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An exploration on animal and public health significance of salmonella from major meat sources in Puducherry, India

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Abstract
A study was framed to isolate the predominant strains of Salmonella prevalence in Puducherry. A total of 150 (Chicken, Chevon and Beef each 50) raw meat samples procured from different retail outlets in and around Puducherry. The study objective included Salmonella identification and PCR confirmation, serotyping, biofilm formation assay and antimicrobial resistance testing with selected antibiotics. Conventionally confirmed isolates were subjected for PCR against *invA* gene. Overall prevalence was 7.4 per cent and chicken was found to harbour highest isolates. On serotyping *Salmonella typhimurium* was the only strain recorded in all meat samples. Recovered *Salmonella typhimurium* were biofilm producers. High antimicrobial resistance were observed to Amoxycillin, Co- trimaxazole and Metronidazole whereas, maximum sensitivity to Azithromycin, Chloramphenicol, Cephalexin, Cefotaxime and Tetracycline. This study indicated the need to take up foodborne disease surveillance in Puducherry under the Indian context and to identify the common high-risk food commodities for microbial contamination and antibiotic resistance.

Keywords: Foodborne illness, raw meat, salmonella, biofilm, antibiotic resistance

Introduction
Meat, in general considered of major protein sources in India. Meat production in India estimated at 6.18 million tones, standing 5th of world meat production [29].Though India stands at 5th position, export in the livestock area is not up to the standards. The expected meat production in 2030 is 3.3 times more than present situation [16]. Over the last 25 years, the global incidence of foodborne infections has markedly increased, with nearly a quarter of the population at a high risk of foodborne diseases [31]. World Health Organization [44] estimated that foodborne and waterborne diarrheal diseases together kill around 2.2 million people annually. The contaminated meat and dairy products are the most common cause of human Salmonellosis outbreaks in worldwide [20]. In India, majority of food borne outbreaks remains underreported or non-investigated and may only be noticed after major health effects or economic damage has occurred. Nevertheless few peoples already have been reported on Salmonella food poisoning [8, 5, 1, 12]. Salmonella survivability also increasing in other side by means of microbial biofilm on the contact surfaces in the food industry or small intestine. The direct contact with raw materials or foodstuffs can cause contamination and due to which the product become unsafe [39]. The biofilm forming isolates are considered an emerging menace to public mainly of resistant to antimicrobial agents [15].
Even though studies on the microbial quality of fresh meat sold in Puducherry, was carried out in Rajiv Gandhi Institute of Veterinary Education and Research, no systematic study has been conducted on the Public and animal health significance of Salmonella. Therefore the present work framed with the objectives of isolation and identification Salmonella from raw meat (chicken, chevon and beef), Serotyping, PCR based confirmation of the *invA* gene, biofilms production and antimicrobial resistance profiling of the isolates.

Materials and methods
Collection and transportation of meat samples
A 100 gram of raw meat samples were collected from different retail meat shops in and around Puducherry (chicken, chevon and beef each 50) and subjected to this study. All the samples

were collected in sterile polyethylene bags and immediately transported to laboratory in ice box for further analysis.
In this study all the chemicals, primers, reagents and culture media were procured from the Merck Limited, Hi media from Mumbai and Bangalore genei. The PCR master mix was procured from Gene Technologies, Chennai

Primary and Selective enrichment
Twenty five gram of each meat sample (chicken, chevon and beef) was homogenized in 225 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 Selenite F Broth (SB) and Rappaport Vassiliadis Soya (RVS) Broth and incubated for 24 hours at 37 ^{0}C for secondary enrichment.

Isolation on selective and differential media
Bacteria growing in SB and RVS broth were streaked onto Mac Conkey's agar for differentiation of lactose and non-lactose fermenters. Non lactose fermenters were streaked onto Xylose Lysine Deoxycholate (XLD) and Bismuth Sulfate agar (BSA) incubated at 37 ^{0}C overnight. Agar plates were examined for the presence of colorless colonies on Mac Conkey's agar, pink colonies with or without black centers on XLD and black colonies surrounded by a black or brownish zone with a metallic sheen on BSA. All the remaining negative samples were decontaminated as per standard procedures.

Identification of Salmonella
The presumptive colonies were subjected to cultural, morphological and biochemical characters as per [9]. Primary identification tests include Gram's staining, Catalase test (Slide test), Oxidase test and Motility test were performed. Secondary identification tests like Indole production, Methyl Red (MR) reaction, Voges-Proskauer (VP) Reaction, Citrate utilization test, Urease activity, Triple Sugar Iron test, Lysine Decarboxylase and Carbohydrate Utilization tests proceeded as per the standard procedures. All biochemical tests were performed at 37 ^{0} C for 24 hours.

Preservation and revival of isolates
A loopful of the isolated organism was added to the sterile DMSO-glycerol broth vials and mixed well in the vortex mixture. The vials were then labeled and stored at – 40 ^{0}C. The isolates were revived once in three months. The vials stored at - 40^{0}C was thawed and streaked on Nutrient agar and incubated at 37 ^{0} C overnight. The isolated organism was also streaked onto MH slants in screw capped test tubes and incubated at 37 ^{0}C for 24 hours. Once the growth was obtained the slants were stored at 4 ^{0}C.

Molecular confirmation by PCR
Extraction of DNA from isolates
The reference strain of *Salmonella* spp was obtained from Institute of Microbial Technology, Chandigar. Template preparation and PCR was carried out for the detection of *invA* gene as per the method described by [34]. Primer, Reaction mixture and PCR conditions details are given in table 5, 6 & 7.

