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Prevalence and antimicrobial resistance for Salmonella

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Abstract

A total of 720 faecal samples from different host were collected. All the samples were processed for isolation and identification of *Salmonella* by various methods and confirmation was made by molecular characterization. Of these, 15 were found positive and 8 isolates were found susceptible to all antibiotics (Pan Susceptible). Three isolates showed resistance towards at least one of the antibiotics used. Only one isolate was found to be Multi Drug resistant (MDR).

Keywords: Prevalence, Antimicrobial resistance, Salmonella

Introduction

Salmonellosis is a direct occupational anthropozoonosis of economic impacts. Food animals harbor a wide range of *Salmonella* serovars and therefore act as a source of contamination for non-typhoid human salmonellosis**.** Most human salmonellosis cases are associated with consumption of contaminated egg, chiken, pork, beef and milk products (Zaki *et al.*, 2009)**.** The antimicrobial resistance of *Salmonella* is an increasing problem and has become a public health issue worldwide**.** Eventually, most of the *Salmonella* isolates have developed resistance against multiple drugs due to their indiscriminate, repeated and abusive applications. Resistant trait of bacteria can transmit from animals to humans through consumption of contaminated meat (Yildirim *et al*., 2011).

Materials and methods

During the period of April 2016- March 2017, a total of 720 meat samples comprising of chicken meat (100), cara-beef (400), pork (10), poultry caeca (100), buffalo intestine (30) and human stool (80) were collected from the areas of Pantnagar, Nagla, Lalkuan, Haldwani, Rudrapur, Kiccha, Kashipur, Dineshpur and Kathgodam of District Udham Singh Nagar and Nainital of Uttarakhand state of India. Isolation and identification was carried out using selective plating media as Brilliant Green Agar (BGA), Xylose Lysine Deoxycholate (XLD) Hekoen Enteric Agar (HEA) plates and Bismuth Sulphite Agar (BSA). Morphological characterization of Salmonella isolates was also carried out (Old, 1996).

The identified salmonella isolates were sent for serotyping to Salmonella Typing Centre, Division of Bacteriology and Mycology, IVRI, Izatnagar, Bareilly (U.P.). These

isolates were also subjected to PCR based molecular characterization with products at 284 bp, 204 bp and 401 bp for the primer pairs targeting invA, ompC and typh gene respectively (Skyberg *et al.,* 2006).

Antibiotic sensitivity test by disc diffusion on Mueller- Hinton agar (CLSI, 2011) was performed with antibiotic as Ciprofloxacin (5 mcg), Norfloxacin (10 mcg), Ampicillin (10 mcg), Gentamicin (10 mcg), Cefalexin (30 mcg), Tetracycline (30 mcg), Nalidixic Acid (30 mcg), Chloremphenicol (30 mcg), Sulphamethizole (300 mcg), Furazolidone (50 mcg), Tobramycin (10mcg), Streptomycin (10mcg) and Amikacin (30mcg).

Results and discussion

In meat, the prevalence was found to be 2.08 %. (Table 1), but lower prevalence of 0.94% was reported by Chandrashekhar, (2012). Highest prevalence (10%) was recorded in pork but sample size was too low although in another Danish study it ranged between 0.9– 1.2 percent (Nielsen *et al*., 2001). The prevalence rate of Salmonella in chicken meat and poultry caeca was recorded as 4.0%, unrelated with Kumar and Lakhera, (2013) who reported 2% but concomitant with a study in US (4.2%) and New Zealand (3%) (Wong *et al.,* 2007). Salmonella organism could not be recovered from stool samples concurrent with a study of 232 samples examined, only one sample (0.43%) yielded Salmonella serovar (Bisht, 2010). The prevalence of Salmonella in carabeef was found to be 1.25% and in buffalo intestine 3.33% contrary to our previous study, where none of the carabeef samples revealed Salmonella (Upadhyay *et al.,* 2016). The reason of such low prevalence could be awareness among animal owners for superior managing practices because of extension activities of public health experts. Furthermore, it may also be due to samples collected from healthy individuals, less number of processed samples, low endemicity of salmonellosis in this area and high-quality management and hygienic practices.

Molecular Characterization

All putative isolates were confirmed as *Salmonella* using *inv*A gene primers which produced a 284 bp amplicon specific for *Salmonella* genus (Figure 1, Table 2). A multiplex PCR was used for simultaneous detection of *Salmonella* genus and *Salmonella* Typhimurium targeting *omp*C and *typh* genes, respectively (Figure 2). All 15 isolates came positive for *omp*C *Salmonella* genus and 10 (66.67%) isolates were confirmed as *S.* Typhimurium on the basis of *typh* gene concomitant to Upadhyay *et al.,* (2016).

Table 1: Prevalence of *Salmonella* in samples collected from different sources.

S. No.	Sample type	No. of Sample	Prevalence (%)
1.	Human stool	80	0
2.	Chicken meat	100	4 (4)
3.	Cara-beef	400	5 (1.25)
4.	Pork	10	1 (10)
5.	Poultry caeca	100	4 (4)
6.	Buffalo intestine	30	1 (3.33)
	Total	**720**	**15 (2.08)**

Table 2: Details of *Salmonella*-specific primers.

