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Screening of salmonella and *Shigella* from foodborne diarrheal cases in puducherry, India

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Abstract
Food borne diarrheal cases are increasingly reported in India. And other side an emerging of different antibiotics resistant pathogens being challenge for the treatment. Of this Salmonella and *Shigella* very commonly isolated in children and adults. On focus of these pathogens, study was framed which includes characterization and PCR confirmation of Salmonella and *Shigella dysenteriae* and antibiotic resistance profiles of isolates from human diarrheal cases reported in Puducherry. Altogether 50 diarrheal samples collected and subjected for this study. A total of three (6%) Salmonella isolates and five (10%) *Shigella* isolates were recovered. Recovered Salmonella serotype was of *Salmonella typhimurium*. Among the *Shigella* isolates four (8%) of *Sh. dysenteriae* and one (2%) identified as *Sh. flexneri*. Salmonella (*invA*) and *Sh. dysenteriae* (*rfpB*) genes were confirmed by PCR. Both the isolates were able to produce biofilm on slime production assay. Maximum sensitivity observed to chloramphenical, azithromycin and maximum resistant were against amoxicillin. This study clearly reveals the public health urgency of awareness on Salmonella and *Shigella* among the Pondicherry people.

Keywords: Foodborne pathogens, Salmonella, *Shigella* Biofilm, antibiotic resistant profiles

1. Introduction
Foodborne diseases remain a major public health problem across the globe. This is severe in developing countries like India due to difficulties in securing optimal hygienic food handling practices. In developing countries 70% of cases of diarrheal disease are associated with the consumption of contaminated food [42]. In general transmission of enteropathogens often occurred directly or indirectly through contaminated with faeces or from inanimate objects [15]. Among the foodborne diseases Salmonella and *Shigella* represent a major health problem worldwide, particularly in developing countries where they recognized as the most frequent causes of morbidity and mortality [2, 23]. These two pathogens have been associated with diarrhea but the severity of the diarrhea varies with the strain [41]. The link between infections and food consumption is supported by the findings of other researchers elsewhere [38, 43].
Salmonella is facultative anaerobe, Gram-negative flagellated rod-shaped bacterium usually found in the intestinal tract of mammals and avian species, more predominantly in poultry [30] and it is endemic in India. There are over 2500 serovars of Salmonella distributed widely in nature and in India more than 235 serovars have been recorded and this number is increasing constantly. Contaminated raw meat, meat products, milk and milk products are the maximum causes of human Salmonellosis outbreaks in worldwide [18]. Nowadays an emergence of non typhoidal Salmonella is responsible for most of the foodborne outbreaks which induce bacterial enteritis in children. There are 1.3 billion cases of gastroenteritis and 3 million deaths occurred worldwide due to Salmonellosis [6]. In developing countries along with Salmonella, *Shigella* is also an important cause of diarrheal deaths. *Shigella* affects all age group people but very common in children and immune compromised individuals. Infection mainly spread by eating contaminated food or by drinking contaminated water [39]. It has been reported that not less than 140 million cases of Shigellosis occur worldwide with 600,000 deaths annually and 60% of such deaths are seen in children below 5 years of age [19]. *Shigella* consists of four species of Gram-negative, non-motile, non-spore-forming, rod-shaped bacteria, namely *Sh. boydii*, *Sh. dysenteriae*, *Sh. flexneri* and *Sh. sonnei* among this *Sh. dysenteriae* causes bacillary dysentery in human, characterized by mild to severe form of dysentery, fever, abdominal cramps and fluid loss. In recent years, the health concern all over the world is about the emergence of numerous antibiotic resistant strains of pathogenic bacteria including Salmonella and *Shigella* in foods [9]. Among the different types of resistant mechanism, beta-lactamases

mediated drug resistance by Gram-negative bacteria is very commonly reported [3]. Based on the report of World Health Organization, bacterial isolates like non typhoidal Salmonella and *Shigella* species are mostly resistant against fluoroquinolones [7]. Many studies on Salmonella and *Shigella* in food [11, 22 & 34] and diarrheal samples [14, 25 & 26] have been reported in India. Nevertheless, systematic approach and public health significance are continuously remains under reporting. Our present study was designed mainly to induce the public health awareness among the Puducherry people.