Serotyping
Isolates of Salmonella were sent to National Salmonella and Escherichia Centre (Central Research Institute, Kasauli, Himachal Pradesh, India) on nutrient agar slants.

Assessment of biofilm production of the isolates
The confirmed Salmonella colonies were further purified on Muller Hinton agar before being subjected for biofilm production assay. Slime production assay was performed as per [41]. Briefly, Brain heart infusion agar supplemented with 5 per cent sucrose and Congo red (0.08 g/l) was prepared and autoclaved. The isolates were inoculated and incubated aerobically for 24 to 48 hours. The ability of the isolates to produce bio-films was indicated by black colonies with a dry crystalline consistency and red colour colonies indicates negative. Procedure was done with the comparison of positive and negative controls.

Antimicrobial resistance profiling of the isolates
The drug susceptibility of Salmonella 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 showed in Table 2.

Results
Isolation of Salmonella
In the present study, peptone water was used as a non-selective pre enrichment medium. The pre enrichments were sub cultured in selective enrichment media Rappaport Vassiliadis Soy (RVS) broth and Selenite F broth (SB). A total of 150 raw meat samples were processed and screened for *Salmonella* spp. Eleven (7.4 per cent) samples positive by culture and all the 11 samples were confirmed by polymerase chain reaction. In total isolates, eight (72.7 per cent) were from Rappaport Vassiliadis Soy (RVS) broth, three (27.2 per cent) were isolated from Selenite F broth.
Out of 11 (7.4 per cent) isolates, five (45.4 per cent) from chicken, four (36.6 per cent) from beef and two (18.1 per cent) from chevon were isolated only by RVS. Among the five isolates from chicken, three (60 per cent) from RVS and two (40 per cent) from SB were recovered (Table 3). Chicken being a natural host for Salmonella the prevalence was high compared to the other meat. The rest of the all samples were negative by culture.

Identification of Salmonella
Out of 150 samples processed, 11 (7.4 per cent) samples were found to show pale colonies on Mac Conkey's agar, pink colonies with black centre in XLD agar and black colonies with metallic sheen on BSA (Plate - 1,2 &3). On Gram's staining, all were found Gram-negative slender rods. Catalase positive cultures were further identified up to subspecies level as per [9]. Results showed in Table 4.

Serotyping of Salmonella
The serotype isolated Salmonella 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.

PCR for primers targeting *invA* gene
All the 11 positive meat isolates of Salmonella by cultural and biochemical tests were subjected for PCR with Salmonella genus virulence specific primers (*invA* gene). DNA was prepared by the boiling-lysis method from the suspected Salmonella culture. PCR products analysed on 1.5 per cent

Agarose gel electrophoresis (along with positive and negative control and 100 bp ladder) revealed the presence of expected 244 bp products under UV translluminator. The primers targeting the *invA* gene successfully amplified the DNA prepared by the boiling-lysis method from all Salmonella culture. The details of results of PCR are depicted in Picture 1.

Assessment of biofilm production

PCR confirmed isolates of Salmonella are subjected for biofilm production test and all were possess the ability to produce black colonies with dry crystalline consistency on modified Congo Red Agar. The results indicated isolates were able to produce biofilm (Plates - 4&5).

Antimicrobial resistance profiles

The details of the results and consolidated antimicrobial sensitivity pattern of 15 antibiotics depicted in Table 8. Salmonella isolates from chicken, were 100 per cent sensitive to azithromycin, chloramphenical and cefotaxime, 100 per cent resistant to amoxycillin and metronidazole, 60 per cent resistant to tetracycline and norfloxacin, 40 per cent resistant to nalidixic acid and 20 per cent resistant to ciprofloxacin.

Out of two isolates of Salmonella from chevon, 100 per cent were sensitive to azithromycin, cephalexin, chloramphenical, timethoprim, tetracycline and cefotaxime, 100 per cent resistant to amoxycillin, co-trimoxazole and metronidazole and 50 per cent resistant to gentamicin, nalidixic acid, ciprofloxacin and norfloxacin. Out of four Salmonella isolates from beef 100 per cent were sensitive to amoxycillin, cephalexin, cefotaxime, perfloxacin and sulphamethizole, 100 per cent resistant to metrinadazole, 75 per cent resistant to norfloxacin and tetracycline, 50 per cent resistant to gentamicin, nalidixic acid and 25 per cent resistant to ciprofloxacin.

Discussion

Food borne diseases always follow the consumption of contaminated food especially from animal products such as meat or carcasses. In developing countries most of the foodborne outbreaks are mainly associated with Salmonella spp. These organisms are subsequently transferred to human through production, handling and consumption of meat and meat products. Food is an important vehicle for human infection of *Salmonella* spp but the number of Salmonella shedding from food is usually low. It is necessary to use pre enrichment media such as peptone water, buffered peptone water or universal pre enrichment broth to assist isolation. This may allow the small number of food or contaminated Salmonella to grow, that may otherwise be killed by the toxic effects of different enrichment media. Pre enrichment normally helps to multiply or resuscitate Salmonella that have been sub lethally damaged. Many studies have documented the use of peptone water for pre enrichment. In the present study peptone water was used as a non- selective pre enrichment media. Peptone water was a preferred pre enrichment media for Enterobacteriaceae family especially Salmonella as it favoured the repair and growth of stressed or sub lethally injured isolates arising from exposure to heat, desiccation, high osmotic pressure or wide temperature fluctuations [2, 13, 47, 26].

