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Studies on Molecular Characterization of *Salmonella spp.* Isolated from Field Samples of Small Ruminants

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A B S T R A C T

Molecular characterization of the *Salmonella* spp isolated from small ruminants in and around Bidar. A total of 140 fecal samples collected were from sheep and goats. Based on the morphological, cultural and biochemical characterization indicated that 8 isolates were *Salomnella spp.* Biochemically confirmed isolates were subjected to i*nvA* gene based PCR and diagnostic amplicon specific to virulence gene of 284 bp was evident in all the 8 *Salmonella* isolates indicating potential carriage of invasion properties linked to virulence.

Introduction

Salmonellae are one of the most common causes implicated in outbreaks of food born infectious disease around the world (Abou-Zeed *et al*., 2000). *Salmonellae* have a wide host range, including, animals, birds and human (Douce *et al*., 1991). *Salmonellae* produce a variety of putative virulence determinants, including adhesions, invasion, fimbriae, exotoxin and endotoxin (Jones *et al*., 1982). Virulence of microorganism is associated with the capacity to attach and colonize at the site of infection, with subsequent damage to the host and is promoted by aggregation that interferes with the host defense (Abou-zeed *et al.,* 2000 and Pasquaii *et al.,* 2004). The isolation and identification of *Salmonellae* by traditional methods is time consuming and laborious.

The polymerase chain reaction (PCR) provides a way of overcoming these difficulties and allows amplification of the target DNA (Murugkar *et al.,* 2003). The aim of this study was to determine virulence patterns of *Salmonella* spp isolated from sheep and goats. All isolates were tested for presence of virulence *invA* gene.

Materials and Methods

Isolation and identification of *Salmonellae*

A total of 140 fecal samples from sheep and goats were collected. The collected samples were added to the pre-enrichment media (Selenite F broth and Tetrathionate broth)

and were incubated at 37°C for 24 hours. The suspected colonies cultivated onto MacConkey's agar, XLD agar, *Salmonella* Shigella agar and BGA. The inoculated plates were incubated for 24 48 hours at 37°C. Suspected colonies were identified primarily as Gram- negative rods. The pure colonies of the isolates were identified biochemically using Sugar fermentation Reaction (Karim *et al*., 2008).

Detection of *Salmonella spp* by PCR

Isolation of DNA

The DNA was extracted from the isolated colony by Snap-chill method (Zaheri *et al*., 2005) and isolated DNA was subjected to PCR by using reported *Salmonella* specific primers (*invA* gene).

Determination of purity and yield of the DNA samples by UV spectrophotometry

The purity and concentration of the extracted genomic DNA was estimated by UV spectrophotometry. An aliquot of 20 µl of DNA sample was dissolved in 0.98 ml of sterile distilled water. The diluted DNA was transferred in to 1ml microcuvette and the optical density (OD) was read at 260nm and 280nm in a UV spectrophotometer. Sterile DW was used as blank. The ratio of 260/280 OD was calculated. A ratio of 1.7 to 1.9 was considered pure. Further, the purity of the DNA sample was checked by electrophoresis on (0.8 %) agarose gel.

The following primers were used

Two oligonucleotide primers were selected for *Salmonella*

Fowared: [5′-GTG AAA TTA TCG CCA CGT TCG GGC AA-3′]

Reverase: [5′-TCA TCG CAC CGT CAA AGG AAC C-3′]

PCR amplification and cycling protocol

DNA samples were amplified in a total of 50µl of the following reaction mixture (Table 1). After mixing the contents, tubes were centrifuged to collect the contents in the bottom. The tubes with cap were placed firmly into the thermal cycler and conditions were set as detailed below (Table 2).

Electrophoresis

The PCR products were visualized by gelelectrophoresis. Samples of final PCR product were mixed with loading dye and loaded onto a 1.5% agarose gel and subjected to electrophoresis for 1 hour at two V. in LX TBE buffer (Murugkar *et al.,* 2003).

Results and Discussion

All suspected colonies of *Salmonella* on the basis of cultural and morphological properties were subjected to biochemical tests (Table 3). Eight isolates were confirmed to be *Salmonella* species after the biochemical results.

PCR based confirmation of *Salmonella* to genus level

The PCR is a highly accurate method which makes it possible to detect nucleic acid amplification products. The results can be obtained rapidly so that they can be used not only to support bacteriological investigation but also to make the result more reliable (Galan and Curtiss, 1991).

In the present study, a PCR was used to amplify *Salmonella* specific target DNA sequences. A new set of oligonucleotides primer taken from the *invA* gene was used for amplification to detect and identify *Salmonella* serovares. The primers are different from those suggested in previous studies of (Baumler *et al.,* 1997). The investigation using PCR for the presence of *invA* gene in this study demonstrated its presence in all examined *Salmonella* isolates. This finding was consistent with previous reports (Galan and Curtiss, 1991) that established the presence of *invA* gene in nearly all *Salmonella* spp.

