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Full Length Research Paper

Incidence and antibiotic resistant profiles of pathogenic *Salmonella* spp. from different environmental and food samples

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Salmonella **bacteria cause one of the leading foodborne infections resulting in gastroenteritis and salmonellosis in people of all ages. They also cause severe invasive disease in infants, elderly persons and immuno-compromised persons. Here, we report the isolation, identification and antibiotic resistant pattern of *Salmonella enterica* isolated from water and sediment samples collected from coastal and river sources as well as poultry and seafood samples from retail shops in Tamil Nadu, India. Sampling was done at different intervals. All the samples were primarily enriched with Selenite F broth and plated on Xylose Lysine Deoxycholate (XLD), Deoxycholate Citrate Agar (DCA) and Brilliant Green Agar (BGA). Out of the 121 samples screened for the presence of *S. enterica*, 97 (80%) samples were found positive. From the 503 suspected *S. enterica* isolates screened, only 402 (80%) strains were confirmed as *S. enterica* through phenotypic characters. However, molecular approaches showed that 371 (74%) isolates were *S. enterica*. Fresh water sample (113), poultry meat (90) and seafood sample (71) had the highest number of *S. enterica* contamination followed by fresh water sediment (53), coastal water sample (46) and coastal sediment (29). Of all the strains, 371 strains (92%) possessed the gene *InvA* which helped in confirming the virulence factor through PCR molecular screening. Among these 23 (6%) isolates which exhibited haemolytic activity, antimicrobial susceptibility pattern was tested by the standard disc diffusion technique. A high level of resistance among all the strains was observed against Rifampicin, Vancomycin and Ampicillin. However, 63 (16%) isolates showed multidrug resistance against more than three antibiotics. Multidrug resistant and frequent isolation of *S. enterica* from various sources of samples are matters of serious concern as they pose threat to public health.**

Key words: *Salmonella enterica*, food borne infection, *InvA* gene, haemolytic activity, multi drug resistance.

INTRODUCTION

Salmonella enterica is considered as one of the major causes of foodborne infection worldwide (Voetsch et al., 2004; De Jong and Ekdahl 2006; Galanis et al., 2006). Currently *Salmonella enterica* comprises over 2500 serotypes and most of them are potentially pathogenic. It may be divided into two groups based on the pathogenic character such as human-restricted Typhoidal and food poisoning group or non-Typhoidal group. Typhoidal group consisting of *Salmonella enterica* Typhi, *Salmonella enterica* Paratyphi A and *Salmonella enterica* Paratyphi B is a host restricted human parasite causing typhoid fever. The food poisoning group, which is essentially an animal parasite can also infect human beings causing gastroenteritis, septicaemia and localized infection.

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Foodborne disease caused by *S. enterica* remains one of the most widespread diseases in industrialized as well as developing countries, even though the incidence varies between countries. A majority of *S. enterica* infection is attributed to consumption of contaminated poultry meat and seafood (Angulo et al., 2000; Raffatellu et al., 2008). These harbour a wide range of *S. enterica* serotypes, which can infect wide range of hosts and frequently reach the human food chain causing foodborne illness. *Salmonella enterica* Typhimurium and *Salmonella enterica* Enteritidis were the ones most frequently isolated from many cases all over the world (WHO, 2005). A number of investigations conducted in India reported that *S. enterica* is most prevalent in seafood (Nambiar and Iyer, 1991; Hatha and Lakshmanaperumalsamy, 1997; Shabarinath et al., 2007; Kumar et al., 2008).

In recent years, distribution of multidrug resistant *S. enterica* in food sample has increased worldwide (WHO, 2000). This made treatment of Salmonellosis difficult due to therapeutic failures (Kare et al., 1999; Zahurul et al., 2003). This may be due to improper and repeated use of antibiotic in humans and animals for treatment purposes (Choi et al., 2005; Wang et al., 2006; Frank et al., 2007; Lassmann et al., 2007; Khan et al., 2009; Vo et al., 2010). These increase the mortality and morbidity leading to high economic impact.