Enrichment media contain additives that selectively permit Salmonella to grow while inhibiting the growth of other contaminated bacteria. Some however, are also relatively toxic to certain serovars of Salmonella, e.g. Selenite inhibits *S. cholera suis*. Elevated temperature has also been used in some laboratories especially with RVS broth, where the recommended temperature for incubation is around 41.5^0C [40]. Rappaport Vassiliadis Soy (RVS) broth and Selenite F broth (SB) were used as selective enrichment media in the present study and better isolation was observed with RVS broth compared to SB. RVS broth also was found to be an effective enrichment media for preparation of template DNA for PCR examination when compared to SB which did not produce good quality of DNA. Authors [4] used buffered peptone water for non - selective enrichment and RVS broth for selective enrichment of Salmonella isolates. [13] Used SB, RVS broth for secondary enrichment of Salmonella. Solid, selective agars permit differential growth to varying degrees. They inhibit the growth of other unwanted bacteria other than Salmonella, which gives the information on some of the principal differential biochemical characteristics usually non lactose fermentation and hydrogen sulphide production. The results were recorded after 24 to 48 hours of culture at 37^0C. Salmonella form characteristic colonies on such media that are usually distinguishable from the colonies of other bacteria with the possible exceptions of Proteus, Pseudomonas and Citrobacter. In this study XLD and BSA were used as selective and differential media for the isolation of Salmonella from the selectively enriched cultures. Mac Conkey's agar was used as a selective and differential media at initial stage for streaking of enriched culture from selective broth and also used to purify the Salmonella suspected colonies from XLD, BSA and Salmonella-Shigella agar. [35] Recommended the Selenite F enrichment broth for selective isolation and Mac Conkey's agar and XLD agar for purification.

Out of 150 raw meat samples processed and screened for the presence of Salmonella in meat, 11 (7.4 per cent) isolates were recovered. Among the 11 isolates five (45.4 per cent) from chicken, two (18.1%) from chevon and four (36.6 per cent) from beef were recorded. This result was found to be higher than [22] who reported a total of ten isolates of *Salmonella* spp from raw meat with an overall prevalence of 5 per cent among food animals in Maharastra, India. An overall prevalence of Salmonella in 7.4 per cent food animals in current study is comparable with the findings [23]. In their study 5 per cent (n=300) Salmonella incidence was reported from the municipal slaughter houses and the retail meat shops in Hyderabad and Karnataka, India. This higher and lower incidence variation may also relay the suitable environment persistence for Salmonella. In present study increased isolates shows the presence of suitable ecology for Salmonella.

In a total of 50 samples screened 5 (10 per cent) were positive in chicken. Similarly, [11] reported the 8 per cent (n=100) incidence in Puducherry. In an earlier study [35] on poultry carcasses reported 8.3 per cent (n=60) incidence in Tamil Nadu. [37] Reported, a total of 40 (n=80) Salmonella isolates from poultry in Assam, India. Chicken is considered as a natural host for *Salmonella* spp hence, the prevalence of Salmonella in the present study is high compared to other meat. However, variations observed between the reported Salmonella prevalence in previous investigations around the world may be due to several factors, including the initial Salmonellosis in live birds, sanitation within the slaughterhouse, possible contamination during poultry processing steps (e.g., the amount of cross-contamination of chicken carcasses by contact with intestinal tracts during slaughter or processing) and differences among isolation

methods applied to detect Salmonella.

Four percent incidence in chevon was recorded in the present study (n= 50). This observation differs from the works done in the other parts of India wherein, 17.6 per cent and 38.7 per cent prevalence of Salmonella were recorded in goats slaughtered at Bareilly and Wardha district of Maharashtra, respectively [11, 28]. The differences in the prevalence reported could be associated with the sampling procedures, type of sample, transportation of samples, isolation techniques or the actual difference in the occurrence and distribution of Salmonella in the study population itself. This increased prevalence than earlier studies clearly indicates the presence of health hazards in chevon.

Out of 50 beef samples processed and screened, 8 per cent isolates were recovered. An overall 8 per cent Salmonella prevalence among beef samples in the current work is higher than a report [22] from 6 per cent (n=50) in Maharashtra, India and also higher than those reported 4 per cent (n=50) in faecal samples from diarrhoeic young animal, from Telangana, Chennai, Maharashtra, Goa, Uttar Pradesh, and Rajasthan [30]. The workers attributed the reason for lower prevalence in young animals and absences of Salmonella in bovine to the limited number of samples included in the study. The higher prevalence in the present study could be due to the actual prevalence of Salmonella in cattle itself or it might be attributed to the unhygienic conditions in slaughtering, dressing, washing and handling the carcasses leading to the cross contamination of the meat samples.

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 from the meat samples 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. This is in accordance with that of Chaiba [7], who had reported that *Salmonella typhimurium* was the predominant and most frequently isolated Salmonella serotypes among the 57 Salmonella isolated from meat samples. The isolation of the serovar *Salmonella typhimurium* in the present study is an indication of a close association of man and meat animals during any of the farm operations or consumption of contaminated meat. Isolation of *Salmonella typhimurium* alone from the meat samples might be attributed to the fact that only a small geographical area was covered in the present study.

A total of 11 (7.4 per cent) isolates of Salmonella were subjected for PCR with *invA* gene (genus specific for Salmonella genus). All the isolates were confirmed to carry the *invA* gene. [35] Recommended PCR for the detection of Salmonella from broiler meat and the incidence was 8.3 per cent (n=60) in Tamil Nadu. In this study primer targeting the *invA* gene was found to be an accurate for the identification of Salmonella. [21] Examined the presence of Salmonella by polymerase chain reaction in poultry carcasses. A total of 60 neck skin swab samples were taken from 12 different broiler flocks in Iran. PCR amplification of *invA* gene was the method for detection of Salmonella. In their study Salmonella was isolated from 11.6 percent of samples by conventional culture method. All positive isolates from conventional culture method were confirmed by PCR amplification and *invA* gene was superior for the confirmation of Salmonella.

