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Biochemical characterization of *salmonella spp*. from diarrhoeic cases of pigs

Ramesh Kankanawadi, Basavaraj Awati, Ambadas and Shakuntala Kage

Abstract
The present study was designed to study biochemical characterization of the *Salmonella spp.* from pigs. A total of 175 faecal samples collected from different pig farms which are located in Bidar, Hassan and Tumakuru districts of Karnataka out of which 24feacal samples were found to be positive for *Salmonella spp.* Based on the morphological and cultural characters, suspected isolates were subjected to biochemical characterization the results revealed indicated that all the isolates were showing the typical characteristics of *Salmonella spp.* All the twenty four isolates showing typical colony characteristics were selected and further subjected to biochemical tests. All the isolates are Catalase-positive, Gram negative isolates were first in streaked on triple sugar iron (TSI) agar). Suspected isolates were inoculated into Tryptone water broth, Urease test, Indole test, methyl red test, Voges-Proskauer's test, Citrate utilization test those showing typical positive *Salmonella spp.* reaction were further subjected to specific biochemical tests are used by *Salmonella spp.* identification test and the obtained results are shown in Table 8 and 9.

Keywords: Tetrathionate broth, tryptone water broth, voges-proskauer's test medium, peptone water, glucose phosphate peptone water, methyl red indicator

Introduction
Pig rearing is one of the traditional activities in India carried out by rural folk. Among various livestock activities, piggery is most efficient way of meat production utilizing kitchen waste, vegetable waste etc. Though initially local breeds have been raised, now-a-days exotic pig breeding is popular and pork from such animal is having wide acceptance. Further, pig farming requires small investment on building and equipments.

A systematic of production pork involves food safety and food standard issues. The pork produced should be safe and wholesome. Food safety hazards caused by food-borne pathogens such as *Salmonella spp.* or serotypes remain a major problem for the food industry. Salmonellosis is an important health problem and a major challenge worldwide having greater significance in developing countries (Wang *et al*., 2008) [21]. Pork and pork products are recognized as an important source for human Salmonellosis (Smith *et al*., 2010) [19]. *Salmonella spp.*is an important cause of food-borne (alimentary) health problems in humans (Pang *et al.*, 1995; De Jong Skierus, 2006; Hernandeza *et al*., 2013) [3, 10]. The risk of *Salmonella spp.* might differ between the production systems, caused by components of the husbandry systems affecting disease development and pathogen shedding or differences in the level of resistance to the pathogen (Zheng *et al*., 2007) [23]. The increased consumption of pork coupled with the high prevalence of enter pathogens in the swine industry suggests a rise in food-borne illness cases which can lead to human food-borne illness and loss of product shelf-life.

The World Health Organization (WHO) reports that the incidence and severity of cases of Salmonellosis have increased significantly (WHO, 2010) [22]. Strains resistant to a wide range of antimicrobials emerged in the 1990s and constitute a serious additional concern for public health (WHO, 2010) [22]. The economic impact of this zoonosis in commercial food production is also substantial and control of *Salmonella spp.*is becoming more challenging with the trend towards cheaper and faster food.

The subspecies of *Salmonella spp.* can be further divided into serotypes, also called serovars, differentiated from each other based on the presence of somatic (O) and flagellar (H) antigens. The number of serotypes that have been identified is continuously increasing, today adding up to more than 2500 (Grimont, 2007) [8]. The majority (1531) of these serotypes belong to *Salmonella enterica* sub sp. *enterica* and were originally given names such as Typhimurium,

Dublin, Infantis *etc*., while the serotypes belonging to other subspecies have been identified by numbers according to their antigenic formulae (Grimont, 2007) [8].

According to WHO and the European Food Safety Authority (EFSA), all serotypes of *Salmonella enterica* are potentially hazardous to human health and thus regarded as pathogens (EFSA, 2010) [5]. However the majority of *Salmonella spp.* infections reported in humans and domestic animals are caused by relatively few of the more than 2500 serotypes.