Materials and methods

Sampling

A total of 50 diarrheal samples were collected from Government hospitals and private hospitals/laboratories in a period of one year (2016). All the samples were collected in sterile disposable plastic containers and transported to the laboratory for further processing. Information related to the samples recorded at the time of collection using questionnaire. An undertaking of no objection was taken from individuals who have voluntarily given the samples.

Chemicals and reagents

In this study all the chemicals, primers, reagents and culture media were procured from the Merck Limited, Hi media from Mumbai and Bangalore genei. PCR master mix was procured from Gene Technologies, Chennai.

Processing of samples

Procured samples was processed in the Biosafety level - II laboratory, present in Department of Veterinary Public Health and Epidemiology, Rajiv Gandhi Institute of Veterinary Education and Research, Kurumbapet, Puducherry. Samples remaining after the tests and negative samples were discarded after decontamination.

Selective enrichment for Salmonella and Shigella

Ten gram samples was transferred to 90 ml of Peptone Water (PW) and incubated at 37 ^{0}C overnight for the metabolic recovery and proliferation of the microbes. One milliliter of pre-enrichment culture was inoculated into tubes containing 9 ml of Rappaport Vassiliadis Soya (RVS) Broth and incubated for 24 hours at 37^{0}C for secondary enrichment.

Isolation of Salmonella on selective and differential media

Bacteria growing in RVS broth were streaked onto Mac Conkey's agar, Xylose Lysine Deoxycholate (XLD) and Bismuth Sulfate agar (BSA), Xylose Lysine Deoxycholate (XLD) and Salmonella-*Shigella* agar (SS), incubated at 37 ^{0}C overnight. After incubation plates were examined for the presence of specific colony morphology of Salmonella and Shigella. The presumptive Salmonella and *Shigella* colonies were subjected to cultural, morphological and biochemical characteristics as per standard procedure [8].

Standardization of PCR

The reference strain *Salmonella* spp obtained from Department of Veterinary Microbiology, Rajiv Gandhi Institute of Veterinary Education and Research, Kurumbapet, Puducherry and *Shigella dysenteriae* from Mahathma Gandhi Medical College and Research Institute, Pillaiyarkupam, Puducherry, used as a standard reference for PCR. Boiling lysis method was followed for DNA preparation [32]. PCR amplification carried out in an automated thermal cycler (Eppendorf Mastercycler, Germany) and products were analyzed by electrophoresis in 1.5% agarose gel in Tris acetate EDTA (TAE) buffer (1X). Details of the Primers, Reaction mixture and Standardization are given in table no.4, 5 & 6.

Serotyping

Isolates of Salmonella after the PCR confirmation were purified and sent to National Salmonella and Escherichia Centre (Central Research Institute, Kasauli, Himachal Pradesh, India), on Nutrient agar slants for serotyping.

Assessment of biofilm production

The confirmed Salmonella and *Shigella* colonies after the PCR were purified on Muller Hinton agar before being subjected for biofilm production assay. Slime production assay was performed using modified Congo red agar [37].

Antimicrobial resistance profiling of the isolates

The drug susceptibility of Salmonella and *Shigella* isolates was performed on Mueller Hinton agar plates by disc diffusion method in accordance with the recommendations of the Clinical and Laboratory Standards Institute [10]. List of antibiotics used are given in table no. 1.

Results

Isolation of Salmonella and Shigella

In this study, for both Salmonella and *Shigella* peptone water used as a non-selective pre enrichment medium. The pre enrichment cultures were sub cultured in selective enrichment media RVS broth. A total of 50 samples processed and three (6%) Salmonella isolates and five (10%) *Shigella* isolates were recovered. Recovered isolates subjected for primary and biochemical tests and identified up to species level. Among the *Shigella* isolates four (8%) of *Sh. dysenteriae* and one (2%) identified as *Sh. flexneri* as primary, secondary and sugar fermentation tests by using standard procedure. Results are shown in table no. 2 & 3.