A total of 11 confirmed isolates of were subjected for biofilm production assay. All were found to produce black colonies with dry crystalline consistency colonies on modified Congo Red Agar. The result indicates that all the isolates were able to produce biofilm. [42] Studied the biofilm formation ability of *Salmonella* isolates from a chicken slaughter plant in China. The isolates have exhibited variation in biofilm forming behaviour with a relatively high biofilm production was observed for *Salmonella agona* compared to *Salmonella typhimurium* isolates.

[27] Assessed the biofilm forming ability of *Salmonella enterica* isolated from meat products in USA. Twenty *Salmonella enterica* subsp *enterica* strains were isolated from meat (n=11) and meat products (n=7). Two (10 percent) *Salmonella enterica* subsp *enterica* strains from ground chicken meat and chicken sausage were defined as biofilm forming but others were considered non biofilm forming. Different studies have been conducted to see the biofilm formation of Salmonella isolates on different surfaces [3]. Various factors (growth medium, incubation period, fixation of adhered cells and staining) affect development of Salmonella biofilm [28]. According to those studies, the source of isolates (from humans, animals or food) did not affect the biofilm [18]. The evidence of biofilm shows the existing survivability of Salmonella in meat samples which indicates the persisting threat to public.

Out of five Salmonella isolates from chicken, 100 per cent were sensitive to azithromycin, chloramphenical and cefotaxime, 100 per cent were resistant to amoxycillin and metronidazole, 60 per cent were resistant to tetracycline and norfloxacin, 40 per cent were resistant to nalidixic acid and 20 per cent were resistant to ciprofloxacin. [14] Screened the raw meat to find out the prevalence of multi drug resistance Salmonella isolates. Among the variety of antibiotics tested, the highest resistance was observed with nalidixic acid followed by tetracycline, trimethoprim, and streptomycin. The isolates of chicken resistant to nalidixic acid, tetracycline, trimethoprim, and streptomycin were 90.6 per cent, 71.9 per cent, 56.6 per cent and 25 per cent respectively.

Out of two isolates of Salmonella from chevon, 100 per cent were sensitive to azithromycin, cephalexin, chloramphenical, timethoprim, tetracycline and cefotaxime, 100 per cent were resistant to amoxycillin, co-trimoxazole and metronidazole and 50 per cent were resistant to gentamicin, nalidixic acid, ciprofloxacin and norfloxacin. A cross sectional study was conducted on 249 apparently healthy slaughtered goats at the municipal abattoir of Dire Dawa to determine the antimicrobial susceptibility pattern of the isolates. Of all the isolates, 41 (93.2 per cent) were multiple antimicrobial resistant and the highest level of resistant was observed for tetracycline (100 per cent). In their study, isolates of Salmonella were showing highest sensitivity to ciprofloxacin [17].

Out of four Salmonella isolates from beef 100 per cent sensitive to amoxycillin, cephalexin, cefotaxime, perfloxacin and sulphamethizole, 100 per cent were resistant to metrinadazole, 75 per cent were resistant to norfloxacin and tetracycline, 50 per cent were resistant to gentamicin, nalidixic acid and 25 per cent were resistant to ciprofloxacin. [6] Author cultured the Salmonella from beef samples in Nigeria, the isolates were moderately sensitive to ciprofloxacin, sulphademethoxazole, chloramphenical. The highest sensitivity was observed for gentamicin and the isolates were completely resistant to tetracycline. Several studies have documented the high level of resistance of Salmonella to the tetracycline [45, 24] which is in agreement with the result obtained in this study. The high resistance of

tetracycline observed in the study could be as a result of the use of tetracycline in food animal production which has led to worldwide spread of tetracycline resistance observed in Salmonella isolates [43, 25, 32].

In this study the resistance of ciprofloxacin, nalidixic acid and cepholexin has also been reported because these antibiotics are commonly used in veterinary and human medicine. Salmonella resistance to the fluoroquinolones (ciprofloxacin) is of great concern to public health as invasive forms of Salmonellosis are treated with these compounds [19, 43, 24]. The absence of resistance to the fluoroquinolones by Salmonella serovars from foods in Nigeria has been documented [33]. Even though the sensitivity with cefotaxime was noticed, it is quite costlier than other commonly available antibiotics. Chloramphenicol is not frequently used nowadays due to many side effects but in this study complete sensitivity of chloramphenicol is noticed. The occurrence of multi-drug resistance Salmonella from food is of global health concern as this could lead to major healthcare challenge since multidrug resistance hinder the possibility of therapeutic treatments. Nowadays most of the human strains are resistant to chloramphenicol, sulphonamides and tetracycline. Recent resistant additions include resistance to trimethoprim and also particular concern to the fluoroquinolones [46]. 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.

Conclusion

Contamination of meat with Salmonella observed in this study might be attributed to the unhygienic and improper handling of meat during slaughter, dressing and evisceration. The usual practice of washing the carcass in the same water, in which the intestines and offal had been washed, was considered as one of the predominant reasons for the increased prevalence of Salmonella in the carcasses. The knowledge on hygienic meat production among the butchers was found poor. Henceforth, there is an urgent need for educating the butchers regarding the significance of Salmonellosis and its public health significance and also regarding clean meat production. Proper cooking and hygienic measures need to be followed by the consumer to avoid these organisms. There is also a need for a comprehensive epidemiological study and control of Salmonella contamination at various levels of meat production and slaughter points in Puducherry.