Table.1 Components of PCR reaction mixture

Sl. No.	Components	Volume per reaction
1	PCR master mix (Taq buffer,Taq polymerase,dNTPs)	25µl
2	Primer Forward	2 µl
3	Primer Reverse	2 µl
4	Template (*Salmonella* DNA)	2 µl
5	Nuclease free water -to make a final volume of 50 µl	19 µl
Total		**50 µl**

Table.2 PCR program for amplification of *invA* gene

	Step		Temperature	Duration	Cycle
1	Initial denaturation		95	1 min	1
2	Cycling	Denaturation	94	1 min	35
		Annealing	55	1 min	
		Extension	72	1 min	
3	Final denaturation		72	5 min	1

Table.3 Summary of the biochemical test results of *Salmonella* isolates

Sl. No.	Biochemical test	G1	G2	G3	G4	G5	G6	G7	G8
1	**Indole formation**	-	-	-	-	-	-	-	-
2	**Methyl red test**	+	+	+	+	+	+	+	+
3	**Voges-Proskauer Reaction**	-	-	-	-	-	-	-	-
4	**Citrate utilization**	+	+	+	+	+	+	+	+
5	**Urea hydrolysis**	-	-	-	-	-	-	-	-
6	**TSI H_2S production**	+	+	+	+	+	+	+	+
7	**Catalase**	+	+	+	+	+	+	+	+

(+) Positive after 48hrs of incubation

(G) Isolates *Salmonella*

Plate.1 PCR amplification of inv-A gene of *Salmonella* isolates; M- 100 bp DNA ladder; Lane 1- Positive control; Lane- 2,3,4- *Salmonella* isolates; Lane 5- Negative control

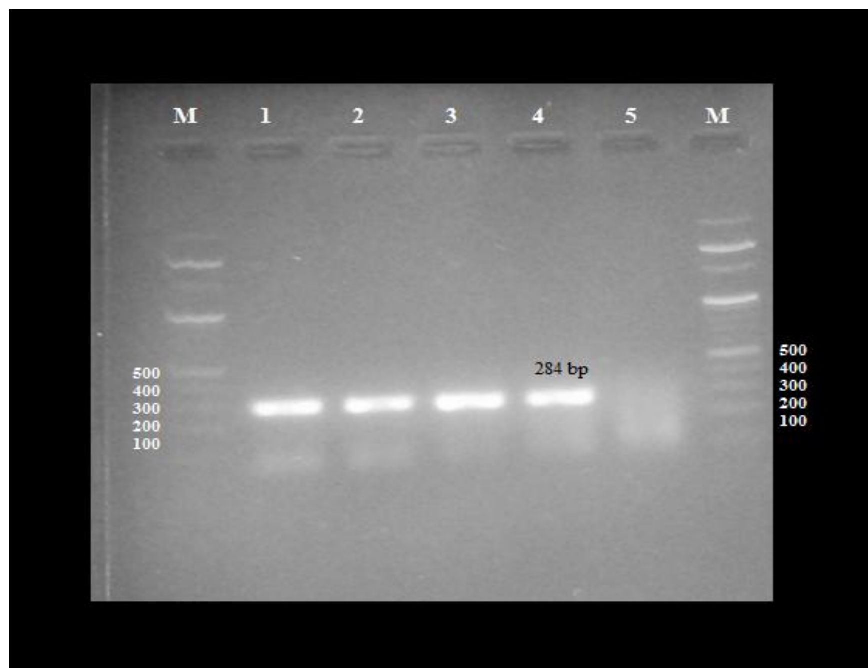
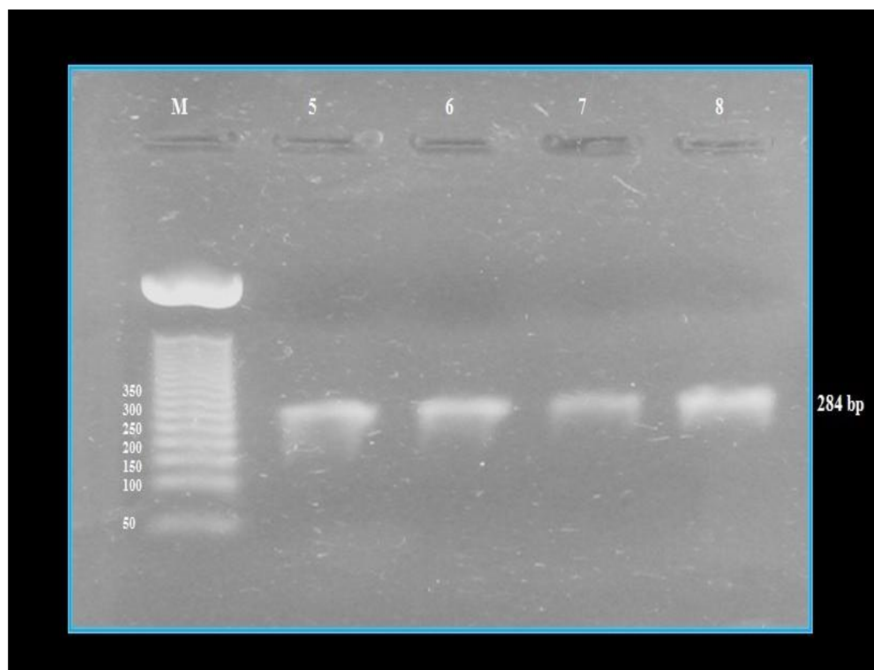


Plate.2 PCR amplification of inv-A gene of *Salmonella* isolates; M- 50 bp DNA ladder; Lane 5,6,7,8- *Salmonella* isolates



The *invA* gene is important in the invasion of phagocytic epithelial cells and entry into the intestinal mucosa (Tanaka *et al*., 2004). Based on the high specificity of primers targeting *invA* gene, they were employed for the specific confirmation of the *Salmonella* isolates.

All the *Salmonella* suspected cultures subjected to PCR amplification generated a product of approximate molecular size 284 bp. 50 and 100 bp DNA marker was used as a molecular weight marker. The band size detected in all the *Salmonella* isolates and analyzed by agarose gel electrophoresis 8 isolates were confirmed to genus level by *invA* gene based PCR (Plate 1 and 2).

From the present studies it was concluded that *Salmonella* is highly pathogenic microorganism affected animal production and cause serious diseases. The virulence effects of *Salmonella* attributed to its abilities to adherence and attachment to the host epithelial cells, also the presence of *invA* gene which responsible for the invasion properties.

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