PCR confirmation of Salmonella and *Sh. dysenteriae*

All three isolates of Salmonella and four isolates of *Sh. dysenteriae* subjected for PCR with primers targeting (*invA)* and *(rfpB).* DNA was prepared by the boiling-lysis method from the culturally confirmed culture. PCR products analyzed on 1.5% Agarose gel electrophoresis (along with positive and negative control and 100 bp ladder) revealed the presence of expected 244 bp (*invA*) and 211 bp (*rfpB*) products under UV translluminator. The primers targeting the gene successfully amplified the DNA prepared by the boiling-lysis method from culture. Picture attached in no. 1 & 2.

Serotyping of Salmonella

Salmonella isolates were identified up to primary and secondary biochemical tests and confirmed by polymerase chain reaction. Confirmed Salmonella isolates were sent to National Salmonella and Escherichia Centre (Central Research Institute, Kasauli, Himachal Pradesh, India) on nutrient agar slants for serotyping. The serotype isolated only of *Salmonella typhimurium* and were found to have the somatic antigens as 4, 12 and flagellar phase I antigen was 'I' and the phase II antigen was 1, 2.

Biofilm production

Isolated Salmonella and *Shigella* colonies were purified on

nutrient agar and subjected for slime production assay qualitatively with negative and positive control. All the isolates of Salmonella and *Shigella* produced black colonies with dry crystalline consistency on modified Congo red agar and clearly shows the ability of biofilms productions.

Antimicrobial resistance profile of the isolates of Salmonella

Out of three isolates, 100% were sensitive to chloramphenical, nalidixic acid, perfloxacin and tetracycline, 100% were resistant to amoxycillin, co-trimaxazole and metronidazole and 66.7% were resistant to cephalexin, ciprofloxacin and gentamicin. Details present in table no. 7.

Antimicrobial resistance profile of the isolates of Shigella

Out of four isolates, 100% were sensitive to azithromycin, ciprofloxacin and gentamicin, 75% were sensitive to cefotaxime and chloramphenicol, 100% were resistant to amoxycillin, co-trimoxazole and metronidazole, 75% were resistant to norfloxacin, 50% were resistant to cephalexin, perfloxacin, tetracycline and trimethoprim and 25% were resistant to nalidixic acid and sulphamethizole. Only one isolate of *Sh. flexneri*, 100% was resistant to amoxycillin, co-trimoxazole and metronidazole and remaining antibiotics were found 100% sensitive. Details present in table no. 7

Analysis of samples

In a total of 50 samples screened for Salmonella and *Shigella* the maximum isolates were recovered from children, four (57.1%), followed by teenage people, three (15.7%) and adult, one (4.1%). All the isolates of Salmonella and *Shigella* were only isolated from stool samples of hotel food eaters 8 (26.7%). Compared to vegetarians, meat eaters were found to harbor high number of isolates eight (20.5%). Details are given in table no. 8

Discussion

Foodborne diseases are being under documented in developing countries like India. Although treatment have been given to the affected individual the sources are not been keen to the public. In general most of the food related disease outbreaks are mainly depends upon the route and load of contamination of microbes. Salmonella and *Shigella* are being continuously reported from the many foodborne outbreaks throughout the world. Even though outbreaks are documented sources are not been highlighted. Many methods had been adapted for the isolation of these pathogens. In this study the samples were subjected to pre enrichment in peptone water followed by selective enrichment in Rapport Vassiliadis Soy broth. Other bacterial contamination level was minimized mainly by the pre enrichment and selective enrichment media. For the recovery of non-lactose fermenters mac conkeys agar and for selective isolation of *Salmonella* spp Bismuth sulphate agar, Salmonella and *shigella* agar and for *Shigella* spp Xylose lysine decorxylate agar were selected and used. The results were comparatively significant [14, 22].