The conventional methods of identification of *Salmonella spp.* including the one used by the Food and Drug Administration (FDA) are the time consuming and labour intensive. According to the Bacterial Analytical Manual (BAM) used routinely by FDA to test-food contaminated with *Salmonella spp.*, the sample is first enriched in general enrichment media for 24 hours. The enriched sample is then selectively enriched for 24 hours and then inoculated in selective and differential agar media for identification. Suspected *Salmonella spp.* colonies are then further tested with biochemical and serological methods for final identification. This identifications process takes about 3-5 days.

Materials and Methods

The material and methods used in the present study are presented in this chapter.

General considerations

The glassware used in this study were of neutral glass of Corning or Borosil India Ltd. make. The culture media, buffers and other biochemical reagents were prepared in MilliQ water (Millipore). The chemicals of molecular biology grade were used for the preparation of various solutions and reagents. Enzymes, DNA isolation kits and PCR reaction mixture and other requirements were obtained from M/s. Biosciences. Merck specialties Pvt. Ltd., Bangaloru; BD, BBL and DIFCO, USA; E-Merck (India) Ltd; Hi-media, Mumbai. Plasticware including micro centrifuge tubes, micropipette tips, cryovials, petri dishes and autoclave bags were procured from M/s. Tarson Products Pvt. Ltd., Kolkata.

Preparation of glassware

The glassware used in the study were prepared by soaking them in detergent solution overnight. The following day, they were washed thoroughly in running tap water, followed by rinse in deionised/distilled water (DW). Then oven dried glassware were packed and sterilized in hot air oven at 160 ºC for 1 hr.

Preparation of plastic ware

The new plastic ware including micro centrifuge tubes and micropipette tips were sterilized by autoclaving at 121 ºC for 15 min at 15 psi.

Media, reagents and stains used

MacConkey agar, Brilliant green agar, HI chrome *Salmonella* agar, Xylose liysine Deoxycholate agar, Nutrient agar, Triple sugar iron agar, Nutrient broth, Buffered peptone water, Selenite broth, Tetrathionate broth, Tryptone water broth, Voges-Proskauer's test medium, Peptone water, Glucose phosphate peptone water, Methyl red indicator, Glycerol, Tris Free base, E.D.T.A Sodium salt, Glacial Acetic acid, Hydrogen peroxide and gram's staining kits were obtained from M/s Hi media Laboratories Ltd., Mumbai. Media and reagents were prepared as recommended by the manufacturer instructions and sterilized by autoclaving.

Source and collection of samples

A total 175 feacal samples collected from different pig farms which are present in Bidar, Hassan and Tumukuru districts of Karnataka. The feacal samples were collected in sterilized transport media swabs (Hi Media) were collected aseptically collected from rectal feacal swabs from the suspected diarrhoeal cases were all collected samples transported to department of veterinary microbiology in thermocol box containing icepacks. All the samples were subjected to bacteriological isolation was carried out in Department of Veterinary Microbiology, Veterinary College, Bidar.

Isolation of *Salmonella spp.*

The study was conducted utilizing the conventional methods for the detection of *Salmonella spp.* following the standard guide lines from ISO 6579:2002. This isolation and identification procedure involved four principal stages: pre-enrichment, selective enrichment, selective plating and confirmation.

The pre-enrichment broth after incubation was mixed and 1 ml of the broth was transferred into a tube containing 9 ml Selenite broth (Himedia) and the inoculated Selenite broth were incubated at 37 ºC for 48 hr.

Plating out and identification of *Salmonella* spp.

