ISSN: 2319-6505

Available Online at http://journalijcar.org

International Journal of Current Advanced Research
Vol 5, Issue 7, pp 1053-1057, July 2016

International Journal of Current Advanced Research

ISSN: 2319 - 6475

RESEARCH ARTICLE

PREVALENCE OF *SALMONELLA* IN FOOD SAMPLES OF DEHRADUN REGION

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ARTICLE INFO

Article History:

Received 20th April, 2016
Received in revised form 19th May, 2016
Accepted 15th June, 2016
Published online 25th July, 2016

Key words:

Salmonella, food-borne disease

ABSTRACT

Salmonella is one of the most common causes of food-borne disease. For this reason, the number of rapid test methods for *Salmonella* has grown rapidly in the last decade. PCR has become powerful tools for the detection of pathogens in food. Many different PCR assays have been developed for *Salmonella*, all with different specificities, accuracies, and detection limits. 20 different food samples (goat intestine, poultry intestine, coriander leaves, mint leaves and pastry) were collected from different locations of Dehradun city. For isolation of enteric pathogens, the samples were enriched and selective isolation was carried out. The results of staining, biochemical characteristics and selective isolation indicated the prevalence of *Salmonella* sp. in 20% of samples. Other enteric isolates were identified to be *Proteus* sp. on the basis of Phenyl Pyruvic Acid (PPA) and Triple Sugar Iron (TSI) test. Further, the enrichment broth was processed for PCR assay by using Salmonella Detection Kit, Which contains the amplification of *Salmonella* sp. specific gene invA (284 bp) using specific primers. The amplified product was detected by agarose gel electrophoresis. The results of PCR indicated the same prevalence (20%) of *Salmonella* sp. therefore, results of the bacteriological test correlated with PCR findings. Hence, the present study concludes considerable prevalence of *Salmonella* sp. in food sample which was confirmed both bacteriologically and PCR assay.

INTRODUCTION

Salmonella is closely related to the *Escherichia* genus and are found worldwide in cold- and warm-blooded animals (including humans), and in the environment. They cause illnesses such as typhoid fever, paratyphoid fever, and foodborne illness.

Typhoid fever, also known as enteric fever occurs worldwide, primarily in developing countries, including Indonesia. Typhoid fever is a systemic infection caused primarily by *Salmonella* serotype Typhi. The disease remains an important public health problem in developing countries. In 2000, it was estimated that over 2.16 million episodes of typhoid occurred worldwide, resulting in 216,000 deaths and that more than 90% of this morbidity and mortality occurred in Asia [1]. The transmission of typhoid fever occurs by oral transmission via food or beverages handled by an individual who chronically sheds the bacteria through stool and via sewage-contaminated water sources which could possibly be due to fecal contamination from human and animal.

The unsanitary practices of food and beverages processes lead to contamination of foods by *Salmonella*. The previous study showed that 25%-50% of beverage samples which are sold on the street food counters in Bogor, Indonesia, were contaminated predominantly by *Salmonella paratyphi* A. The contamination of bacteria possibly comes from the uncooked water [2]. The increased frequency of food-borne *Salmonella* has been causing recurring outbreaks, sometime with fatal infections.

The exceedingly variable manifestations of typhoid fever have lead to the development of numerous diagnostic techniques. The routine detection of *Salmonella* in the environment including in foods and beverages is a necessary component of public health programs. Standard cultural methods for detection of *Salmonella* are sensitive enough to detect *Salmonella* in food samples. However, the cultural methods also require multiple sub-culturing stages followed by biochemical and serological confirmatory tests with can take up to seven days to get a confirmed positive result. Therefore, these methods may be too time-consuming in cases where rapid pathogen identification is critical. In addition, sensitivity of cultures can be affected by antibiotic treatment, inadequate sampling, variations of bacteremia and a small number of viable organisms in samples [3].