Table 1: Sources of raw meat samples

S. No	Place of sample collection	Number of Samples		
		Chicken	Chevon	Beef
1	Mettupalayam	2	3	-
2	Muthirapalayam	2	3	-
3	Kurumbapet	2	-	-
4	Dharmapuri	2	-	-
5	Kathirkamam	2	2	-
6	Thilasupet	1	2	-
7	Thattanchavady	2	3	-
8	Kalapet	1	3	3
9	Lawspet	2	3	-
10	Iyankuttipalayam	2	-	-
11	Reddiyarpalayam	2	2	1
12	Moolakulam	1	1	1
13	Manaveli	2	2	2
14	Arumbathapuram	2	2	2
15	Ariyankuppam	2	4	1
16	Thavalakkuppam	-	3	6
17	Othiyanpet	2	1	4
18	Koonichampet	2	2	4
19	Pillaiyarkuppam	2	1	1
20	Karikkalampakkam	2	1	4
21	Bahour	3	3	1
22	Thirukkanur	3	3	4
23	Thirubuvanai	2	1	4
24	Vazhuthavur	2	1	-
25	Sedarapet	2	1	2
26	Sulthanpet	3	3	10
	Total	50	50	50

Table 2: List of antibiotics used in this study.

Sr.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 3: Comparison of RVS and SB broth

Samples (n=150)	Salmonella		
	Total isolates	RVS	SB
Chicken (50)	5 (7.4%)	3 (60%)	2 (40%)
Chevon (50)	2 (4%)	2 (4%)	-
Beef (50)	4 (8%)	4 (8%)	-

Table 4: Primary and secondary biochemical test results

Characteristics	Sample No.										
	Chicken					Chevon		Beef			
	1	6	7	14	21	1	16	17	23	26	25
Primary and secondary biochemical tests											
Shape	R	R	R	R	R	R	R	R	R	R	R
Motility	+	+	+	+	+	+	+	+	+	+	+
Catalase	+	+	+	+	+	+	+	+	+	+	+
Oxidase	-	-	-	-	-	-	-	-	-	-	-
Urease	-	-	-	-	-	-	-	-	-	-	-
Simmon's citrate	+	+	+	+	+	+	+	+	+	+	+
Indole	-	-	-	-	-	-	-	-	-	-	-
MR Test	+	+	+	+	+	+	+	+	+	+	+
VP Test	-	-	-	-	-	-	-	-	-	-	-
H2S production in TSI slant	+	+	+	+	+	+	+	+	+	+	+
Lysine decarboxylase	+	+	+	+	+	+	+	+	+	+	+
Ornithine decarboxylase	+	+	+	+	+	+	+	+	+	+	+
ONPG	-	-	-	-	-	-	-	-	-	-	-
Sugar fermentation tests											
Dulcitol	+	+	+	+	+	+	+	+	+	+	+
Dextrose	+	+	+	+	+	+	+	+	+	+	+
Maltose	+	+	+	+	+	+	+	+	+	+	+
Xylose	+	+	+	+	+	+	+	+	+	+	+
Trehalose	+	+	+	+	+	+	+	+	+	+	+
Arabinose	+	+	+	+	+	+	+	+	+	+	+

+ : Positive; - : Negative

Table 5: Primer sequence for *invA* gene

Organisms	Target gene	Primer sequence (5'- 3')	Expected Product size (bp)	References
Salmonella spp	*invA*	**F**:GTGAAATTATCGCCACGTTCGGGCAA **R**:TCATCGCACCGTCAAAGGAACC	244	Rahn *et al.* (1992)

Table 6: Details of the Reaction mixture

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

Table 7: Details of the PCR conditons

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

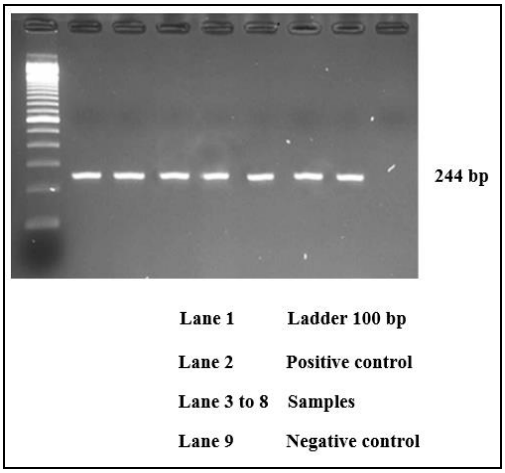

Table 8: Consolidated antimicrobial sensitivity pattern (n=11)

List of antibiotics	Chicken (%)			Chevon (%)			Beef (%)		
	R	I	S	R	I	S	R	I	S
Amoxycillin	100	-	-	100	-	-	100	-	-
Azithromycin	-	-	100	-	-	100	-	25	75
Co – trimoxazole	80	20	-	100	-	-	100	-	-
Cephalexin	-	20	80	-	-	100	-	-	100
Ciprofloxacin	20	20	60	50	-	50	25	75	-
Chloramphenical	-	20	100	-	-	100	-	25	75
Cefotaxime	-	-	100	-	-	100	-	-	100
Gentamicin	20	20	60	50	-	50	25	25	50
Metronidazole	100	-	-	100	-	-	100	-	-
Norfloxacin	60	20	20	50	50	-	75	25	-
Nalidixic acid	40	60	-	50	50	-	25	-	75
Perfloxacin	-	40	60	-	50	50	-	-	100
Sulphamethizole	20	20	60	50	-	50	-	-	100
Trimethoprim	20	-	80	-	-	100	50	-	50
Tetracycline	60	-	40	-	-	100	50	-	50