After incubation for 18-24 hrs., a loop-full of material from the Selenite broth was transferred and streaked separately onto the surface of Brilliant green agar, HI Chrome *Salmonella spp.* agar, Xylose lysine deoxycholate agar, Mac Conkey agar, and plates separately. The plates were incubated at 37 ºC for 18-24 hrs. After incubation, the plates were checked for growth of typical *Salmonella spp.* colonies. Non lactose fermenter *Salmonella spp.* colonies appear colourless and transparent on Mac Conkey agar plates and typically did not alter appearance of the medium. Transparent colonies with black centres on HI Chrome *Salmonella spp.* agar, pink colonies with or without black centre on XLD agar and colourless or red to pinkish or opaque-white colonies aften surrounded by pink or red zone on BGA agar because the bacterium does not ferment lactose or sucrose. The pure cultures were streaked on triple sugar iron (TSI) agar slant and incubated at 37 ºC for 24-48 hrs. Those producing typical reaction on TSI (pink slant and black butt with H_2S production-blackening of agar). The suspected colonies were confirmed by staining characters and biochemical tests (BAM, 2007) [24].

Morphological characterization by Gram's staining method: The representative *Salmonella spp.* colonies were characterized microscopically using Gram's stain (Himedia) done as per the standard methods.

Biochemical confirmation

Suspected *Salmonella spp.* colonies were checked for their biochemical profiles. Here used two methods, one is laboratory tube prepared by using agar and broth and second is KB011 Hi *Salmonella*™ Identification kit (Himedia). These two methods involves biochemical tests as mentioned in the table-3.The commonly used biochemical tests included Catalase, Triple sugar iron (TSI) agar, Trypton water broth,

Indole, Methyl red, Voges Proskauer's test and Citrate test. The biochemical testing was done according to standard protocol.

Tryptone water broth

Tryptone water broth	17.29 gm
Distilled water	1000 ml

Heated if necessary to dissolve the medium completely. Dispensed and sterilized by autoclaving at 15 lbs. pressure (115 °C) for 15 min. Transfer the sterile tryptone water broth into sterilized test tubes and inoculated suspected colony from HI Chrome *Salmonella spp.* agar and incubation for 48 hrs. At 37 °C. After incubation there is a change of colour from purple to yellow colour. Here yellow colour is considered as positive and where same appeared as purple colour considered as negative.

Catalase test

A well grown 18-24 hrs. Old culture on HI Chrome *Salmonella spp.* agar plate was picked up and spread on clean glass slide, a drop of 3 per cent hydrogen peroxide (H_2O_2) added on the smear and observed for the immediate development of bubbles which was considered as positive for Catalase.

Rapid Urease Test Broth

Rapid Urease Test Broth	20.30 gm
Distilled water	1000 ml

Mixed well and sterilized by filtration. Did not autoclave or heat the medium. Dispensed in sterile tubes as desired.

Appearance: Light yellow to light pink homogeneous free flowing powder.

Colour and Clarity of prepared medium: Yellowish orange coloured clear solution in tubes.

Table 1: Biochemical characteristics of *Salmonella* species

SL No	Name of test	Typical *Salmonella* reaction
1.	Catalase	+
2.	H_2S production on TSI	+
3.	Indole test	-
4.	Methyl red(MR) test	+
5.	Voges Proskaur(VP) test	-
6.	Citrate test	+
7.	Urease test	-
8.	Lysine decarboxylation	+
9.	ONPG	+
10.	Trypton water broth	+
11.	Acid from sugars	
	a. Lactose	-
	b. Arabinose	+
	c. Maltose	+
	d. Sorbotol	+
	e. Dulcitol	V

Note: +: 90 % strains positive, -: 90 % strains negative, and V: variable reaction

A well grown 18-24 hrs. old culture of each presumed-positive colonies on HI Chrome *Salmonella spp.* agar were colony was inoculated into tubes of urea agar and incubated at 24 hrs. At 37 °C. The agar turns purple-red in positive test and yellow in negative test.

Indole test

Tryptone broth	15 gm
Distilled water	1000 ml

A loopful of culture was inoculated into a test tube containing tryptone broth and incubated for 48 hrs. At 37 °C. Later on, 5 drops of Kovac's reagent were added. A positive reaction was noticed by development of bright red coloured ring and negative reaction by the yellow coloured ring.

