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Isolation, phage typing & antibiogram of *Salmonella* from man & animals in northeastern India

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***Background & objectives*: *Salmonella* is an important zoonotic pathogen and its prevalence in the animals acts as a continuous threat to man. The present study was carried out to report the isolation along with the serotypes, phage types and antibiogram pattern of *Salmonella* among man, livestock and poultry in the northeastern India.**

***Methods*: A total of 654 samples from diarrhoeic livestock and humans were processed for the isolation of *Salmonella*. All the isolates were subjected to antibiogram studies against 15 antimicrobials. Representative isolates of *S.* Typhimurium and *S.* Enteritidis were phage typed.**

***Results*: Ninety five isolates of *Salmonella enterica* belonging to 5 serotypes- *S.* Typhimurium, *S.* Enteritidis, *S.* Gallinarum, *S.* Paratyphi B and *S.* Bareilly were obtained with an overall prevalence rate of 14.40 per cent. *S.* Typhimurium isolates were distributed among four phages- DT003, DT004, DT096 and DT193 and all the *S.* Enteritidis isolates belonged to a single phage type, PT13a/7. Interspecies sharing of the phages was observed. Norfloxacin, enrofloxacin, gentamycin and ciprofloxacin were most effective, whereas, doxycycline, ampicillin, amoxycillin and tetracycline were relatively less effective.**

***Interpretation & conclusion*: Our findings showed that three of the five serovars as well as some of the phage types of these serovars were shared by animals and humans indicating the zoonotic potential of the organism. Thus, it is imperative that salmonellosis control measures adopted for humans should give adequate importance to its control in the animals particularly their products.**

Key words Antibiogram - humans - livestock - phage typing - prevalence - salmonellosis

Enteric *Salmonella* infection is a global problem both in man and animals, and has been attributed to be the most important bacterial aetiology for enteric infections worldwide[1]. Salmonellosis is endemic in India and its importance, as potential zoonosis needs no emphasis. Depending upon the species predilection of individual *Salmonella* serovars, these are either grouped as host adapted (*e.g.*, *S.* Typhi in humans, *S.* Choleraesuis in pigs, *etc*.) or non host adapted (*e.g.*, *S.* Typhimurium, *S.* Enteritidis, *etc*.).

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Although reports are available on the prevalence of salmonellosis in livestock and poultry in the region[2,3], information regarding involvement of the non host adapted serotypes in humans in this region is meager and needs to be evaluated in relation to their prevalence in livestock to get a clear epidemiological picture. This study was undertaken to describe the prevalence of *Salmonella* among humans and various livestock and poultry in the northeastern region along with their phage typing and antibiogram.

Material & Methods

Collection and processing of samples: During April 2003 to April 2004, stool samples from patients with diarrhoea were collected from diagnostic laboratories in Guwahati and Shillong. Cloacal swabs from diarrhoeic poultry and rectal swabs from diarrhoeic pigs and cattle (Table I) from various organized, unorganized and government farms in Arunachal Pradesh, Meghalaya and Assam were collected aseptically in sterile test tubes and immediately brought to the laboratory for processing. Kauffmann tetrathionate broth (TTB) (Oxoid Ltd., UK) containing brilliant green in the final concentration of 1:1,00,000 was used as a selective enrichment broth for primary isolation of Salmonella. Brilliant green agar (BGA) (Oxoid Ltd., UK) was used as selective medium for primary isolation and MacConkey's lactose agar (MLA) (Oxoid Ltd., UK) was used for purification of suspected colonies. Characterization and preliminary identification of suspected *Salmonella* cultures were made on the basis of morphology, cultural characteristics, and biochemical reactions[4]. Agglutinability of the suspected *Salmonella* culture with *Salmonella* polyvalent 'O' sera (BBL laboratories, USA) was tested using slide agglutination test[5].

Serotyping and phage typing of the organisms: The isolated strains of *Salmonella* were serotyped at National Salmonella and Escherichia Centre, Central Research Institute, Kasauli, Himachal Pradesh (India). Of the confirmed and serotyped isolates (n=95), 40 comprising 27 *S.* Typhimurium and 13 *S.* Enteritidis were phage typed at Robert Koch Institute, Germany.