R – Resistant; S- Sensitive and I – Intermediate

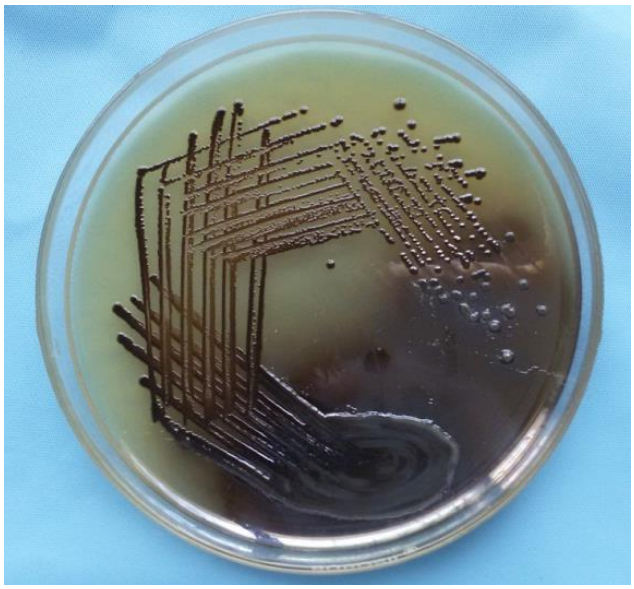

Plate 1: Salmonella – BSA

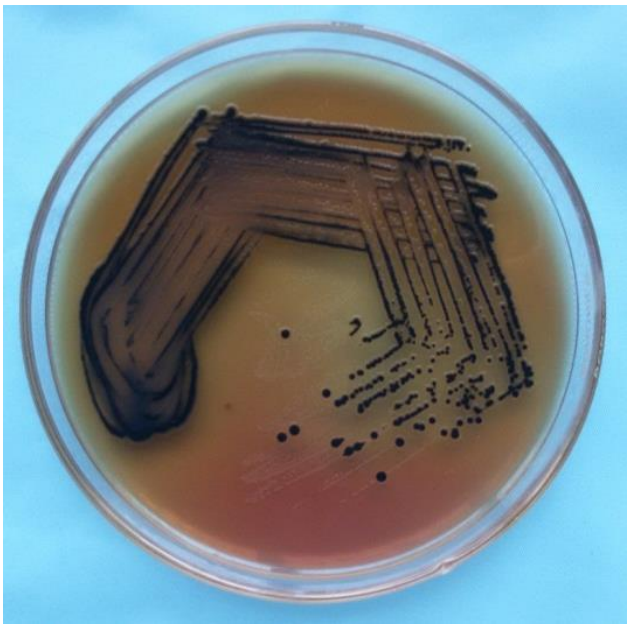

Plate 2: Salmonella – SSA

Plate 3: Salmonella – XLD

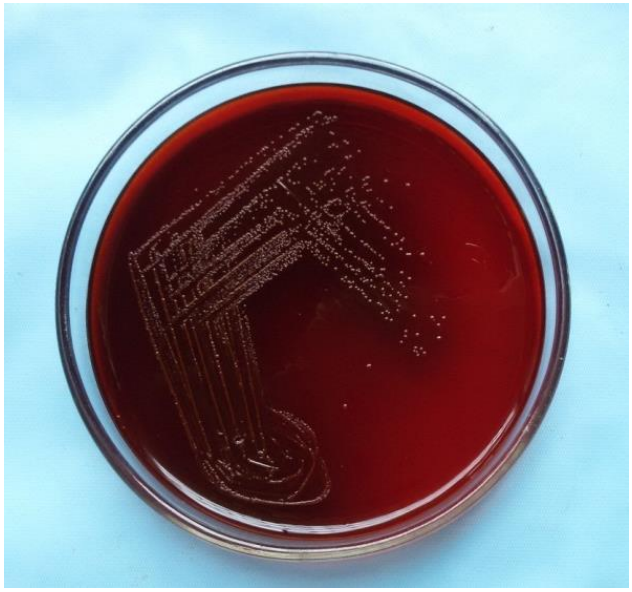

Plate 4: Biofilm positive

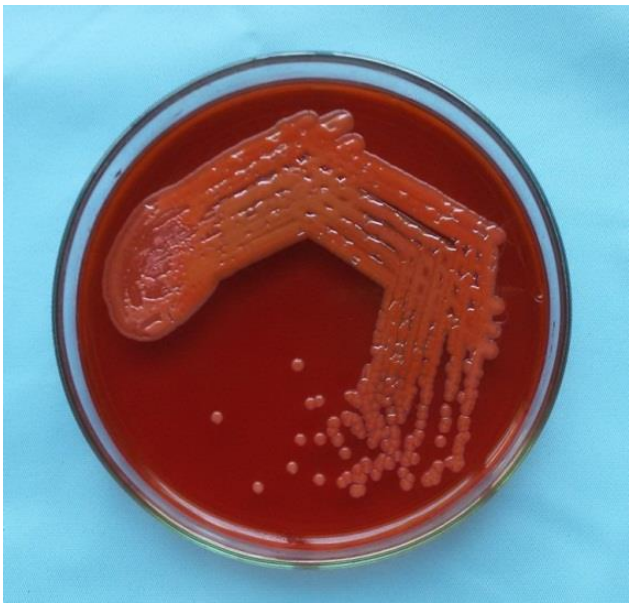

Plate 5: Biofilm negative

References

1. Antony B, Dias M, Shetty AK, Rekla B. Food poisoning due to Salmonella enterica serotype weltevreden in Mangalore. Indian Journal of Medical Research. 2009; 27(3):257-258.
2. Ali NK, Amber F, Adnan K, Ameera YK, Shahana UK. Microbial contamination of raw meat and its environment retail shops in Karachi, Pakistan. Journal of Infection in Developing Countries. 2010; 4(6):382-388.
3. Aggarwal P, Singh SM, Bhattacharya MM. An outbreak of food poisoning in a family due to Salmonella weltevreden at New Delhi. Journal of Diarrheal Disease Research. 1995; 3:224-225.
4. Ahmed A, Shimamoto T. Isolation and molecular characterization of Salmonella enterica, Escherichia coli O157:H7 and Shigella spp. from meat and dairy products in Egypt. International Journal of Food Microbiology, 2013, 168-169.
5. Agarwal R, Singh S, Bhilegaonkark, Singh V. Optimization of microtitre plates assay for the testing of biofilm formation on ability in different Salmonella

serotypes. International Food Research Journal. 2011; 18:1493.
6. Bata SI, Karshima NS, Yohanna J, Dashe M, Pam VA, Ogbu KI. Isolation and antibiotic sensitivity patterns of Salmonella species from raw beef and quail eggs from farms and retail outlets in Jos, Plateau State, Nigeria. Journal of Veterinary Medicine Animal Health. 2016; 8(4):29-34.
7. Chaiba A, Fouzia RF, Abdelkader C, Rachida SB, Mouloud Z. Occurrence of Salmonella in chicken carcasses and giblets in Meknes – Morocco. Pakistan Journal Nutrition. 2008; 7:231-233.
8. Choudhary SP, Narayan KG, Saxena SN, Mago ML, John PC. Isolation of Salmonella Bornum (6, 7, 14:238) for the first time in India. Indian Journal of Medical Science. 1985; 39(3):45-46.
9. Cowan ST. (3rd Ed.). Cowan and Steels'. Manual for the identification of medical bacteria. Cambridge University Press, Cambridge, 1974, 97-164.
10. Clinical & Laboratory Standards Institute (CLSI). Performance Standards for Antimicrobial Susceptibility Testing; Twenty second informational supplement, 2012, M100S22.
11. Chandra M, Singh BR, Shankar H, Agarwal M, Agarwal RK, Sharma G *et al*. Prevalence of Salmonella antibodies among goats slaughtered for chevon in Bareilly (northern India). Preventive and Veterinary Medicine. 2007; 80:1-8.
12. Dikid T, Hpalya SS, Thakur JS, Tanya N, Thapur R, Kumar R. Salmonella food poisoning outbreaks in Karar town of Punjab. Indian Journal of Public Health. 2009; 53(4):265.
13. Dabassa A, Bacha K. The prevalence and antibiogram of Salmonella and Shigella isolated from abattoir, Jimma town South West, Ethiopia. International Journal of Pharmacology and Biological Research. 2012; 3:143-7.
14. Dallal MMS, Mahnaz T, Latif G, Shabnam M, Maryam S, Rounak B *et al*. Characterization of antibiotic resistant patterns of Salmonella serotypes isolated from beef and chicken samples in Tehran. Journal of Microbiology. 2009; 2(4):124-131.
15. Dhanalakshmi G, Prabha R, Jagadheeshkumar D. Prevalance, antibiotic resistance and biofilm producing meat isolates in and around Namakkal, TN, India, International Journal of Advances in Interdisciplinary Research. 2015; 2(2):6-10.
16. FAO STAT. Food and Agriculture Organization htt://fao.org/default. 2012.
17. Ferede B, Dessica F, Feleke A, Tadesse G, Moje N. Prevalence and antimicrobial susceptibility of Salmonella isolates from apparently healthy slaughtered goats at Dire Dawa municipal abattoir Eastern Ethiopia. Journal Microbiology and Antimicrobials. 2015; 7(1):1-5.