Methyl Red test

MR-VP Medium (Glucose Phosphate Broth)	17gm
Distilled water	1000 ml

Heated if necessary to dissolve the medium completely. Distributed in test tubes in 5 ml amounts and sterilized by autoclaving at 15 lbs. pressure (115 °C) for 15 min.

Appearance: Cream to yellow homogeneous free flowing powder.

Colour and Clarity of prepared medium: Light yellow coloured clear solution without any precipitate.

A loopful of culture was inoculated into a test tube containing 5 ml MR-VP medium (Glucose Phosphate Broth) and incubated for 48 hrs. At 37 °C. Later on, 5 drops of methyl red indicator were added. A positive reaction was noticed by development of bright red colour and negative reaction by the yellow colour.

Voges-Proskauer (VP) reaction

MR-VP Medium (Glucose Phosphate Broth)	17gm
Distilled water	1000 ml

Heated if necessary to dissolve the medium completely. Distributed in test tubes in 5 ml amounts and sterilized by autoclaving at 15 lbs. pressure (115 °C) for 15 min.

To the test tube containing 5 ml of sterile MR-VP medium (Glucose Phosphate Broth), a loopful of young culture was inoculated and incubated at 37 °C. After 48 hrs. Add few drops Barritt's A (α-napthol) and Barritt's B (40% potassium hydroxide) were added and mixed well. The tubes were kept at room temperature in slanting position and examined after 15 min and 1 hr intervals. In positive reaction a pink or crimsoned colour was developed, while in negative reaction there was no colour change.

Citrate Utilization test

Simmon's Citrate Agar slants were inoculated by the suspected colony of *Salmonella spp.* from HI Chrome *Salmonella spp.* agar plate and incubated at 37 °C for 48 hrs. After incubation the slants was examined for growth and colour changed. Growth on the medium is accompanied by a rise in pH to change the medium from its initial green colour to deep blue was considered as positive tests. All the isolates showed positive reactions in biochemical tests were further employed for characterization by PCR using various virulence associated genes and antimicrobial resistance patterns.

Sugar fermentation tests

Sugar fermentation tests was performed production of acid

gas from ONPG, Arbinose, Lactose, Maltose Sorbitole and Dulcitol respectively, these test are done by using KB011 Hi*Salmonella*™ identification kit manufactured by Himedia colour change in positive purple or dark purple and other all test yellow colour in positive reaction and in case of negative colour appear as same colour.

Motility test

Motility test medium	20 gm
Distilled water	1000 ml

Motility of the organism was detected by inoculating in motility test medium by straight wire and incubating at 37 ºC for 24 hrs. After the incubation positive reaction growth away from stab line causing turbidity.

Standard / reference strain

The standard strain of *Salmonella spp.* (*S.* Typhimurium No. Ref 0180 P) used study was obtained from 11 rue Emile 20LA B.P. 2332 38033 Grenoble Cedex 2 France. Microbiologic 200 Cooper Avenue North St. Cloud MN 56303, www.microbiologics.com, LIT: 2128, Rev F (Himedia laboratory Pvt. Ltd. Mumbai). Were this was inoculated into sterile nutrient broth and incubated at 37 ºC for 24hrs. After incubation streaked onto selective media HI Chrome *Salmonella spp.* agar were incubated at 24 hrs. at 37 ºC. After incubation pure colony picked up and inoculated into 20 % nutrient-glycerol preservative were incubated at 37 ºC for 24 hrs. After, incubation stored at -20 ºC.

Results

The present study was carried out with an objective of isolation, identification and molecular characterization of the *Salmonella spp.* from suspected diarrheic cases in pigs from different pig farms in Bidar, Hassan and Tumkuru districts of Karnataka. The result obtained during the programme of research work was documented as follows.

