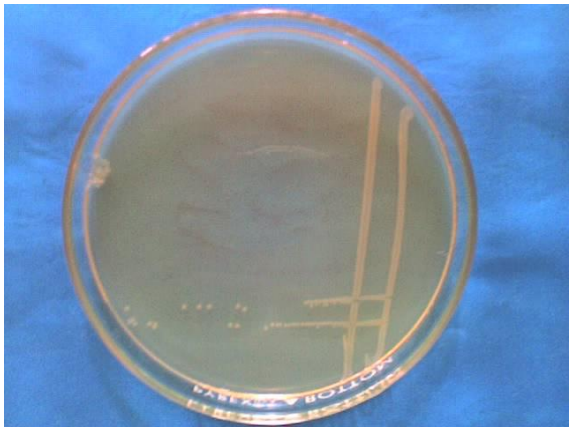

Fig. 2. Colourless, pale, transparent colonies onto McConkey agar.

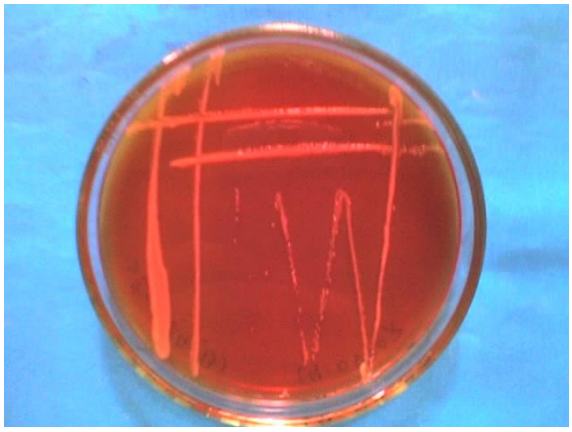

Fig. 3. Pink colour colonies with a reddish background onto BG agar.

Table 10. Results of biochemical tests of the isolated *Salmonellae*.

Isolate	Dextrose	Maltose	Lactose	Sucrose	Mannitol	Indole	MR	VP	H_2S
D_1	+	+	-	-	+	-	+	-	+
D_2	+	+	-	-	+	-	+	-	+
D_3	+	+	-	-	+	-	+	-	+
D_4	+	+	-	-	+	+	+	-	+
D_5	+	+	-	-	+	-	+	-	+
D_6	+	+	-	-	+	-	+	-	+
H_1	+	+	-	-	+	-	+	-	+
H_2	+	+	-	-	+	-	+	-	+
H_3	+	+	-	-	+	+	+	-	+
H_4	+	+	-	-	+	-	+	-	+

H1 = Isolate from healthy calf of dairy farm; H2 = Isolate from healthy calf of Char Nillokhiya; H3 and H4 = Isolate from healthy calf of Veterinary clinic; D1 = Isolate from diarrhoeic calf of BAU dairy farm; D2, D3 and D4 = Isolates from diarrhoeic calf of Veterinary clinic; D5 and D6 = Isolates from diarrhoeic calves of Char Nillokhiya; MR = Methyl Red; VP = Voges-Proskauer; (+) = Positive; (-) = Negative; H2S=Hydrogen sulfide.

Table 11. Results of pathogenicity test in white mice.

Group no.	Experimental. Animal No.	Death of experimental animals after inoculation						Remarks
		Hours						
		12	24	36	48	60	72	
A Inoculated with isolate No. H_1	1	...	...	...	...	...	died	Orally.
	2	...	...	...	...	...	...	
	3	...	died	...	...	...	...	I/P
	4	...	...	...	died	...	...	
B Inoculated with isolate No. H_2	5	...	...	...	...	died	...	Orally
	6	...	...	...	died	...	...	
	7	...	died	...	...	...	...	I/P
	8	...	...	died	...	...	...	
C Inoculated with isolate No. D_1	9	...	...	...	...	...	died	Orally.
	10	...	...	...	...	...	...	
	11	...	...	...	...	...	...	I/P
	12	...	...	...	...	died	...	
D Inoculated with isolate No. D_2	13	...	...	...	...	died	...	Orally
	14	...	...	...	...	...	...	
	15	...	...	...	died	...	...	I/P
	16	...	...	died	...	...	...	
F Inoculated with Nutrient broth	17	...	...	...	...	...	...	Orally
	18	...	...	...	...	...	...	
	19	...	...	...	...	...	...	I/P
	20	...	...	...	...	...	...	