18. Ghasemmahdi H, Hossein T, Mehran M, Karim M, Rojan M, Armen B *et al*. Antibiotic Resistance Pattern and Biofilm Formation Ability of Clinically Isolates of *Salmonella enterica* Serotype typhimurium. International Journal of Enteric Pathogens. 2015; 3(2):27372.
19. Gordon D. Ceftriaxone - resistant Salmonella infection from antibiotic- treated cattle. Gastroenterology. 2000; 119:3.
20. Herikstad H, Motarjemi Y, Tauxe RV. Salmonella surveillance, a global survey of public health serotyping. Epidemiology of Infection. 2002; 129:1-8.
21. Jamshidi A, Zahraei-Salehi T, Afshari-Nic S. Detection of Salmonella spp contamination of carcasses slaughtered in poultry abattoir in Mashhad, Iran. Archives of Razi Institute. 2016; 62(4):229-233.
22. Kalambhe DG, Zade NN, Chaudhari SP, Shinde SV, Khan W, Patil AR. Isolation, antibiogram and pathogenicity of *Salmonella* spp. recovered from slaughtered food animals in Nagpur region of Central India. Veterinary World. 2016; 9(2):176-181.
23. Kumara P, Rao J, Haribabuc Y, Manjunath D. Microbiological quality of meat collected from municipal slaughter houses and retail meat shops from Hyderabad and Karnataka region, India. APCBEE Procedia. 2014; 8:364-369.
24. Learn-Han L, Yoke-Kqueen C, Shiran MS, Sabrina S, Noor Zaleha AS, Sim JH *et al*. Molecular characterization and antimicrobial resistance profiling of Salmonella enteric subsp. Enteric isolated from 'Selom' (*Oenanthest olonifera*).International of Food Research Journal. 2009; 16**:**191-202.
25. Logue CM, Sherwood JS, Olah PA, Elijah LM, Dockter MR. The incidence of Antimicrobial - resistant Salmonella spp. on freshly processed poultry from US Midwestern processing plants. Journal of Applied Microbiology. 2003; 94:16-24.
26. Makabanyane IN, Ndou RV, Ateba CN. Genotypic Characterization of Shigella Species Isolated from Abattoirs in the North West Province, South Africa Using PCR Analysis. Journal of Food and Nutrition Research. 2015; 3(2):121-125.
27. Milan CA, Agostinetto RCS, Conceiçao HL, Gonzalez CDT. Sanitizer resistance of biofilm-forming Salmonella isolated from meat products. Arquivo Brasileiro de Medicina Veterinária eZootecnia. 2015; 67:642-646.
28. Moon AH. Studies on Prevalence and antibiogram of Salmonella species of polluted meat origin. Asiatic Journal Biotechnology Resources. 2011; 2(04):447-453.
29. National Livestock Policy & 19th Livestock Census 2012, Department of Animal Husbandry, Dairying & Fisheries, 2013
30. Nair A, Balasaravanan T, Malik SVS, Mohan V, Kumar M, Vergis J *et al*. Isolation and identification of Salmonella from diarrheagenic infants and young animals, sewage waste and fresh vegetables. Veterinary World. 2015; 8(5):669-673.
31. Oliver SP, Jayarao BM, Almeida RA. Foodborne pathogens in milk and the dairy farm environment: food safety and public health implications. Food borne Pathogens and Diseases. 2005; 2:115-129.
32. Parveen S, Taabodi M, Schwarz JG, Oscar TP, Harter-Dennis J, White DG. Prevalence and antimicrobial resistance of Salmonella recovered from processed poultry. Journal of Food Protection. 2007; 70:2466-2472.
33. Raufu IA, Zongur L, Lawan FA, Bello HS, Adamu MS, Ambali AG *et al*. Prevalence and antimicrobial profiles of Salmonella serovars from vegetables Maiduguri, North Eastern Nigeria. Sokoto. Journal of Veterinary Science. 2014; 12:23-28.
34. Rahn K, Grandis SAD, Clarke RC, Curtiss R, Gyles CL. Amplification of an invA gene sequence of *Salmonalla typhimurium* by polymerase chain reaction as a specific method of detection of Salmonella. Molecular and Cellular Probes, 1992, 271-279.
35. Shanmugasamy M, Velayutham T, Rajeswar J. InvA