Collection of samples

A total 175 samples were collected from different pig farms of Karnataka (Table 7). The samples were processed in the Dept. of Veterinary Microbiology, Veterinary College, Bidar by standard protocols.

Isolation of *Salmonella spp.*

All the samples were subjected to bacteriological isolation, A total of 24 (13.71 %) *Salmonella spp.* isolates were obtained in the present study (Table 7).

Cultural and morphological characterization

Samples were positive for *Salmonella spp.* organisms was identified by cultural and biochemical characterization. The colony characters showed on different selective media are HI chrome *Salmonella* agar showed transparent colonies with black centres due to production of H_2S_4, pink colonies with black centre on XLD agar due to production of H_2S_4, colourless or red to pinkish white colonies after surrounded by pink or red zone on BGA agar because the bacterium does not ferment lactose or sucrose and Non lactose fermenter *Salmonella spp.* colonies appear colourless and transparent on MacConkey agar plates and typically did not alter appearance of the medium and colourless with transparent colonies on nutrient agar Depending on cultural characters samples out of the total 175 samples were suspected for *Salmonella spp.* organisms and from these 24 were confirmed to be *Salmonella spp.*by biochemical characteristics. The rest samples were confirmed to be other *Enterobacteriaceae* on the basis of their biochemical properties.

On Gram's staining, the morphologically isolated bacteria was small rod shaped, Gram negative, single or paired in arrangement.

Table 2: Collection of feacal samples from different farms of Karnataka

SL No	Details of pig framing Karnataka	Suspected diarrhoeic cases	Total no. of isolates	Isolates (%)	Overall prevalence %
1.	Krishna farm shivanagar, Bidar	41	5	12.2	13.71
2.	Slaughter house Bidar	34	5	14.7	
3.	Kumar Naik, Hassan(D)	15	2	13.3	
4.	Konehalli farm, Tumukur (D)	10	1	10	
5.	KVK, Hassan	17	2	11.7	
6.	ILFC, HVC, Hassan	58	9	15.5	
Total		175	24	13.71	

Biochemical characterization of isolated *Salmonella* species

All the twenty four isolates showing typical colony characteristics were selected and further subjected to biochemical tests. All the isolates are Catalase-positive (Plate 15), Gram negative isolates were first in streaked on triple sugar iron (TSI) agar (Plate 9) and & 9). Suspected isolates were inoculated into Tryptone water broth (Plate 8), Urease test (Plate 7), Indole test (Plate 10), methyl red test (Plate 11), Voges-Proskauer's test (Plate 12), Citrate utilization test (Plate 13) those showing typical positive *Salmonella spp.* reaction were further subjected to specific biochemical tests are used by *Salmonella spp.* identification test (Plate 14) and the obtained results are shown in Table 8 and 9.

Table 3: Biochemical characteristics of isolated *Salmonella* species

SL No	Name of biochemical test	Shown reaction	Negative reaction
1.	Catalase (+)	Presence of bubbles	Absence of bubbles
2.	H_2S production on TSI (+)	Black Butt and pink slant	Orange yellow
3.	Indole test (-)	Bright red	Yellow
4.	Methyl red (MR) test (+)	Red	Yellowish-orange
5.	Voges-Proskaur's (VP) test (-)	Pinkish red	Colourless/ slight copper
6.	Citrate test (+)	Blue	Green

7.	Urease test (-)	Pink	Orange yellow
8.	Lysine decarboxylation (+)	Purple/ dark purple	Yellow
9.	ONPG (+)	Yellow	Colourless
10.	Trypton water broth (+)	Yellow	Purple
11.	**Acid from sugars**		
	a. Lactose (-)	Yellow	Red/Pink
	b. Arabinose (+)	Yellow	Red/Pink
	c. Maltose (+)	Yellow	Red/Pink
	d. Sorbotol (+)	Yellow	Red/Pink
	e. Dulcitol (V)	Yellow	Red/Pink