In view of increasing concern about the antibiotic resistance, research has been focused on the alternative to antibiotic treatment to reduce the pathogen load in food. In this connection, bacteriophage studies have been initiated. Application of bacteriophage is a preventive step in the preharvest stage of food production. Phage exhibits high degree of specificity against the target bacteria. Particularly, gastrointestinal microbial populations such as *Salmonella enterica*, *Campylobacter* spp, *Listeria* spp and *E. coli O157:H7* get eliminated by using phage (Smith and Huggins, 1983; Alisky et al., 1998; Loc Carrillo et al., 2005). The present investigation was undertaken to isolate, identify and characterize *S. enterica* from various samples such as poultry meat, seafood and environmental samples and an attempt was also made to control *S. enterica* by using bacteriophage.

MATERIALS AND METHODS

Sample collection

Over a period of six months, a total of 121 samples including poultry meat (18), seafood (23) and environmental (80) samples (water and sediment) were collected. Twenty water and sediment samples each were collected from coastal and fresh water environments. Meat and seafood samples were procured from retail shop and fish market at Kanchipuram and the environmental samples were collected from different places such as Chennai, Kanchipuram, Mahabalipuram, Kovalam and Pichavaram mangrove in Tamil Nadu.

Isolation and identification of *Salmonella enterica*

All the samples were analysed for the presence of *S. enterica* following the method given in Food and Drug Administration (USFDA, 2011). Twenty five gram of meat, seafood and sediment samples were aseptically weighed and pre-enriched with buffered peptone water. For water samples, 25 ml was inoculated into 225 ml of pre-enrichment broth. After 16-20 h of incubation at 37°C, 0.2 ml of sample was transferred aseptically into 10 ml Selenite F broth for enrichment and incubated for 18-24 h at 37°C. Two or three loopful of culture were streaked onto different selective media such as Xylose Lysine Deoxycholate Agar (XLD), Deoxycholate Citrate Agar (DCA) and Brilliant Green Agar (BGA), and incubated for 24 h at 37°C. Suspected *S. enterica* colonies were biochemically characterized following the WHO protocol (WHO, 2010), which included Triple Sugar Iron agar reaction (TSI), Urease production assay, Lysine Iron agar reaction (LIA), Motility Indole Ornithine test (MIO) and Citrate Utilization test. All the biochemically confirmed *Salmonella enterica* isolates were again confirmed for the presence of invasive (*InvA*) gene through PCR identification molecular method using *InvA* specific primers *InvA* F-5'-GTGAAATTATCGCCACGTTCGGGCAA-3' and *InvA* R-5'-TCATCGCACCGTCAAAGGAACC-3' (Rahn et al., 1992). The reference strain of *S. enterica* serovar Typhimurium was used as positive control in the present study. This was kindly donated by Dr. T. Ramamurthy, Deputy Director (Senior Grade) of NICED, Kolkata, India. A negative control containing the same reaction mixture except the DNA template was included. Amplified PCR products were separated on a 1.5% agarose gel, stained with ethidium bromide (0.5 µg/ml) and DNA images were taken using a gel documentation system (Gel Doc 2000, Bio-Rad).

Determination of antibiotic resistant pattern

All the confirmed *S. enterica* isolates were tested for the presence of antibiotic resistant pattern using disc diffusion technique according to National Committee for Clinical Laboratory Standard (NCCLS, 2004). Fresh bacterial isolates were inoculated in 0.8% NaCl suspension to a turbidity equivalent to 0.5 McFarland Standard. Using a sterile cotton swab, the culture was swabbed onto the Muller Hinton Agar (MHA) plate. The antibiotics used to determine the resistant pattern of *S. enterica* isolates were Aztreonam (30 mcg), Cefotaxime (30 mcg), Rifampicin (5 mcg), Tetracycline (30 mcg), Vancomycin (30 mcg), Ampicillin (10 mcg), Nalidixic acid (30 mcg), Ciprofloxacin (5 mcg) and Trimethoprim (25 mcg).

Haemolytic activity of *Salmonella enterica* isolates

Haemolytic activity patterns of all the isolates were determined by using blood agar plate supplemented with blood erythrocytes. The test isolates were spot inoculated on to blood agar plates and incubated at 37°C for 18-24 h and the plates were examined after incubation for the haemolysin production.