Antibiotic sensitivity test: *In vitro* susceptibility of the organisms to various antimicrobial agents was determined by the disc diffusion technique[5]. The antimicrobial agents (concentration in μg) used were: ampicillin (Ap, 25), amoxycillin (Am, 10), cephalexin (Cp, 30), chloramphenicol (Ch, 30), chlortetracycline (Ct, 30), ciprofloxacin (Cf, 30), doxycycline (Do, 10), enrofloxacin (Ex, 10), gentamicin (Gm, 30), kanamycin (Km, 10), nalidixic acid (Na, 30), nitrofurantoin (Nf, 100), norfloxacin (Nx, 10), tetracycline (Tc, 30), and trimethoprim (Tr, 30) (Hi-Media Laboratories, Mumbai, India).

Results & Discussion

There are more than 2500 *Salmonella* serovars distributed throughout the world; some of these *viz*., *S.* Typhi, *S.* Gallinarum, *S.* Dublin and *S.* Choleraesuis are host specific, the majority are non adapted and can cause infection in man and animals alike[6]. In the present study, 95 (14.5%) *Salmonella* isolates belonging to 5 serovars- *S.* Typhimurium, *S.* Enteritidis, *S.* Gallinarum, *S.* Paratyphi B and *S.* Bareilly were isolated from 654 samples from diarrhoeic cases in humans, poultry, pigs and cattle (Table I). Non-typhoidal salmonellosis is an important enteric infection in humans, particularly in the neonates and younger children[7]. It has been estimated that approximately 13 million cases of salmonellosis occur worldwide annually, of which about 70 per cent reports come from China, India and Pakistan[8]. In this study, 23 (20.5%) *Salmonella* were recovered from 112 human stool samples examined. The potential hazard of the organism can be viewed from the fact that virtually one in every five stool samples collected from diarrhoeic patients was positive for *Salmonella*. All the salmonellae isolated from humans in the present study, *viz*., *S.* Enteritidis, *S.* Typhimurium and *S.* Paratyphi B were non host-specific and their role in human salmonellosis has been well established[9,10].

Poultry is known to be the largest single reservoir of *Salmonella*[11]. In the present investigation, 34 (14.7 %) of the 231 cloacal swab samples from diarrhoeic birds showed presence of *Salmonella*. The isolation rate was higher than that reported by other workers in the region[2,3], and could be attributed to the fact that the swabs from only the diarrhoeic birds were processed in our study. Four serovars, *viz*., *S.* Typhimurium (35.2%), *S.* Gallinarum (35.2%), *S.* Enteritidis (23.5%) and *S.* Paratyphi B (5.8%) were recorded. *S.* Gallinarum, the causative agent of fowl typhoid, is the most prevalent host-adapted *Salmonella*

Table I. Isolation of *Salmonella* from human and animals

Source	No. of samples	No. positive for *Salmonella* (%)	Serotypes of *Salmonella* isolated	
			Serotypes	No. of isolates
Human (stool samples)	112	23 (20.5)	*S.* Typhimurium	10
			S. Enteritidis	11
			S. Paratyphi B	2
Poultry (cloacal swabs)	231	34 (14.7)	*S.* Typhimurium	12
			S. Gallinarum	12
			S. Enteritidis	8
			S. Paratyphi B	2
Piglets (rectal swabs)	176	25 (14.2)	*S.* Typhimurium	12
			S. Enteritidis	11
			S. Paratyphi B	1
			S. Bareilly	1
Cattle (rectal swabs)	135	13 (9.6)	*S.* Typhimurium	5
			S. Enteritidis	6
			S. Bareilly	2
Total	654	95 (14.5)		95