Table 4: Biochemical characteristics of *Salmonella spp.* isolates from feacal samples

SL No	Isolate No.	Urease	TWB	TSI (H_2S)	Catalase	Indole	MR	VP	CUT	LDC	ONGP	Lactose	Arabinose	Maltose	Sorbotol	Dulcitol
1	F3	-	+	+	+	-	+	-	+	+	+	-	+	+	+	+
2	F4	-	+	+	+	-	+	-	+	+	+	-	+	+	+	+
3	F6	-	+	+	+	-	+	-	+	+	+	-	+	+	+	+
4	F23	-	+	+	+	-	+	-	+	+	+	-	+	+	+	+
5	F36	-	+	+	+	-	+	-	+	+	+	-	+	+	+	+
6	F43	-	+	+	+	-	+	-	+	+	+	-	+	+	+	+
7	F51	-	+	+	+	-	+	-	+	+	+	-	+	+	+	+
8	F63	-	+	+	+	-	+	-	+	+	+	-	+	+	+	+
9	F66	-	+	+	+	-	+	-	+	+	+	-	+	+	+	+
10	F70	-	+	+	+	-	+	-	+	+	+	-	+	+	+	+
11	F77	-	+	+	+	-	+	-	+	+	+	-	+	+	+	+
12	F84	-	+	+	+	-	+	-	+	+	+	-	+	+	+	+
13	F91	-	+	+	+	-	+	-	+	+	+	-	+	+	+	+
14	F105	-	+	+	+	-	+	-	+	+	+	-	+	+	+	+
15	F111	-	+	+	+	-	+	-	+	+	+	-	+	+	+	+
16	F124	-	+	+	+	-	+	-	+	+	+	-	+	+	+	+
17	F129	-	+	+	+	-	+	-	+	+	+	-	+	+	+	+
18	F135	-	+	+	+	-	+	-	+	+	+	-	+	+	+	+
19	F137	-	+	+	+	-	+	-	+	+	+	-	+	+	+	+
20	F147	-	+	+	+	-	+	-	+	+	+	-	+	+	+	+
21	F153	-	+	+	+	-	+	-	+	+	+	-	+	+	+	+
22	F157	-	+	+	+	-	+	-	+	+	+	-	+	+	+	+
23	F163	-	+	+	+	-	+	-	+	+	+	-	+	+	+	+
24	F167	-	+	+	+	-	+	-	+	+	+	-	+	+	-	-

F: Feacal sample

Plate 1: Urease test: 1. Test sample, 2. Positive control, 3. Negative control

Plate 2: Tryptone water broth: 1.Test sample, 2.Positive control, 3. Negative control

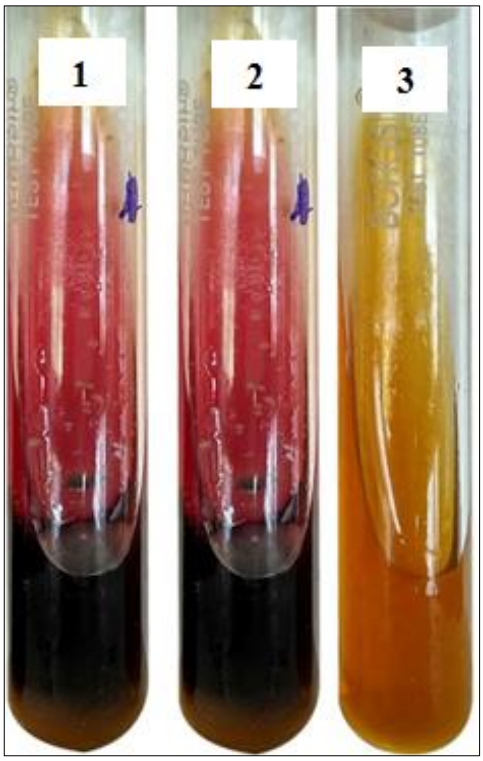

Plate 3: H_2S gas on TSI agar: 1.Test sample, 2. Positive control, 3. Negative control