A total of 3 (6%) Salmonella isolates were recovered from 50 samples processed and screened for Salmonella. Authors [22] screened diarrheic samples for presence of Salmonella in Tamil Nadu, India. Out of 118 diarrheic samples screened the incidence of Salmonella was 18.6%. This result is higher than the present findings and authors also randomly focused on the sample collection. This may also contributed to the high number of isolates then our study. Increased isolates indicates the maximum chances of contamination of Salmonella. In our study mainly food poisoning cases were selected and screened. [12] Screened the infant diarrheal samples in Ethiopia for Salmonella. Among 372 stool samples cultured, four (1%) Samples were positive for Salmonella. The prevalence mainly depends on seasonal influence also; the presence of climatic changes may be one of the reasons in Nigeria for the less number of isolates. In our study increased isolates indicates the suitable environment persisting in Puducherry.

Isolates of Salmonella were sent to National Salmonella and Escherichia Centre (Central Research Institute, Kasauli, Himachal Pradesh, India) on nutrient agar slants for serotyping. The serotype isolated was *S. typhimurium*. All the isolates were found to have the somatic antigens as 4, 12 and flagellar phase I antigen was 'I' and the phase II antigen was 1,2. Presence of this serotype in all the isolates indicates most of the Salmonella may belong to this serotype. [34] Studied on Salmonella from meat sources and could isolate only *Salmonella typhimurium* with same antigenic properties. This evidence may suggest that maximum possibilities of meat contamination among the consumers in Puducherry.

Shigella, one of the hiding organisms were it is under reported than Salmonella in many developing countries. The clinical symptoms sometimes mimic the Salmonella were its goes undignified. In most of the cases symptomatic treatment been adopted and underreporting still persisting. A total of five (10%) isolates were recovered from the 50 samples, processed and screened for Shigella. Among the five isolates, four (80%) were *Sh. dysenteriae* and one (20%) confirmed as *Sh. flexneri*. No other *Shigella* species could able to recover. Prevalence of *Shigella* comparatively higher than Salmonella. In general water considered the maximum route contamination of *Shigella* and increased isolates may suggest that water plays an important vehicle in contamination process. In our present findings the prevalence of *Sh. dysenteriae* was more than *Sh. flexneri*. This is showing that chances of many dysentery cases may be increased in future. This was more significant than earlier findings and clearly indicates that the emergence of *Sh. dysenteriae* than other *Shigella* species. [31] Reported the presence of *Shigella* from sudden continuous dysentery cases of children in hospitalized in New Delhi. *Shigella* isolated from 23 cases (38.3%) and *Sh. flexneri* was the predominant species isolated 73.9% followed by *Sh. dysenteriae* 13.1%. Many authors have also reported a high prevalence of *Sh. flexneri* in human stool samples [4,5]. The identified *Shigella* species were of late lactose fermenters. This result is in agreement with the report of Mandal [26] who cultured the stool samples from Puducherry and reported the late lactose fermenting *Shigella* colonies. The variation of the incidence of *Shigella* may vary with the food habits and also type of food consumed by the people in Puducherry.

The primers targeting the *invA* gene for *Salmonella* and *rfpB* gene of *Sh. dysenteriae* successfully amplified the DNA prepared by boiling-lysis method from the culture. In boiling-lysis method of DNA preparation, centrifugation and washing steps were found to remove the PCR inhibitors from the culture medium. Boiling the bacterial cells and then immediately cooling was efficient for releasing the DNA from the cells. Resulting supernatant was directly subjected to the PCR assay without further purification steps [21].

Isolates of Salmonella are of biofilm producers it indicates the presence virulence gene. Pathogenic strains of Salmonella are able to produce biofilm and adhere on various abiotic contact

surfaces including glass, stainless steel, rubber and polypropylene have documented [17, 24]. Different studies have been conducted to see the biofilm formation of Salmonella isolates on different surfaces [1]. Various factors (growth medium, incubation period, fixation of adhered cells and staining) affect development of Salmonella biofilm [33]. According to those studies, the source of isolates (from humans, animals or food) did not affect the biofilm [16].

All isolates of *Shigella* had been produced biofilm on modified Congo red agar. Thus, this is in agreement with earlier study [29], who studied the Congo red uptake from solution by the different *Shigella* species and also reported the severity of the disease mainly mediated by virulence of *Shigella* species. In their results *Shigella* showed a distinct decline of binding in the order *Sh. dysenteriae*, *Sh. flexneri*, *Sh. boydii*, and *Sh. sonnei*. Modified Congo red agar also find suitable for detection of slime production assay than other modified agars. Presence of biofilm producing isolates may be an added threat to the public. Mainly it goes antibiotic resistance in human on the prediction site and cleaning agent resistance in food production industries/slaughter houses.