Bacteriophage isolation

Waste samples were collected from local fish market in the study area and bacteriophage was enriched by using Decca strength broth. The enrichment mixture was centrifuged at 5000 rpm for 10 min and the supernatant was filtered through membrane filter. A combination of 1 ml filtrate and 100 µl of *S. enterica* (10^7) was added to the soft agar. Then, the soft agar was overlaid onto the Trypticase Soy Agar (TSA) and the plates were incubated at 37°C. After 6-8 h of incubation, the plates were checked for plaque formation (Atlas et al., 1995).

Screening of phage

Screening of phage was done by using 2 g of fish which were taken in two beakers separately, one beaker containing raw fish and another containing fish with phage filtrate. Both were incubated at 37°C for 24 h then homogenized and plated in selective media by using spread plate technique. After the incubation period, the plates were compared for the presence of phage.

RESULTS

Distribution of *S. enterica* in water, sediment, poultry meat, fish and crustaceans is presented in Table 1. Out of the 121 samples examined for the presence of *S. enterica*, only 97 samples were positive for *S. enterica*. The break up was poultry meat (18), fresh water sediment (19), fresh water (18), coastal water (16), coastal water sediment (16) and seafood sample (10). Among the 6 categories of samples, poultry meat, fresh water and fresh water sediment sample harboured high number of *S. enterica*. Low incidence of *S. enterica* was found in seafood samples. Out of the 503 isolates, 402 strains were confirmed as *S. enterica* through phenotypic characteristics. However, only 371 strains possessed *InvA* gene, which is virulence as well as identification character through molecular technique (Figure 1).

Antibiotic resistant pattern of *Salmonella enterica* isolates

The pattern of resistance to various antimicrobial substances of *S. enterica* isolates is given in Tables 2 and 3. All the isolates were tested against nine antibiotics and almost all isolates were found to have resistance against Rifampicin, Vancomycin and Ampicillin. No resistance pattern was observed in Ciprofloxacin and Trimethoprim. For the remaining antibiotics, the isolates showed different resistant patterns.

Haemolytic activity

All the isolates were screened for their ability to lyse blood erythrocytes in blood agar plates. Totally, 23 isolates exhibited haemolytic activity. Among the selected strains, 11 isolates showed alpha haemolytic activity, 12 showed beta haemolysis and the remaining isolates failed to produce haemolysin in blood agar plates.

Screening of bacteriophage

Bacteriophage against the selected *S. enterica* isolates was done. The results are presented in Figure 2A. Plates with phage isolates showed less number of colonies when compared to the plate without phage isolates. Figure 2B clearly shows that phage had a control over *S. enterica*.

DISCUSSION

The purpose of this study was to assess the *S. enterica* prevalence in food, water and other environmental samples. In the present investigation, a total of 121 poultry meat, seafood, water and sediment samples were tested for the occurrence of *S. enterica*. The distribution of *S. enterica* was predominant in the meat (poultry) sample collected from retail shop as well as water and sediment collected from fresh water area. This is in strong agreement with the findings of Davies and Evison (1991) and Guard-Petter (2001). This might be due to the secondary contamination by the handlers. Meat and environmental samples are considered as a major reservoir of *S. enterica* (Tauxe et al., 1997; Angulo et al., 2000; Hsueh et al., 2004; Barrow et al., 2012). In both developing and industrialized countries, the incidence of *S. enterica* infection in humans and the prevalence of *S. enterica* in many food products have increased significantly (Saha et al., 2001). Byrd et al. (2002) reported that transportation and processing could be the main source of cross contamination in addition to the carrier state. Many *S. enterica* serovar have been frequently isolated from natural environment such as soil, estuarine environment contaminated by humans and animal feces (Baudart et al., 2000; Winfield and Groisman, 2003; Abulreesh et al., 2005). In the present study, 43% of seafood samples were found to be contaminated with *S. enterica*. This is because of secondary contamination by *S. enterica* as they are frequently handled when transported from one place to another and the use of poor quality ice, water and some other raw materials (Badonia et al., 1988; Hatha and Lakshmanaperumalsamy, 1997).