The development of molecular methods for diagnosis of infectious diseases has improved the sensitivity, specificity, quality and availability of diagnosis and treatment. Several polymerase chain reaction (PCR) assays for detection of

Salmonella have been developed, and different targets DNAs for amplification have been applied. PCR enables the detection of *Salmonella* in different sources, such as human or animal feces [4],[5] soil[6] environmental water samples and other sources[7],[8]. PCR studies have also been carried out to evaluate the specificity of *invA* primers to detect *Salmonella* by PCR technique. [9],[10],[11],[12],[13],[14] The oligonucleotide primer pairs were developed according to the sequences of the chromosomal *invA* gene[9] which is essential in the invasion of *Salmonella* to enter the epithelial cells. [15],[16] reported that the *invA* primers were able to discriminate between *Salmonella* and non-*Salmonella* species.

The detection limit was 300 cfu/mL of pure culture; however they did not evaluate the methods on environmental samples. [17] Demonstrated that the *inv A* primers were specific for the detection of *Salmonella* in drinking and surface waters and the limit of detection of PCR was 2.6 x 104 cfu/mL. PCR analysis offers several advantages including the specificity and rapidity. The present study was done to find out the prevalence of *Salmonella* Species in food samples of Dehradun region and their confirmation through molecular biology techniques.

MATERIAL AND METHODS

Samples

Samples were collected from Dehradun region. Total twenty samples were taken. The five samples each were collected from poultry intestine and goat intestine.Pastreis creamy part from 5 different bakeries were taken as sample. Three samples of coriander leaves and two samples of Mint (Pudina leaves) were taken.

Sample collection

Sample was collected in a sterile container or container was sterilized by autoclaving.Approx. 10-20 g of each sample was taken.They were transported to laboratory in ice box, without any delay.

Sample processing

The samples were either processed using pestle-mortar or mixer grinder.Pestle mortar or grinder jar was disinfected with 70% alcohol.Minimal quantity of Buffered Peptone Water was added for proper homogenization of sample. The suspension was made be as smooth as possible.Using a cut sterile tip 1g or 1 ml (approx) of homogenized sample was transferred into 10 ml of Buffered Peptone Water (for viability of injured *Salmonella).*It was incubated at 37°C for 18-24 hrs. 1ml of this broth was transferred to 10 ml of Tetra thionate broth for enrichment. It was *i*ncubated at 37°C for 24 hrs.For requiring culture, a loopful of enriched broth was streaked on the plate of Salmonella Shigella Agar. It was observed for colonies and the cultural characteristics were noted.

Biochemical characterization of isolates

Isolates were characterized using Triple Sugar Iron test and Phenyl pyruvic acid (PPA) test.

Detection of Salmonela by PCR

HiMedia's *Salmonella d*etection kit is used.It is a qualitative conventional PCR kit which contains the amplification of *Salmonella* spp. specific gene inv A (284 bp) using specific primers. The amplified target is detected by using agarose gel electrophoresis.

RESULT

Totally 20 different food samples were collected from different locations of Dehradun city. Out of 20, culture was found to be positive for 15 samples while remaining 5 samples gave negative results.

Table 1 Prevalence of Enteric pathogens in different types of food sample

Types of food sample	Number	Sample No.	Prevalence of Enteric pathogens (%)
Goat intestine (GI)	5	GI 1 – GI5	100
Poultry intestine (PI)	5	PI 1 – PI 5	100
Coriander leaves (CL)	3	CL 1 – CL 3	100
Mint leaves (MI)	2	ML 1 – ML 2	100
Paestry (PS)	5	PS 1 – PS 5	0
TOTAL	20		75

Upon selective isolation colonies were obtained in Salmonella ShigellaAgar (SS Agar) which was presumptively identified on the basis of cultural characteristics and staining.

Table 2 Cultural Characteristics of Isolate (Gi 2) On Ss Agar

Size	2 mm – 3 mm
Shape	Circular
Colour	Black centre
Margin	Smooth
Elevation	Convex
Optical Characteristics	Translucent
Consistency	Easily picked with needle

Table 3 Staining characteristics of Isolate (GI 2)

Gram character	Gram negative
Morphology	Bacille
Arrangement	Singly arranged

After biochemical characterization, the enteric Pathogens were differentiated into *Proteus* sp. (86.66%) and *Salmonella* sp. (13.33%).