Out of three Salmonella isolates, 100% were sensitive to chloramphenical, nalidixic acid, perfloxacin and tetracycline, 100% were resistant to amoxycillin, co-trimaxazole and metronidazole and 66.7% were resistant to cephalexin, ciprofloxacin and gentamicin. [27] Isolated and characterized the antibiotic susceptibility pattern of Salmonella in children with acute diarrhea in Ethiopia. The high sensitivity was observed against ciprofloxacin (91.3%). This clearly reveals the increasing resistance of other antibiotics. Maximum resistant noticed against chloramphenical and tetracycline. In our study chloramphenical and teteracycline were showing sensitivity against isolates. Most of the commonly used antibiotics are slowly developing resistance against the Salmonella may put pressure on discovering of new antibiotics. Recent resistant additions include resistance to trimethoprim and also particular concern to the fluoroquinolones [44]. In this study also presence trimethoprim resistance isolates considered the emerging problems among foodborne pathogens. Equally the use or misuse of antibiotics in human for example also leads to the development of antibiotic resistant. Drug resistant Salmonella emerge in response to antimicrobial usage in humans and in food animals so, selective pressure from the use of antimicrobials is a major driving force behind the emergence of resistance.

Out of four isolates Shigella, 100% were sensitive to azithromycin, ciprofloxacin and gentamicin, 75% were sensitive to cefotaxime and chloramphenicol, 100% were resistant to amoxycillin, co-trimoxazole, 50% were resistant to cephalexin, tetracycline and trimethoprim. Emerging of multi - drug resistance *Shigella* possess the continuous threat to the public and epidemiological investigation need to be applied to assess the widespread resistance. Only one isolate of *Sh. flexneri*, shows 100% resistant to amoxycillin, co-trimoxazole and metronidazole and remaining antibiotics were 100% sensitive. Nalidixic acid is the first-line antimicrobial for shigellosis treatment in the WHO guidelines for both adults and children [39]. However, this antibiotic is no longer effective for the treatment of shigellosis because of the development of resistance. In the present study, 25% of MDR *Shigella* isolates showed resistance to nalidixic acid.

Treatment of *Shigella* dysentery with specific antibiotics only reduces the diarrheal mortality in humans [13]. An antibiogram study was conducted by Ud - Din [36] in Bangladesh for *Shigella* isolates recovered from human stool samples. Isolates were resistant to commonly used antibiotics including trimethoprim - sulfametho xazole (89.5%), nalidixic acid (86.5%), and ciprofloxacin (17%), respectively. All isolates were susceptible to cefotaxime. In our study maximum chances were of *Sh. dysenteriae*. [26] Cultured diarrheal samples (n=210) in Puducherry for the presence of *Shigella* spp. The majority (79%) of the isolates of *Shigella* were resistant to ampicillin and co- trimoxazole, while 50% were resistant to ciprofloxacin. In present study ciprofloxacin find to have better sensitivity than other antibiotics.

A total of 50 samples collected from volunteers and correlated with the laboratory investigation for the presence of Salmonella and Shigella. The distribution of Salmonella and *Shigella* based on sampling was high in children (57.1%) followed by teenage people (15.7%) and adult (4.16%). This result is in agreement with the findings of Sudershan and team [35] who reported the prevalence of foodborne diseases more in children. Of the sex wise prevalence of Salmonella and Shigella, male (19.5%) was higher than females (0%). In general male get more access to eat food from outside compared to females. This reason may also attribute to the causes. Based on the food habit of the volunteers, those consumed the hotel food had the prevalence of 26.7% compared to the home made food consumptions (0%). Many times hotel foods are not hygienically maintained this also may contribute to the factors. When compared to the vegetarians (0%), non-vegetarians were more prone for infection based on the present study (20.5%). [35] Authors also highlighted most of the foodborne isolates are recovered from the meat eaters, in Hyderabad, India. In general meat harbors the high number of microorganism if not been processed hygienically. Increased prevalence of infection in non vegetarians may also be a chance of getting from meat and meat food sold in Puducherry. These results also insist that lack of hygienic awareness among the peoples in Puducherry. The community based extension activities may reduce the exposure to infections.