strain in India[11]. *S.* Typhimurium and *S.* Enteritidis are not only involved in severe outbreaks of avian salmonellosis and economic losses to the poultry industry[12,13], these serovars also pose a definite zoonotic hazard as poultry are known to be the major transmitters of non host-adapted salmonellosis in humans[14]. The northeastern region of India has a sizeable pig population and there are numerous reports on the isolation of *Salmonella*[15,16]. Of the 176 samples from pigs, *Salmonella* was recovered in 25 (14.2%) samples. The isolates recovered from pig samples were *S.* Typhimurium (12) and *S.* Enteritidis (11), *S.* Paratyphi B (1) and *S.* Bareilly (1) (Table I). Various workers have documented the significance of *S.* Typhimurium[15,16], *S.* Enteritidis[17] and *S.* Paratyphi B[15] in porcine diarrhoea. Pigs are easily exposed to *Salmonella* owing to their scavenging habit and consequently act as an important reservoir and a source of human salmonellosis in many parts of the world. A total of 13 (9.7%) *Salmonella* isolates were recovered from cattle diarrhoeic rectal swabs comprising *S.* Enteritidis (6), *S.* Typhimurium (5), and *S.* Bareilly (2). *S.* Bareilly, isolated from pigs and cattle in the study has also been reported to be involved in salmonellosis outbreaks in the children[18].

One of the characteristic features observed during the study was that human as well as the livestock and poultry in the region shared most of the serovars indicating the potential hazard of interspecies sharing of these organisms. It has been reported that livestock and their products can contribute to as much as 96 per cent of the total *Salmonella* infection in humans[19]. Involvement of these serovars emphasizes the need to control their transmission from one generation to the next and horizontal spread within the herds/flocks as well as interspecies transmission[20].

The phage typing results of the representative isolates of *S.* Typhimurium and *S.* Enteritidis revealed that of the 27 *S.* Typhimurium isolates, four were untypable and of the remaining, nine belonged to phage type DT193, seven to DT003, six to DT004, and one human isolate belonged to phage type DT096 (Table II). Though phage type DT003 was found to be present in both human and poultry isolates, phage types DT004 and DT193 were found in poultry, pigs and cattle. Presence of phage type DT003 in poultry has also been reported earlier[21]. The sharing of phage types among various species indicated the interspecies transmission of organism[22,23] and re-emphasised the need to control salmonellosis at every step. All the 13 isolates of *S.* Enteritidis (six from humans, three from poultry and four from pigs) were found to belong to a single phage type PT 13a/7. The presence of this phage type among different species was also reported by other workers[21,23,24].

Table II. Phage typing pattern of *Salmonella* isolated from various sources

Serotype	Source	No. of isolates phage typed	Phage type	No. of isolates
S. Typhimurium	Human	6	DT003	5
			DT096	1
	Poultry	12	DT003	2
			DT004	2
			DT193	5
			UT	3
	Pig	6	DT004	4
			DT193	2
	Cattle	3	DT 193	2
			UT	1
S. Enteritidis	Human	6	PT13a/7	6
	Poultry	3	PT13a/7	3
	Pig	4	PT13a/7	4
Total		40		40

UT, Untypable

In recent years, antibiotic resistance in *Salmonella* has assumed alarming proportions worldwide[19,25]. Monitoring drug resistance pattern among the isolates not only gives vital clues to the clinician regarding therapeutic regime to be adopted against individual cases, but is also an important tool to devise a comprehensive chemoprophylactic and chemotherapeutic drug schedule on herd basis within a geographical area. In the present study, highest number of isolates showed resistance against doxycycline (58; 61.05 %), followed by ampicillin (49; 51.57%), amoxycillin (43; 45.26%) and tetracycline (42; 44.21%), chlortetracycline (36; 37.89%), nitrofurantoin (40; 42.11%), chlortetracycline (36; 37.89%), kanamycin (31; 32.63%), cephalexin (24; 25.26%), nalidixic acid (17; 17.9%), chloramphenicol (15; 15.79%), trimethoprim (9; 9.5%), ciprofloxacin (8; 8.4%), gentamicin (6; 6.3%), enrofloxacin (2; 2.1%) and norfloxacin (1; 1.1%) (Table III). All the human isolates were resistant to at least one of the 15 antibiotics tested. This could be due to the wide and varied use of different antibiotics by human patients with simultaneous evolution of newer antibiotics that have precipitated into pathogens of multiple drug resistance. Moreover, the presence of antibiotic residues in foods of animal origin may result in increased drug resistance amongst human isolates[25].