Table 1. Distribution of *Salmonella* in different samples.

S/N	Source (N)	Type of sample examined	No. of positive sample obtained	Total number of isolates		
				***Salmonella* suspected isolates**	**Biochemically confirmed *Salmonella***	**Molecular confirmed *Salmonella***
1	Water sample (40)	Coastal water (20)	16 (80%)	56	46	40
		Mahabalipuram (10)	8 (80%)			
		Kovalam (10)	8 (80%)			
		Fresh water	18 (90%)	142	113	107
		River water[a] (10)	8 (80%)			
		Pond water[a] (10)	10 (100%)			
2	Sediment sample (40)	Coastal water	16 (80%)	37	29	27
		Mahabalipuram (10)	8 (80%)			
		Kovalam (10)	8 (80%)			
		Fresh water	19 (95%)	66	53	48
		River water[a] (10)	9 (90%)			
		Pond water[a] (10)	10 (100%)			
3	Seafood sample[b] (23)		10 (43%)	89	71	66
		Finfishes (13)	6 (46%)			
		Crustacean (10)	4 (40%)			
4	Meat[c] (18)	Poultry (18)	18 (100%)	113	90	83
	Total (121)		97 (80%)	503	402	371

[a]River and pond water collected from Chennai and Kanchipuram.
[b]Seafood sample collected from fish market in Kanchipuram.
[c]Meat sample collected from retail meat shop in Kanchipuram.

Multidrug resistant *S. enterica* remain a matter of deep concern in disease control program and it is predominantly isolated from chicken with *Salmonellae* bacteraemia infection (Kariuki et al., 2002; Mwangi et al., 2002). In the present study, all isolates showed resistance to at least three antibiotics and most of the multidrug resistant isolates were obtained from poultry and seafood samples. The multidrug resistant strain was found resistant against more than three tested antibiotics. All isolates were sensitive to Ciprofloxacin and Trimethoprim. This could be due to exhaustive use of the first group of antimicrobials like Ampicillin, Tetracycline, Rifampicin and Vancomycin, while the other group is a newly introduced one in the medical field, and not used for animal treatment. In the previous study, most of the *S. enterica* was found resistant to Streptomycin, Ampicillin, Tetracycline, Spectinomycin and Sulfisoxazole (Zewdu and Cornelius, 2009). The prevalence of antibiotic

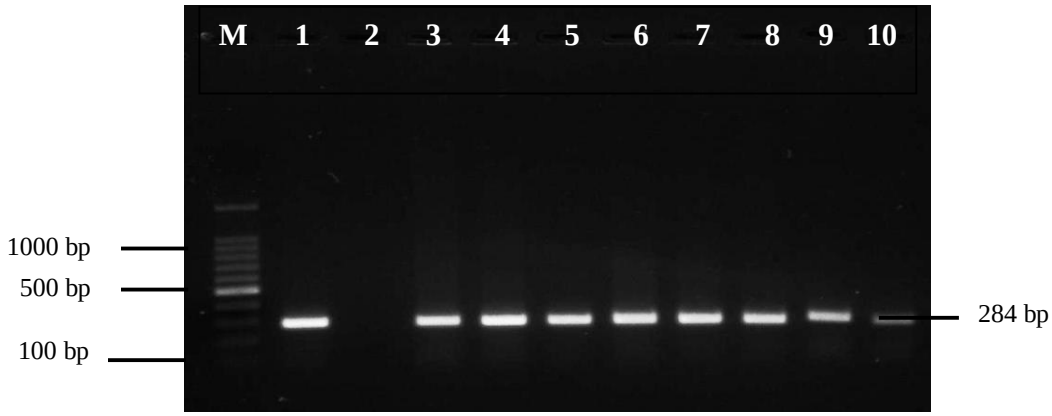

Figure 1. Simplex PCR analysis for identification of *Salmonella enterica* isolated from different food and clinical samples. Lane M - DNA marker (100 bp); Lane 1: *Salmonella enterica* serovar Typhimurium - Reference strain (positive control); Lane 2: Reaction mixture without template DNA (Negative control); Lanes 3 and 4: *Salmonella enterica* isolates from poultry samples; Lanes 5 and 6: *Salmonella enterica* isolates from fresh water and sediment samples; Lanes 7 and 8: *Salmonella enterica* isolates from coastal water and sediment samples; and Lanes 9 and 10: *Salmonella enterica* isolates from seafood samples.