Table 4 Presumptive Identification of *Salmonella* sp. and *Proteus* sp. based on PPA Test and TSI Reaction

Sample code No.	PPA Test	TSI Reaction	Presumptive Identification of *Salmonella* sp. And *Proteus* sp.
GI 1	+	A/A, H_2S +	*Proteus* sp.
GI 2	-	K/A, H_2S +	*Salmonella* sp.
GI 3	+	A/A H_2S +	*Proteus* sp
GI 4	+	A/A H_2S +	*Proteus* sp
GI 5	+	A/A H_2S +	*Proteus* sp
PI 1	+	A/A H_2S +	*Proteus* sp
PI 2	+	A/A H_2S +	*Proteus* sp
PI 3	+	A/A H_2S +	*Proteu s*sp
PI 4	-	K/A H_2S +	*Salmonella* sp.
PI 5	+	A/A H_2S +	*Proteus* sp
CL 1	+	A/A H_2S +	*Proteus* sp
CL 2	+	A/A H_2S +	*Proteus* sp
CL 3	+	A/A H_2S +	*Proteus* sp
ML 1	+	A/A H_2S +	*Proteus* sp
ML 2	+	A/A H_2S +	*Proteus* sp

Detection of Salmonella sp. by PCR Method

After the PCR amplified product was electrophoresed, the band for invA gene (284 bp) was observed only for two food samples i.e. GI 2 and PI 4.

Table 5 Results of invA gene amplification by PCR

Sample	Result
GI 1	-
GI 2	+
GI 3	-
GI 4	-
GI 5	-
PI 1	-
PI 2	-
PI 3	-
PI 4	+
PI 5	-
CL 1	-
CL 2	-
CL 3	-
ML 1	-
ML 2	-

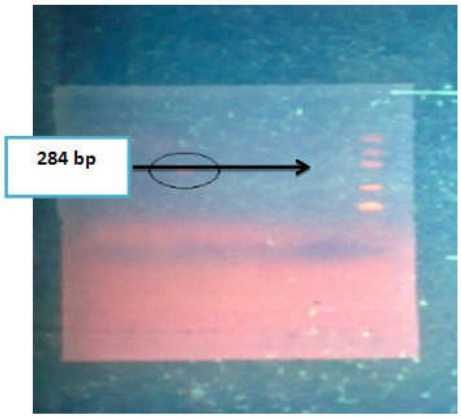

Figure 1

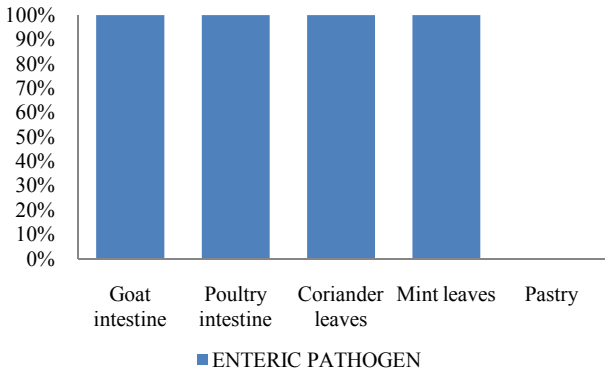

Figure 2 Prevalence of Enteric pathogens

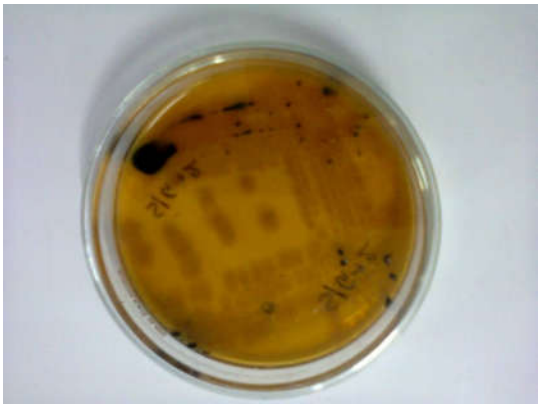

Figure 3 Presumptive Identification of *Salmonella* Sp and *Proteus* sp.