Serotypic variation in drug sensitivity was shown by the isolates in the present study. In addition to the quinolone group of antibiotics, *S.* Typhimurium was also observed to be sensitive against gentamicin, trimethoprim and chloramphenicol in the present study and it showed higher resistance against doxycycline, amoxycillin, chlorteracycline, tetracycline and kanamycin. Antibiotic resistance has been reported to be more common in Typhimurium than the other serovars[24]. Resistance to some of the aminoglycosides such as kanamycin is of great concern since it has been a major drug of choice in the treatment of enteric infections[27]. *S.* Gallinarum showed high resistance to tetracycline, nitrofurantoin, ampicillin, chloramphenicol, amoxycillin and nalidixic acid. *S.* Gallinarum has been the major host specific serotype affecting the poultry for decades[6]. Most of these antibiotics are added in the poultry feed as supplements and the obvious lack of control on the antibiotic usage may be the probable cause for their high resistance[25]. *S.* Enteritidis showed higher resistance to doxycycline, ampicillin, amoxycillin and nitrofurantoin. Least resistance by the isolates in the present study was observed in case of norfloxacin, enrofloxacin and ciprofloxacin. The findings are concurrent to the observations of other workers[27,28]. Fluoroquinolone group of antibiotics have rapid and prompt bactericidal action at a very low minimum

Table III. Antibiotic resistance pattern of *Salmonella* isolates from various sources

	No.	No. of *Salmonella* isolates resistant to various antibiotics														
		Nf	Nx	Km	Do	Ex	Ap	Ct	Cp	Gm	Tc	Am	Na	Ch	Cf	Tr
Poultry:																
S. Typhimurium	12	8	0	9	9	0	8	10	1	1	9	6	2	4	2	4
S. Gallinarum	12	10	0	5	3	0	9	6	4	0	11	7	7	8	0	1
S. Enteritidis	8	4	0	2	4	0	4	4	1	0	6	4	0	0	1	2
S. Paratyphi B	2	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
Total	34	22	0	17	16	0	21	20	6	1	26	17	9	12	3	7
Pigs:																
S. Typhimurium	12	0	0	0	7	0	3	6	1	1	7	1	0	0	0	0
S. Enteritidis	11	3	0	0	9	0	7	4	2	0	6	8	3	0	0	0
S. Paratyphi B	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
S. Bareilly	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
Total	25	3	0	0	18	0	10	10	3	1	13	9	3	0	0	0
Humans:																
S. Typhimurium	10	2	1	1	3	2	3	1	2	1	0	4	2	2	2	2
S. Enteritidis	11	7	0	2	10	0	9	2	6	3	1	8	2	0	1	0
S. Paratyphi B	2	0	0	1	2	0	1	0	2	0	1	1	0	0	2	0
Total	23	9	1	4	15	2	13	3	10	4	2	13	4	2	5	2
Cattle:																
S. Typhimurium	5	2	0	3	4	0	2	2	3	0	1	1	1	1	0	0
S. Enteritidis	6	4	0	6	5	0	3	1	2	0	0	3	0	0	0	0
S. Bareilly	2	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
Total	13	6	0	10	9	0	5	3	5	0	1	4	1	1	0	0
Total	95	40	1	31	58	2	49	36	24	6	42	43	17	15	8	9

Nf, nitrofurantoin; Nx, norfloxacin; Km, kanamycin; Do, doxycycline; Ex, enrofloxacin; Ap, ampicillin; Ct, chlortetracycline; Cp, cephalexin; Gm, gentamicin; Tc, tetracycline; Am, amoxycillin; Na, nalidixic acid; Ch, chloramphenicol; Cf, ciprofloxacin; Tr, trimethoprim

inhibitory concentrations against salmonellae[29]. However, caution is warranted against their indiscriminate use as is evident from the resistance problems being faced by many developed countries, where more than ten-fold increased resistance has been observed against the quinolones during a two decade study[30]. Excessive or inappropriate use of antibiotics in the rearing of farm animals represents a major factor in the emergence, persistence and spread of resistant salmonellae even in the humans who are the *cul-de-sac* of the food chain[26,27]. Hence, it is imperative that judicious use of antibiotics in the treatment and prophylaxis, after *in vitro* testing, be practiced to sustain the usefulness of the antibiotics in controlling salmonellosis on long-term basis.

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