Target gene	Sequence of primer(5'-3')	Amplicon	Reference
*inv*A	GTG AAA TTA TCG CCA CGT TCG GGC AA TCA TCG CAC CGT CAA AGG AAC C	284 bp	Olivera *et al.* (2003)
ompC	ATC GCT GAC TTA TGC AAT CG CGG GTT GCG TTA TAG GTC TG	204 bp	Alveraz *et al.* (2004)
typh	TTGTTCACTTTTTACCCCTGAA CCCTGACAGCCGTTAGATATT	401 bp	Alveraz *et al.* (2004)

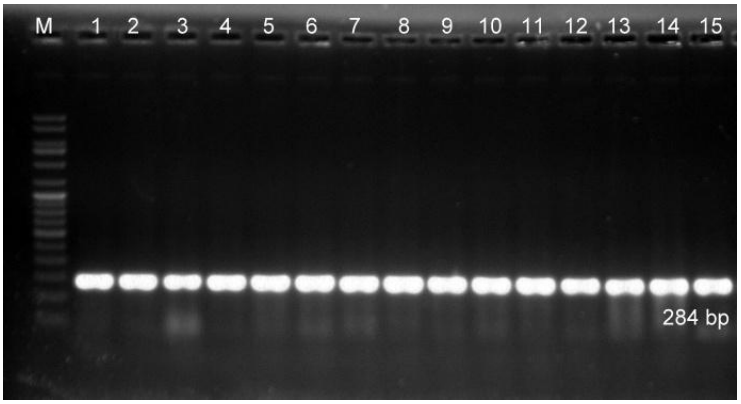

Figure 1: *inv*A gene PCR for *Salmonella* genus confirmation.
Lane M: 100 bp DNA ladder; Lane 1-15: Positive *Salmonella* isolates (284 bp)

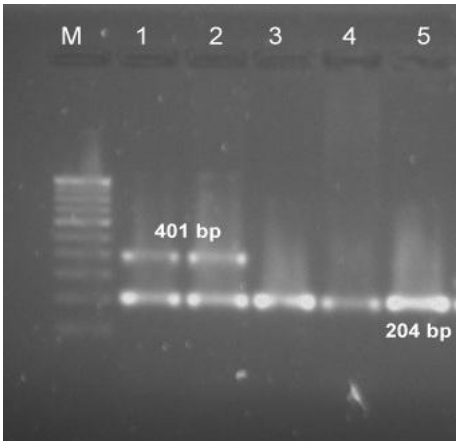

Figure 2: Multiplex PCR for *Salmonella* genus and *S*. Typhimurium identification.
Lane M: 100 bp DNA ladder; Lane 1-2: Positive S. Typhimurium isolates; Lane 3-5: *Salmonella* isolates

Table 3: Antibiotic sensitivity results of *Salmonella* isolates (n = 15).

S. No.	Antibiotic	Sensitive (%)	Intermediate (%)	Resistant (%)
1	Ampicillin (AMP)	14 (93.3)	0	1 (6.6)
2	Amoxyclav(AMC)	15 (100)	0	0
3	Chloramphenicol (C)	15 (100)	0	0
4	Cefotaxime (CTX)	15 (100)	0	0
5	Tetracycline (T)	11 (73.3)	3 (20)	1 (6.6)
6	Norfloxacin (NX)	13 (86.6)	2 (13.3)	0
7	Co-trimoxazole (COT)	15 (100)	0	0
8	Gentamicin (GEN)	15 (100)	0	0
9	Kanamycin (K)	15 (100)	0	0
10	Streptomycin (S)	15 (100)	0	0
11	Amikacin (AK)	14 (93.3)	1(6.6)	0
12	Sulfafurazole (SF)	14 (93.3)	0	1 (6.6)
13	Ciprofloxacin (CIP)	14 (93.3)	1 (6.6)	0
14	Levofloxacin (LE)	15 (100)	0	0
15	Cefazolin (CZ)	12 (80)	3 (20)	0
16	Cefoxitin (CX)	15 (100)	0	0
17	Ceftriaxone (CTR)	13 (86.6)	2 (13.3)	0
18	Nalidixic Acid (NA)	13 (86.6)	0	2 (13.3)

Antibiotic sensitivity test of *Salmonella* isolates

The antibiotic discs (Hi-media, Mumbai) used were Ampicillin, Amoxy-clav, Chloramphenicol, Cefatoxime, Tetracycline, Norfloxacin, Co-trimoxazole, Gentamicin, Kanamycin, Streptomycin, Amikacin, Sulfafurazole, Ciprofloxacin, Levofloxacin, Cefazolin, Cefoxitin, Ceftriaxone and Nalidixic acid. Strains were evaluated as susceptible, intermediate or resistant (Table 3). Eight isolates were found susceptible to all antibiotics (Pan Susceptible). Kumar and Lakhera (2013) reported Nalidixic Acid to be 100% sensitive against all the isolates. Extensive awareness programme of public health experts in this area contributed to little resistance against antibiotics over the last few years as only five isolates were found resistant to Ampicillin, Tetracycline, Nalidixic Acid and Sulfafurazole. Chuanchuen and Padungtod, (2009) observed presence of resistance genes in almost 78% of the *Salmonella enterica* isolates from poultry and pig meat. There is a shoddier prevalence of multi-drug resistance in the Salmonella, which might be due to judicious use of antimicrobials in studied geographical areas.

Summery

The overall prevalence of Salmonella in different meat samples was 2.08%. Higher prevalence was reported in pork (10%) followed by chicken meat as well as poultry caeca (4%). Low antimicrobial-resistant against commonly used in Salmonella is regarded achievement of purpose. Antimicrobials are being used judiciously in livestock and poultry since the problem of resistance has taken a serious turn. However, Salmonella shows change in resistance from one place to another.

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