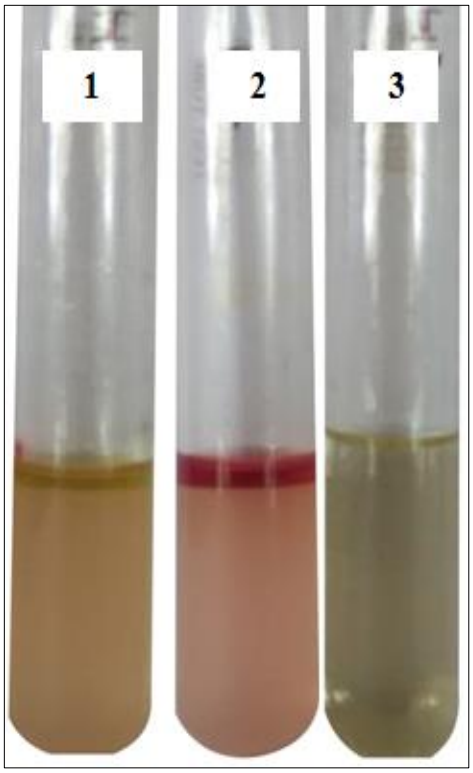

Plate 4: Indole test: 1. Test sample, 2. Positive control, 3. Negative control

Plate 5: Methyl red test: 1. Test sample, 2. Positive control, 3. Negative control

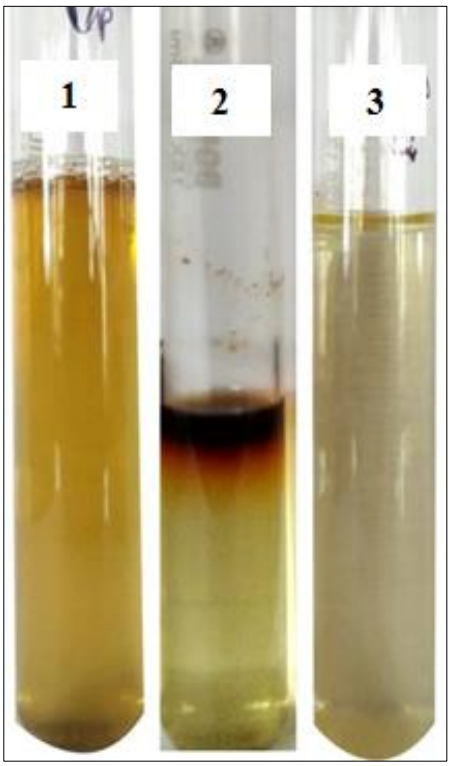

Plate 6: Voges- Proskauer's test: 1. Test sample, 2. Positive control, 3. Negative control

Plate 7: Citrate utilization test: 1.Test sample, 2.Positive control, 3. Negative control

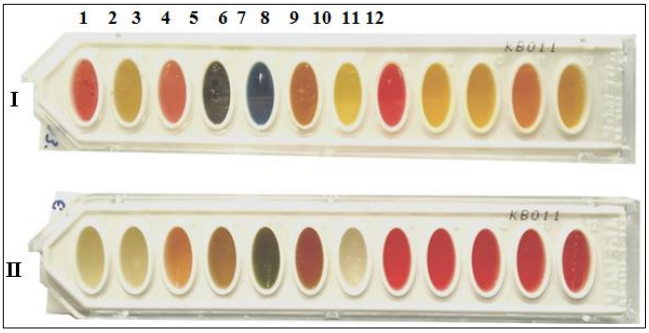

Plate 8: I. Test sample, II. Negative control. Hi *Salmonella spp.* identification kit showing biochemical characteristics of *Salmonella spp.*