H1 = Isolate from healthy calf of BAU dairy farm; H2 = Isolate from healthy calf of Char Nillokhiya; D1 = Isolate from diarrhoeic calf of BAU dairy farm; D2 = Isolate from Diarrhoeic calf of BAU veterinary clinic; I/P = Intraperitonially; (...) = Not died.

Table 12. Antibiotic sensitivity pattern of isolated *Salmonellae*.

***Salmonella* isolates**	**CIP**	**S**	**AML**	**GN**	**SP**	**P**	**RL**	**OT**
D_1	+++	+	+	+++	+++	-	-	++
D_2	+++	++	++	+++	+++	-	-	++
D_3	+++	++	+	+++	++	-	++	++
D_4	+++	+++	++	++	+++	-	+	++
D_5	+++	+	++	+++	++	-	+	++
D_6	+++	+++	+	+++	+++	-	-	++
H_1	+++	++	++	+++	+++	-	-	+
H_2	+++	++	+	+++	++	+	++	++
H_3	+++	+++	++	++	+++	-	+	++
H_4	+++	+	++	+++	++	-	+	++

H1 = Isolate from healthy calf of dairy farm; H2 = Isolate from healthy calf of Char Nillokhiya; H3 and H4 = Isolate from healthy calf of veterinary clinic; D1 = Isolate from diarrhoeic calf of BAU dairy farm; D2, D3 and D4 = Isolates from diarrhoeic calf of veterinary clinic; D5 and D6 = Isolates from diarrhoeic calves of Char Nillokhiya; CIP = Ciprofloxacin; AML = Amoxycillin; P = Penicillin; SP = Spiramycin; OT = Oxytetrcycline; GN = Gentamycin; S = Streptomycin; RL = Sulphamethoxazole; (+++) = Highly sensitive; (++) = Moderately sensitive; (+) = Less sensitive; (-) = Resistant.

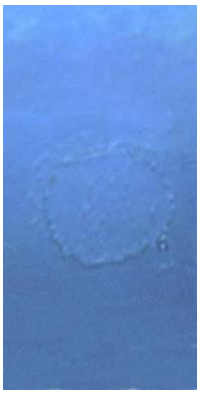

Fig. 4. Serum agglutination test of isolate no. D4.

Fig. 5. Inoculation of broth culture produced death in day old mice.

Within 24-72 hours of inoculation, the inoculated mice which died revealed symptoms of weakness, emaciation and diarrhoea before death. The other mice did not reveal any signs of illness and survived finally. No symptoms or death was observed in any of the control group of animal. On postmortem examination gross pathological lesions observed in almost all the white mice were more or less similar (Figure 6). The animals those died comparatively earlier revealed the congestion of blood vessels at the site of inoculation. In most of the animals pin point hemorrhages and congestion were commonly observed in liver, lung, kidney and heart muscle. Focal necrosis in liver and distention of gall bladder with bile was observed in animals which died after 24 hours of inoculation. The animals those died even later period of inoculation revealed the presence of hemorrhage in the intestine, watery faeces, excessive serosanguinous peritoneal fluid in many cases but in rare cases accumulation of fluid in the pericardium was revealed. The organism was reisolated from the most of the specimens like peritoneal fluid, heart muscle, blood, liver, lung, gall bladder, intestinal contents etc. of the animals died after inoculation (Table11).