Acknowledgement

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Table 1: List of antibiotics used in this study.

S. No	List of antibiotics	Content/disk
01	Amoxycillin	10µg
02	Azithromycin	15µg
03	Co – trimoxazole	30µg
04	Cephalexin	30µg
05	Ciprofloxacin	30µg
06	Chloramphenical	30µg
07	Cefotaxime	10µg
08	Gentamicin	120µg
09	Metronidazole	50µg
10	Norfloxacin	15µg
11	Nalidixic acid	30µg
12	Perfloxacin	5µg
13	Sulphamethizole	30µg
14	Trimethoprim	25µg
15	Tetracycline	30µg

Table 2: Primary and secondary identification tests

S. No	Identification tests	Salmonella	Shigella
1	Shape	Rod	Rod
2	Motility	Motile	Non-motile
3	Catalase	Positive	Positive
4	Oxidase	Negative	Neagtive
5	Urease	Negative	Positive
6	Simmon's citrate	Positive	Neagtive
7	Indole	Negative	Neagtive
8	MR Test	Positive	Neagtive
9	VP Test	Negative	Neagtive
10	H_2S production in TSI slant	Positive	Neagtive
11	Lysine decarboxylase	Positive	Neagtive
12	Ornithine decarboxylase	Positive	Neagtive
13	ONPG	Negative	Positive

Table 3: Sugar Fermentation tests

Sr No	Sugars	Salmonella	Shigella
1	Acid from	Positive	Positive
2	Dextrose	Positive	Neagtive
3	Dulcitol	Positive	Neagtive
4	Maltose	Positive	Neagtive
5	Xylose	Positive	Neagtive
6	Trehalose	Positive	ND
7	Arabinose	Positive	ND
8	Mannitol	ND	Positive (only *Sh. flexneri*)

ND Not done

Table 4: Details of the primers

Organisms	Target gene	Primer sequence (5'- 3')	Expected Product size (bp)	References
Salmonella spp	*invA*	F:GTGAAATTATCGCCACGTTCGGGCAA R:TCATCGCACCGTCAAAGGAACC	244	Rahn *et al.* [35]
Shigella dysenteriae	*rfpB*	F:TCTCAATAATAGGGAACACAGC R:CATAAATCACCAGCAAGGTT	211	Ojha *et al.*[28]

Table 5: Details of the Reaction mixture

S. No	Reaction mixture	Salmonella *(invA)*	*Sh. dysenteriae* (*rfpB*)
1	Template DNA	5 µl	5 µl
2	Primer	20 pmol each primer	20 pmol each primer
3	Master mix	12.5 µl	12.5 µl
4	Triple distilled water	5.5 µl	5.5 µl

Table 6: Details of the Reaction mixture

S. No	PCR Programme	Salmonella (*invA*)	*Sh. dysenteriae* (*rfpB*)
1	Initial Denaturation	95^0C for 5 minutes	94^0C for 3 minutes
2	Denaturaation	94^0C for 30 seconds	94^0C for 30 seconds
3	Annealing	56^0C for 30 seconds	60^0C for 30 seconds
4	Extension	72^0C for 2 minutes	72^0C for 30 seconds
5	Final extension	72^0C for 5 minutes	72^0C for 3 minutes

Table 7: Consolidated antimicrobial sensitivity pattern of isolates isolates (n=8)