Table 2. Frequency of antibiotic resistance in *Salmonella* isolated from different environmental samples.

S/N	Antibiotics	Coastal water (46)	Fresh water (113)	Coastal sediment (29)	Fresh water sediment (53)
1	Aztreonam	0	19 (17)	0	8 (15)
2	Cefotaxime	0	0	0	0
3	Rifampicin	46 (100)	113 (100)	29 (100)	53 (100)
4	Tetracycline	11 (25)	37 (33)	8 (25)	8 (15)
5	Vancomycin	46 (100)	113 (100)	29 (100)	53 (100)
6	Ampicillin	46 (100)	113 (100)	29 (100)	53 (100)
7	Nalidixic acid	3 (6)	44 (39)	5 (17)	28 (53)
8	Ciprofloxacin	0	0	0	0
9	Trimethoprim	0	0	0	0

Table 3. Frequency of antibiotic resistance in *Salmonella* isolated from different raw food samples.

S/N	Antibiotics	Seafood (71)	Meat (90)	Total (%)
1	Aztreonam	7 (10)	40 (44)	47 (29)
2	Cefotaxime	57 (80)	45 (50)	102 (63)
3	Rifampicin	71 (100)	90 (100)	161(100)
4	Tetracycline	36 (50)	30 (33)	66 (41)
5	Vancomycin	71 (100)	90 (100)	161 (100)
6	Ampicillin	71 (100)	90 (100)	161 (100)
7	Nalidixic acid	57 (80)	55 (61)	112 (70)
8	Ciprofloxacin	0	0	0
9	Trimethoprim	0	0	0

resistance is ecologically very important because it is associated with transferrable plasmid (R plasmid) and it may get transferred to different strains among the bacterial populations.

Detection of invasive and pathogenic *S. enterica* in food and water sample may pose serious health hazards. Invasive non-typhoidal *S. enterica* is frequently reported in Africa. It has emerged as the most common bloodstream infection in Sub-Saharan Africa and it causes mortality up to 25% (Feasey et al., 2012). The haemolytic activities have been related to the virulence in *S. enterica.* Of the tested isolates, 23 were positive for haemolytic activity and high prevalence was observed in poultry meat sample. However, if the poultry meat is consumed raw or uncooked, it leads to major public health concern.

The high incidence of these pathogens in commercially important species of sea food and meat is to be noted with concern and in public health point of view. In order to control multidrug resistance *S. enterica*, we tried phage

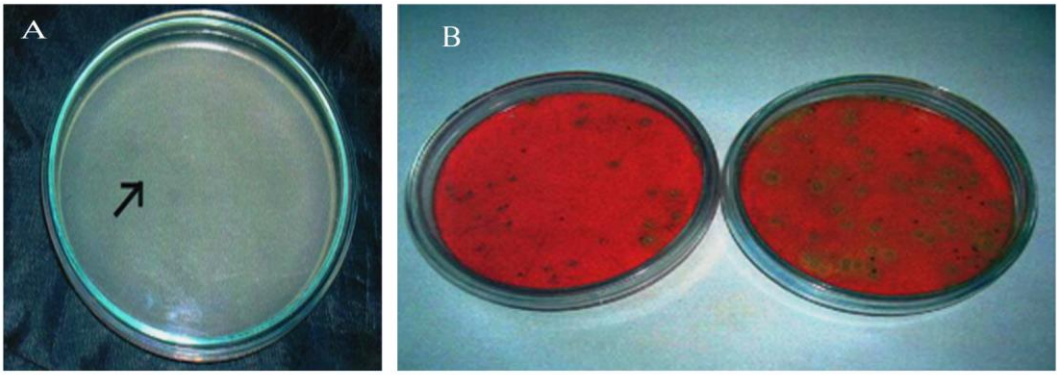

Figure 2. Growth of bacteriophage and their activity against *Salmonella enterica*. A. Bacteriophage in TSA; B. Control of *Salmonella* spp. by bacteriophage.