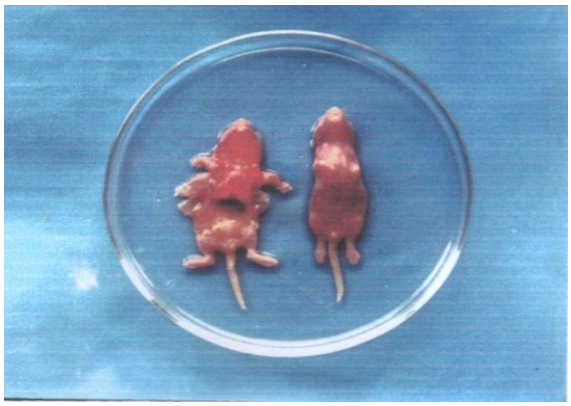

Fig. 6. Post mortem examination of dead mice.

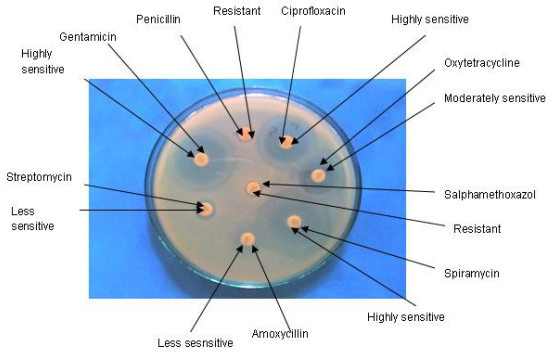

Fig. 7. Result of antibiogram study of isolate no. D1 on nutrient agar media.

From the antibiogram study, it was revealed that all the isolates of Char Nillokhyia (isolate no. H2, D5 and D6) were highly sensitive to ciprofloxacin and gentamicin (Table 12). Isolate no.D5 was moderately sensitive to spiramycin, amoxycillin and oxytetrcycline whereas less sensitive to streptomycin and sulphamethoxazole (Table 12). All the isolates of Char Nillokhyia except H2 were resistant to penicillin (Table 12). In addition to the isolates of Char Nillokhyia all other isolates were also highly sensitive to ciprofloxacin and gentamicin. Most of the isolates were resistant to penicillin and Sulphamethoxazole.

Among the isolates of BAU veterinary clinic (D2, D3, D4, H3 and H4) D2, D3 and H4 were highly sensitive to ciprofloxacin and gentamicin; H3 and D4 were moderately sensitive to gentamycin and amoxicillin (Table 12). All the isolates were moderately sensitive to oxytetracycline whereas resistant to penicillin. Most of the isolates of BAU veterinary clinic were less sensitive to Sulphamethoxazole. Isolate no.D2 was resistant to Sulphamethoxazole (Table 12).

In the present study, it was found that isolate no.D1 was highly sensitive to Ciprofloxacin, Spiramycin, gentamycin; moderately sensitive to Oxytetrcycline (Figure 7). Both isolate D1 and H1 were resistant to penicillin and Sulphamethoxazole (Table 12).

Above all most of the isolates were highly sensitive to ciprofloxacin, gentamycin and spiramycin, moderately sensitive to amoxycillin, streptomycin and oxytetracycline and nearly resistant to penicillin and slulphamethoxazole (Table 12). This finding satisfy the result of Sato *et al.,* 1975; Chugh and Suheir, 1983; Zhang *et al.*,1998; Banani *et al*., 2003 and Kobayashi *et al.,* 2007. The present study will provide an overall idea about the antibiotic sensitivity pattern of the local *salmonella* isolate which will guide the Veterinarians and Physicians in selecting the most effective drug against Salmonellosis in both human and animals.

Acknowledgements

The authors gratefully acknowledged to the authority of Bacteriology laboratory of the department of Microbiology and Hygiene, Bangladesh Agricultural University, Bangladesh for providing the facilities to carry out this research work.

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