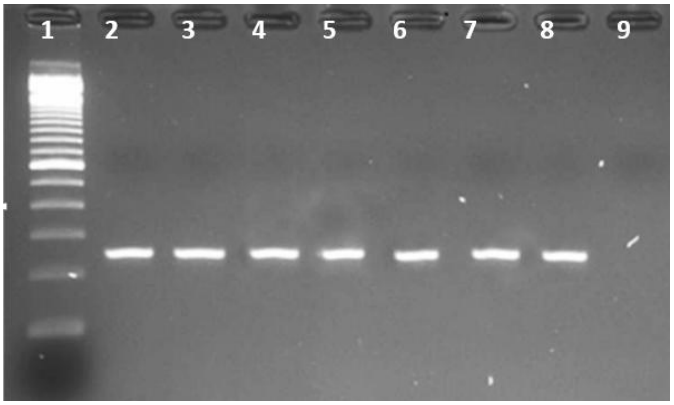
List of Antibiotics	*Salmonella* spp (%) (n=3)			*Sh. dysenteriae* (%) (n=4)			*Sh. flexneri* (%) (n=1)		
	R	I	S	R	I	S	R	I	S
Amoxycillin	100	-	-	100	-	-	100	-	-
Azithromycin	-	33.4	66.6	-	-	100	-	-	100
Co – trimoxazole	100	-	-	100	-	-	100	-	-
Cephalexin	66.7	-	33.4	50	-	50	-	-	100
Ciprofloxacin	66.7	-	33.4	-	-	100	-	-	100
Chloramphenical	-	-	100	-	25	75	-	-	100
Cefotaxime	-	66.7	33.4	-	25	75	-	100	-
Gentamicin	66.7	33.4	-	-	-	100	-	-	100
Metronidazole	100	-	-	100	-	-	100	-	-
Norfloxacin	33.4	-	66.7	75	-	25	-	-	100
Nalidixic acid	-	-	100	25	25	50	-	-	100
Perfloxacin	-	-	100	50	-	50	-	-	100
Sulphamethizole	-	66.7	33.4	25	50	25	-	-	100
Trimethoprim	-	33.4	66.7	50	25	25	-	-	100
Tetracycline	-	-	100	50	-	50	-	-	100

R- Resistant; I- Intermediate; S-Sensitive

Table 8: Details of human stool samples

S. No	Study parameters	Frequency N=50	Positive N=8	Negative N=42
1.	Age			
	Children	7(14%)	4(57.1%)	3(42.8%)
	Teenage Adult	19(38%)	3(15.7%)	16(84.2%)
		24(48%)	1(4.16%)	23(95.8%)
2.	Sex			
	Male	41(82%)	8(19.5%)	33(80.4%)
	Female	9(18%)	0(0%)	9(100%)
3.	Food habit			
	Home made	20(40%)	0(0%)	20(100%)
	Hotel	30(60%)	8(26.7%)	22(73.4%)
4.	Food type			
	Meat eaters	39(78%)	8(20.5%)	31(79.4%)
	Vegetarian	11(22%)	0(0%)	11(100%)

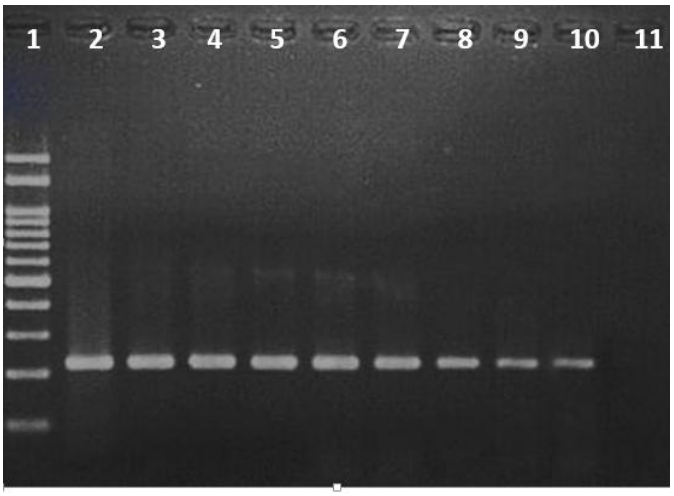
N – Indicates total no. of Samples



244 bp

Fig 1: Result of PCR for detection of *invA* for *Salmonella* spp

Lane 1 Ladder 100 bp
Lane 2 Positive control
Lane 3 to 8 Samples
Lane 9 Negative control



211 bp

Fig 2: Standardization of PCR for detection of *Shigella dysenteriae* (*rfpB*)

Lane 1 Ladder 100 bp
Lane 2 Positive control
Lane 3 to 10Samples
Lane 11 Negative control

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