Isolation, plasmid profiling and antibiogram of *Salmonella* from poultry meat and environmental sources

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ABSTRACT

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 poultry meat and environmental sources in the India. A total of two hundred samples from poultry meat (100), poultry feaces (100) were processed for the isolation of *Salmonella*. All the isolates were subjected to antibiogram studies against 16 antimicrobials. Representative isolates of isolated *Salmonella* were phage typed. Out of two hundred samples only three (one poultry meat and two poultry feaces) were positive for *Salmonella*. The confirmed isolates were subjected to serotyping at National Salmonella Centre (Vet), India. The results indicated that *S.* Rough was found in poultry meat and *S.* Typhimurium and *S.* Berta was found in poultry feaces. A plasmid of 21 kb was consistence in all the isolates. All the isolates obtained in the present study were subjected to antimicrobial susceptibility testing against 16 different antibiotics employing disk diffusion technique in which ampicillin and sulphafurazole showed 100% resistance in comparison to furazolidone. On the other hand all isolates were sensitive to nalidixic acid. Highest level of antimicrobial resistance was recorded for isolates from poultry feaces. Fifty percent or more resistance was observed among these isolates for as many as 5 antimicrobials including sulphafurazole (100%), colistin (100%), ampicillin (100%), co-trimaxazole (50%) and furazolidone (50%).

Keywords: Salmonella, *antibiotics, resistance*

Salmonellae are a large group of enteric bacteria with a broad range of hosts and recognized worldwide as major zoonotic pathogens for both animals and humans (Humphrey, 2000). Salmonellosis is an important foodborne disease of worldwide economic significance. The incidence of foodborne infection by *Salmonella* continues to increase (Rabsch *et al*., 2001). In India, salmonellosis is endemic and its importance, as potential zoonosis needs no emphasis. It causes heavy economic losses every year (Rahman, 2002). The majority of salmonellae infections are caused by contaminated food such as undercooked animal products and cross-contamination with fruits and vegetables (Pang *et al*., 1995; Tauxe, 1991). In major food borne out

breaks of salmonellosis world over, the most common vehicle of infections are foods of animal origin. Poultry and poultry products have been the most commonly implicated food (Leach *et al*., 1999; Humphrey, 2000). Poultry meat and its products have been implicated in a large number of salmonellosis cases in humans (Tietjen and Fung, 1995), accounting for nearly one third of deaths among foodborne illnesses in the US (Mead *et al*., 1999). Raw poultry and meat products remain the principal source of *Salmonella* in many countries (Bansal *et al*., 2006).

During the last decade, antibiotic resistance and multidrug-resistance of *Salmonella* spp. have increased a great deal. The cause appears to be the increased and indiscriminate use of antibiotics in the treatment of humans and animals and the addition of growth-promoting antibiotics to the food of breeding animals.

MATERIALS AND METHODS

A total of 200 samples (100 each of poultry meat, poultry feaces) were collected from the various sources of Bareilly (India) by adopting the standard aseptic measures.

Isolation and identification of bacteria

Salmonella isolation and identification were performed as described Briefly, each sample (10g) was placed in separate sterile plastic bags and put in 90 ml buffered peptone water (BPW) and a swab samples of poultry feaces inoculated in 10 ml buffered peptone water, homogenized mixed thoroughly and incubated for 16-18 h at 37^0C. Pre-enriched samples were inoculated in Rappaport-Vassiliadis (RV) and tetrathionate (TT) enrichment broths for which 1 ml of pre-enrichment inoculum was transferred to 10 ml TT broth and 0.1 ml into 10 ml RV broth and after 24 h incubation, samples were streaked on hektoen enteric agar (HEA) and bismuth sulphite agar (BSA) media. The inoculated Petri plates were incubated at 37^0C for 24 h. Suspected *Salmonella* colonies were picked up and confirmed biochemically by triple sugar iron, urea and agglutination test.

Serotyping and antibiogram

Serotyping of the isolated *Salmonella* was carried out with the antisera available in National Salmonella Centre, Indian Veterinary Research Institute, Bareilly, India, by standard protocol of the *Salmonella* centre. A total of 16 antimicrobial agents currently used in research area were used. By the disc diffusion method, the isolates were tested against Ofloxacin (5 mcg), Amoxycillin(10 mcg), Co-Trimoxazole(1.25 mcg), Sulphafurazole(300 mcg), Nalidixic acid (30 mcg), Gentamicin (10 mcg), Nitrofurantoin (300 mcg), Doxycycline hydrochloride (30 mcg), Furazolidon (50 mcg), Chloramphenicol (30 mcg), Amikacin (30 mcg), Cephataxime (30 mcg), Colistin (10 mcg), Imipenem (10 mcg), Ampicillin (10 mcg) and Enrofloxacin (10 mcg).