therapy. Few studies reported bacteriophage as a controlling agent for gastrointestinal disease causing bacteria (Higgins et al., 2006). Bacteriophages are natural compounds of gastrointestinal microbial population. It does not cause any side effect to humans (Bruttin and Brussow, 2005). Hence, it will be useful in the control of *S. enterica*. Further investigation to trace the load of human pathogen in general and *S. enterica* in particular in meat and seafood from all centres is highly imperative. It seems virtually impossible to protect raw seafood and meat from such defilement but with proper care in handling, processing and cooking, the “infection” can be controlled to a very large extent.

Conclusion

The present study concluded that *S. enterica* contamination in poultry meat and seafood is due to poor sanitation condition in slaughter houses in Tamil Nadu, which may lead to enteric fever and Salmonellosis. It also became clear that unless the ecology of the *Salmonella enterica* is fully understood, complete control of the disease will not be possible. To confirm the sources of these bacteria, further examination of the genetic traits of the isolates is needed.

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REFERENCES

Abulreesh HH, Paget TA, Goulder R (2005). Waterfowl and human-pathogenic bacteria at amenity sites: a review. Proceedings of the 3rd World Waterfowl Conference, 3-7 November, Guangzhou, China. 356-362. Published by World’s Poultry Sciences Association (WPSA), China Branch, South China Agricultural University.

Alisky J, Iczkowski K, Rapoport A, Troitsky N (1998). Bacteriophage show promise as antimicrobial agents. Journal of Infection., 36: 5-15.

Angulo FJ, Johnson KR, Tauxe RV, Cohen ML (2000). Origins and consequences of antimicrobial-resistant nontyphoidal *Salmonella*: implications for the use of fluoroquinolones in food animals. Microb. Drug Resist., 6: 77-83.

Atlas RM, Brown AE, Parks LC (1995). Experimental Microbiology Laboratory Manual, Mosby-Year Book Inc., printed in USA.565.

Badonia B, Ramachandran A, Sankar TV (1988). Quality problems in fish processing. J. Indian Fish. Assoc., 18: 283-287.

Barrow PA, Jones MA, Smith AL, Wigley P (2012). The long view: *Salmonella*– the last forty years. Avian pathology: J. WVPA., 41: 413–420.

Baudart J, Lemarchand K, Brisabois A, Lebaron P (2000). Diversity of *Salmonella* strains isolated from the aquatic environment as determined by serotyping and amplification of the ribosomal DNA spacer regions, Appl. Environ. Microbiol., 66: 1544–1552.

Bruttin A, Brussow H (2005). Human volunteers receiving *Escherichia coli* phage T4 orally: a safety test of phage therapy. Antimicrob. Agents Chemother., 49(7): 2874-2878.

Byrd JA, Hargis BM, Corrier DE, Brewer RL, Caldwell DJ, Bailey RH, McReynolds JL, Herron KL, Stanker LH (2002). Fluorescent marker for the detection of crop and upper gastrointestinal leakage in poultry processing plants. Poultry Science., 81(1): 70-74.

Choi SH, Woo JH, Lee JE, Park SJ, Choo EJ, Kwak YG, Kim MN, Choi MS, Lee NY, Lee BK, Kim NJ, Jeong JY, Ryu J, Kim YS (2005). Increasing incidence of quinolone resistance in human non-typhoid *Salmonella enterica* isolates in Korea and mechanisms involved in quinolone resistance. J. Antimicrob. Chemother., 56: 1111-1114.