Plate 9: Catalase test: Bubbles formation showing Positive reaction

Discussion

Salmonellosis is one of the most important zoonotic diseases and worldwide problem. It is assuming greater significance in developing countries like India. Salmonellosis has been recognized and studied mainly in industrialized countries while occasional outbreaks of non-typhoidal Salmonellosis due to food contamination have been detected in other countries. A number of reports have indicated that the occurrence of organism in various foods *viz*. poultry, beef, pork, eggs, milk, cheese, fish, shellfish, fresh fruit and juice and vegetables have been found to be epidemiologically associated with *Salmonella spp.* infection. The presence of *Salmonella spp.* in feacal sample (rectal swab) from different pig farms with the overall incidence rates varying from 0 to 82 per cent have been reported (Venkateswaran *et al*., 1988; Ejeta, 2004; Van *et al*., 2007 and Kuhn *et al*., 2013) [20, 6, 14].

The incidence of food-borne illness *Salmonella spp.*continue to be an important problem throughout the world. The economic losses associated with *Salmonella spp.*infection have attracted increasing attention in developing countries in recent year although Salmonellosis is endemic in nature and responsible for heavy economic loss in India every year. Pork products are contaminated with harmful, pathogenic and spoilage bacteria by infected stocks, cross contamination due to improper handling and during storage or improper cooking which can lead to human food-borne illness and loss of product shelf-life. Epidemiological data are needed to monitor trends over time. Food-borne Salmonellosis is a notifiable condition in many countries including US and UK (Choudhary *et al*., 2015 [25] and Kumar *et al*., 2008) [18].

Some studies have provided evidence that the virulence plasmid plays a significant role in human disease (Guiney *et al*., 1994) [9]. *Salmonella spp.* induced diarrhoea is a complex phenomenon on involving several pathogenic mechanisms, including production of enterotoxin. This enterotoxin production is mediated by the *stn* thus, it plays a significant role in causing gastroenteritis by producing enterotoxin (Chopra *et al*., 1987) [2]. Hence, the present study was undertaken to isolate, identify, antibiotic sensitivity test and discriminating power of PCR based methods to identify *Salmonella spp.* from suspected diarrhoeic feacal samples to know the prevalence in the different pig farms from different places of Bidar, Hassan and Tumakuru districts of Karnataka.

5.2 Isolation and identification of *Salmonella spp.*

The feacal samples of pigs were analyzed for the presence of *Salmonella spp.* organisms using the standard protocol of two steps pre-enrichment in buffered peptone water, enrichment in selenite broth and selective plating on Hichrome *Salmonella spp.* agar, XLD agar, BGA and MacConkey agar, the isolates obtained were having similar characters as reported by Rajeshwari. (2013) [17]; Paramesh. (2015) [17] and Choudhary *et al*. (2015) [25] have shown isolates with similar characteristics shows in present study. Hektoen Entric agar and XLD agar were highly selective and differential media (Fagerberg and Avens, 1976) [7]

5.3 Biochemical characterization of *Salmonella* species

In the present study, specific biochemical tests were used for the detection of *Salmonella spp.* based upon the morphological, colony characters, and biochemical tests all the *Salmonella spp.* This serotype is one of the most commonly identified serotype in animals next to *S. Enteritidis*. All of the isolates were trypton water broth (Downes and Ito, 2001) [4], Indole negative, Methyl red positive, VP negative, Citrate positive, Urease negative, Lysine decarboxylase test positive, ONGP test positive, Catalase test, TSI positive and other sugar fermenters *viz*. lactose negative, arabinose positive, maltose positive, sorbitol positive and dulcitol positive. Based on the biochemical tests our findings were similar as previously suggested by the authors (Iwade *et al.*, 2006; Sivakumar *et al*., 2012; Kalambhe *et al*., 2015; Megha *et al*., 2015; Choudhary *et al*., 2015 [25]

and Karthik *et al*., 2016) [11, 18, 12, 13, 15].

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