Plasmid profiling

Plasmid DNA isolation was performed from single colony of bacteria grown overnight in 3 ml LB broth and isolated by E.Z.N.A Plasmid Miniprep Kit (Omega, Bio-tek, USA) as per the manufacturer's protocol.

RESULTS AND DISCUSSSION

Isolation and identification of bacteria

A total of 200 samples comprising poultry meat (100) and poultry feaces (100) were screened for the presence of *Salmonella* spp. Out of two hundred samples only three (one poultry meat and two poultry feaces) (1.5%) were positive for *Salmonella* by culture method. All the confirmed isolates were subjected to serotyping at National Salmonella Centre (Vet), IVRI, Izatnagar, Bareilly (UP). The isolation rates from various food varied from 0 (Ramasastry *et al.*, 1999; Lilabati and Vishwanath, 1999) to 100% (Sharma *et al.*, 1989; Kamat *et al.*, 1991). Two percent of chicken feacal samples were found to be positive in present study. On the other hand, Kisiela *et al.* (2005) reported higher isolation rate of 22.2% from chicken feacal samples. In the present study, 2% of chicken meat samples were positive for *Salmonella*. In other studies, varying prevalence rate of *Salmonella* (16 to 21%) have been reported from chicken meat (Plummer *et al.*, 1995).

Serotyping and antibiogram

All the isolates obtained in the present study were subjected to antimicrobial susceptibility testing against 16 different antibiotics employing disk diffusion technique. Results indicate that ampicillin and sulphafurazole showed 100% resistance in comparison to furazolidone. Our results are also similar to the results of Mahon *et al.* (1994) in which ampicilin and furazolidone showed more then 50% antibiotic resistancy. On the other hand all isolates were sensitive to nalidixic acid. Highest level of antimicrobial resistance was recorded for isolates from poultry feaces. Fifty percent or more resistance was observed among these isolates for as many as 5 antimicrobials including sulphafurazole (100%), colistin (100%), ampicillin (100%), co-trimaxazole (50%) and furazolidone (50%). Among the 3 isolates of *Salmonella* 3 resistance pattern were observed (Table 1). The results indicated that of the 3 *Salmonella* isolates, 1 belonged to *S*. Typhimurium (1,4,12,27;i;1,2), 1 to *S*. Berta (1, 9,12: [f],g, [t]:-) and other one to the *S*. Rough.

Table 1: Antibiotic resistance pattern of *Salmonella* isolates.

S.No.	Source	Resistance Pattern	MARI	Average MARI
1.	Chicken (n=1)	Sf, Nf, Fr, A	0.250	0.250
2.	Poultry feaces (n=2)	Co, Sf, Cl, A	0.250	0.250
		Sf, Fr, Cl, A	0.250	

Plasmid profiling

Plasmid profile analysis has been used as a rapid and useful method for serotyping of some *Salmonella* serotypes (Threlfall and Frost, 1990). Plasmid of all the *Salmonella* isolates obtained in this study were extracted through Hames and Higgins method and run on 1% agarose gel. The plasmid profiles differed greatly among different isolates of *Salmonella* isolated from different sources. A plasmid of 21 kb was consistence in all the isolates. The size of these plasmids varies with serovars, ranging from 21 to 180kb (Castro *et al.*, 1992).

Cytotoxic potential of all the 3 *Salmonella* isolates were studied on vero cell line. Microscopic examination of different cells following exposure to CFS prepared from *Salmonella* isolates revealed number of changes including rounding and shriveling of cells, loss of cytoplasmic extension, disorganization of cell sheets, shrinking of cytoplasm etc. All the isolates from poultry meat and poultry feaces were found to be cytotoxic at 1:2 dilution. None of the isolate from poultry meat and poultry feaces samples showed any cytotoxicity to vero cells at 1:4 dilution (Table 2).

Table 2: Cytotoxicity of *Salmonella* isolates on vero cell lines.

S.No.	Source	Ctoytoxicity (%)	
		1:4 dilution	1:2 dilution
1.	Chicken (1)	-	1 (100%)
2.	Poultry faeces (2)	-	2 (100%)

CONCLUSION

Isolated *Salmonella* samples studied for serotypes, phage types and antibiogram from poultry meat and environmental sources in the India. Out of these samples only three (one poultry meat and two poultry feces) were positive for *Salmonella*. Ampicillin and sulphafurazole showed 100% resistance in comparison to furazolidone. On the other hand all isolates were sensitive to nalidixic acid.

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