De Jong B, Ekdahl K (2006). The comparative burden

of salmonellosis in the European Union member states, associated and candidate countries. BMC Public Health., 6(4): 1-9.
Davies CM, Evison, LM (1991). Sunlight and the survival of enteric bacteria in natural waters. J. Appl. Bacteriol., 70: 265-274.
Feasey NA, Dougan G, Kingsley RA, Heyderman RS, Gordon MA (2012). Invasive non-typhoidal *Salmonella* disease: an emerging and neglected tropical disease in Africa. Lancet., 379: 2489–2499.
Frank MA, Rene SH, Jana L, Katie G, Kathryn T, Patrick FM, David GW, Henrik H, Gitte S, Aroon B, Srirat P, Chaiwat P, Frederick JA, Peter G-S (2007). International Spread of Multidrug-resistant *Salmonella* Schwarzengrund in Food Products. Emerg. Infect. Dis., 13 (5):726–731.
Galanis E, Lo Fo Wong DM, Patrick ME, Binsztein N, Cieslik A, Chalermchikit T, Aidara-Kane A, Ellis A, Angulo FJ, Wegener HC (2006). Web-based surveillance and global *Salmonella* distribution, 2000-2002., Emerg. Infect. Dis., 12:381–388.
Guard-Petter J (2001). The chicken, the egg and *Salmonella* enteritidis. Environ. Microbiol., 3: 421-430.
Hatha AAM, Lakshmanaperumalsamy P (1997). Prevalence of *Salmonella* in fish and crustaceans from markets in Coimbatore, South India. Food Microbiol., 14: 111–114.
Higgins JP, Andreatti Filho RL, Higgins SE, Wolfenden AD, Tellez G, Hargis BM (2008). Evaluation of *Salmonella*-lytic properties of bacteriophages isolated from commercial broiler houses. Avian Dis., 52:139–142.
Hsueh PR, Teng LJ, Tseng SP, Chang CF, Wan JH, Yan JJ, Lee CM, Chuang YC, Huang WK, Yang D, Shyr JM, Yu KW, Wang LS, Lu JJ, Ko WC, Wu JJ, Chang FY, Yang YC, Lau YJ, Liu YC, Liu CY, Ho SW, Luh KT (2004). Ciprofloxacin-resistant *Salmonella enterica* Typhimurium and Choleraesuis from pigs to humans, Taiwan. Emerg. Infect. Dis., 10(1): 60-68.
Kare M, Dortelaubaggesen, Frank MA, Jens ME, Jorgen E, Kai F, Peter G-S, Andreas MP, Henrikc (1999). An outbreak of multidrug-resistant, quinolone-resistant *Salmonella enterica* serotype Typhimurium dt104. The New England J. Medicine. 341: 1420-1425.
Kariuki S, Revathi G, Gakuya F, Yamo V, Muyodi J, Hart CA (2002). Lack of clonal relationship between non-typhi *Salmonella* strain types from humans and those isolated from animals living in close contact. FEMS Immunol. Med. Microbiol., 33: 165–171.
Khan AA, Ponce E, Nawaz MS, Cheng CM, Khan JA, West CS (2009). Identification and characterization of Class 1 integron resistance gene cassettes among *Salmonella* strains isolated from imported seafood. Appl. Environ. Microbiol., 75(4):1192–1196.
Kumar Y, Sharma A, Sehgal R, Kumar S (2008). Distribution trends of *Salmonella* serovars in India (2001-2005). Trans. R. Soc. Trop. Med. Hyg., 103: 390-394.
Lassmann B, Gustafson DR, Wood CM, Rosenblatt JE (2007). Re-emergence of anaerobic bacteremias. Clin. Infect. Dis., 44:895–900.
Loc Carrillo C, Atterbury RJ, El-Shibiny A, Connerton PL, Dillon E, Scott A, Connerton IF (2005). Bacteriophage Therapy to Reduce *Campylobacter jejuni* Colonization of Broiler Chickens. Appl. Environ. Microbiol., 71(11): 6554-6563.
Mwangi I, Berkley J, Lowe B, Peshu N, Marsh K, Newton CR (2002). Acute bacterial meningitis in children admitted to a rural Kenyan hospital: increasing antibiotic resistance and outcome. Pediatr. Infect. Dis. J., 21: 1042–1048.
Nambiar VN, Iyer KM (1991). Distribution of *Salmonella* serotypes in fish in retail trade in Cochin. Fish. Technol., 28: 33–37.
National Committee for Clinical Laboratory Standards (NCCLS) (2004). Performance standards for antimicrobial susceptibility testing. Fourteenth informational supplement. Document M100-S14. Wayne, PA.
Raffatellu M, Wilson RP, Winter SE, Bäumler AJ (2008). Clinical pathogenesis of typhoid fever. J. Infect. Dev. Ctries., 2(4): 260–266.
Rahn K, DeGrandis SA, Clarke RC, McEwen SA, Galan JE, Ginocch OC, Curtissiii R, Gyles CL (1992). Amplification of an *invA* gene sequence of *Salmonella* Typhimurium by polymerase chain reaction as a specific method of detection of *Salmonella*. Molecular and Cellular Probes., 6: 271–279.
Saha MR, Saha D, Dutta P, Mitra U, Bhattacharya SK (2001). Isolation of *Salmonella enterica* Serotypes from Children with Diarrhoea in Calcutta, India. J. Health Popul. Nutr., 19(4): 301-305.
Shabarinath S, Kumar SH, Khushiramani R, Karunasagar I, Karunasagar I (2007). Detection and characterisation of *Salmonella* associated with tropical seafood. Int. J. Food Microbiol., 114: 227–233.
Smith HW, Huggins MB (1983). Effectiveness of phages in treating experimental *Escherichia coli* diarrhoea in calves, piglets, and lambs. J. Gen. Microbiol., 129: 2659-2675.
Tauxe R, Kruse H, Hedberg C, Potter M, Madden J, Wachsmuth K (1997). Microbial hazards and emerging issues associated with produce: a preliminary report to the national advisory committee on microbiologic criteria for foods. J. Food Prot., 60: 1400 –1408.
U.S. Department of Agriculture, Food Safety and Inspection Service (2011). Isolation and identification of *Salmonella* from meat, poultry, pasteurized egg and catfish products. MLG 4.05. Effective date 20 January 2011. U.S. Department of Agriculture, Food Safety and Inspection Service, Washington, DC.
Vo AT, Van Duijkeren E, Gaastra W, Fluit AC (2010). Antimicrobial resistance, class 1 integrons, and