gene specific PCR for detection of Salmonella from broilers. Chennai, TN. Veterinary World. 2011; 4(12):562-564.
36. Stalin M, Antony PX, Mukhopadhyay HK, Pillai RM, Thanislass J. Isolation, Characterization and Serotyping of Salmonella from chicken meat sold in retail outlets in Puducherry. Journal of Meat Science. 2011; 7(2):30-33.
37. Sharma I, Das K. Detection of invA Gene in Isolated Salmonella from Marketed Poultry Meat by PCR Assay. Journal of Food Process Technology. 2016; 7:564.
38. Stepanovic S, Circovic I, Ranin L, Svabic VM. Biofilm formation of *Salmonella* spp and Listeria monocytogenus on Plastic surface. Letters in Applied Microbiology. 2004; 38(5):428-32.
39. Vlkova H, Babak V, Seydlova R, Pavlik I, Schlegelova J. Biofilms andhygiene on dairy farms and in the dairy industry: sanitation chemical products & their effectiveness on biofilms – a review. Czech Journal of Food Science. 2008; 26:309-323.
40. Voogt N, Raes M, Wannet WJB, Henken NM, Vande GAW. Comparison of selective enrichment media for the detection of Salmonella in poultry faeces. Letters in Applied Microbiology. 2001; 32:89-92.
41. Vijayalakshmi P, Lalitha. Biotyping of coagulase negative and biofilm producing wound isolates of S. aureus. IJSID. 2013; 3(3):376-385.
42. Wang H, Ye K, Wei X, Cao J, Xu X, Zhou G. Occurrence, antimicrobial resistance and biofilm formation of Salmonella isolates from a chicken slaughter plant in China. Food Control. 2013; 33:378-384.
43. White DG, Zhao S, Sudler R, Ayers S, Friedman S, Chen S *et al*. The isolation of antibiotic-resistant Salmonella from retail ground meats. New England Journal of Medicine. 2001; 345:1147-1154.
44. WHO. Sixty-third World Health Assembly. Document WHA63/A63/11.2010 (Accessed 12th April 2013)
45. Yoke - Kqueen C, Learn-Han L, Noorzaleha AS, Son R, Sabrina S, JIun - Horng S. Characterization of multiple-Antimicrobial resistant Salmonella enteric subsp. Enteric isolated from indigenous vegetables and poultry in Malaysia. Letters in Applied Microbiology. 2008; 46:318-32.
46. Zelalem A, Nigatu K, Zufan S, Haile A, Alehegne W, Tesfu K. Prevalence and antimicrobial resistance of Salmonella associated from lactating cows and in contact humans in dairy farms of Addis Ababa: a cross sectional study. BMC infectious Diseases. 2011; 11:222.
47. Zhao HK, Wang WJ, Zhao P, Zhang HB, Kong N, Zhou MD *et al*. Simultaneous Detection of Salmonella, Listeria Monocytogenes and Shigella in Poultry Samples by Triplex PCR. Journal of Bacteriology and Mycology. 2014; 1(1):5.