genomic island 1 in *Salmonella* isolates from Vietnam. PLoS One., 5(2): e9440.1-8.
Voetsch AC, Van Gilder TJ, Angulo FJ, Farley MM, Shallow S, Marcus R, Cieslak PR, Deneen VC, Tauxe RV (2004). FoodNet estimate of the burden of illness caused by nontyphoidal *Salmonella* infections in the United States. Clin. Infect. Dis., 38(3): S127–S134.
Wang JY, Hwang JJ, Hsu CN, Lin LC, Hsueh PR (2006). Bacteraemia due to ciprofloxacin-resistant *Salmonella enterica* serotype Choleraesuis in adult patients at a university hospital in Taiwan, 1996–2004. Epidemiol. Infect., 134: 977–84.
Winfield E, Groisman A (2003). Role of non-host environments in the lifestyles of *Salmonella* and *Escherichia coli*. Appl. Environ. Microbiol., 69: 3687–3694.
World Health Organization (2000). Overcoming Antimicrobial Resistance. Available at: (http://www.who.int/infectious-diseasereport/2000/index.html).
World Health Organization (2010). Global Foodborne infections Network. Laboratory Protocol: “Biochemical identification of *Salmonella/Shigella* using an Abbreviated Panel of Tests” January.
World Health Organization. Global *Salmonella* Survey, Progress Report (2005). Accessed on Dec.16, 2011: http://www.who.int/salmsurv/links/GSSProgressReport2005.pdf.
Zahurul H, Asna SM, Ashraful Haq J, Mushfequr Rahman MD (2003). Nalidixic acid-resistant *Salmonella enterica* serovar Typhi with decreased susceptibility to ciprofloxacin caused treatment failure: a report from Bangladesh. Jpn. J. Infect. Dis., 56:32–33.
Zewdu E, Cornelius P (2009). Antimicrobial resistance pattern of *Salmonella* serotypes isolated from food items and personnel in Addis Ababa, Ethiopia. Trop. Anim. Health. Prod., 41: 241-249.