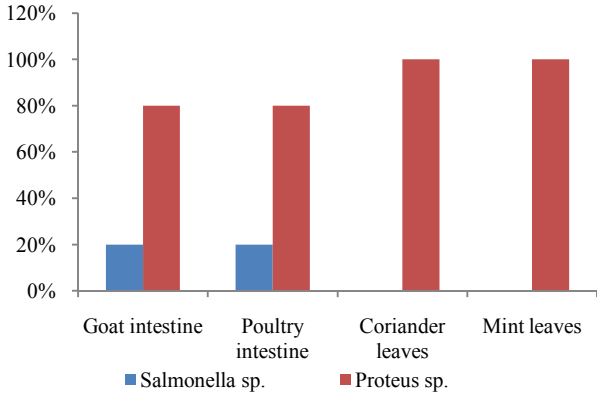

Figure 4

DISCUSSION

The present study highlights the considerably high prevalence of *Salmonella* spp. in intestine of goat and poultry, in which 20% of each goat intestinal samples and poultry samples were contaminated with *Salmonella* sp. However, *Salmonella* was not observed in coriander leaves, mint leaves and pastry. The contamination indicates a lesser breakdown of hygiene at various stages of the food processing and distribution chain and/or a lack of refrigeration of meat. The result for *Salmonella* contamination in poultry samples (20%) was not in close agreement with that of Van *et al*. (2005), who reported that 53.3% of the poultry samples were contaminated with *Salmonella* spp. in the Ho Chi Minh City, Vietnam. [18]

The reported rates of *Salmonella* contamination in goat and poultry are higher in more developed countries. In this study, 20% of poultry and goat samples were contaminated with *Salmonella*, compared to only 23 to 29% in the United Kingdom [19],[20] 2.8 to 26.4% in Ireland, [21],[22]13.2% in The Netherlands,[23] 35.8% in Spain[25], 36.5% in Belgium, [26] and 36% in Korea [27]. However, the rate was much higher, 60% in Portugal [28]. Phan *et al.*, in 2005 reported that 21% of the retail poultry samples were contaminated with *Salmonella* spp. in the Mekong Delta, Vietnam. [29] The differences noted may include difference of two different countries and a longer time to market of products. The exception of Portugal may also be related to climate and temperature of food storage. Different sampling procedures, sample types, and bacterial isolation and identification methods could affect the detected prevalence of *Salmonella* spp. More effective use of refrigeration in meat transport in developed countries could also help to reduce cross contamination of meats.

This study found the agreement between detection of *Salmonella* by bacteriological methods and conventional PCR assay in different food samples. Overall, 20 samples were taken from different locations of Dehradun city. 20% of samples were found to be positive for *Salmonella* by conventional PCR. Over the past 15 years there has been an important evolution in molecular approaches for the rapid detection of food borne pathogens rather than relying on their biochemical and phenotypic characteristics. Foremost among these tools is the Polymerase Chain Reaction (PCR), a technique based on the specific amplification of a short target DNA sequence. [30] Briefly, extracted DNA is first subjected to heat denaturation into single stranded DNA. Next, specific short DNA fragments (primers) are annealed to the single

DNA strands, followed by extension of the primers complementary to the single stranded DNA with the aid of a thermostable DNA polymerase, such as Taq polymerase, an enzyme originally isolated from the bacterium *Thermus aquaticus* (Chien *et al*. 1976). Each new double-stranded DNA is then a targeted during a new thermal cycle and thus the exponential amplification of the specific DNA sequence is achieved. The amplified product is then separated by gel electrophoresis and visualized by staining with fluorescent ethidium bromide. This type of conventional or endpoint PCR, although sensitive and specific under optimized conditions, is time consuming and labour intensive due to post amplification steps, not sensitive enough to measure the accumulated DNA copies accurately, and can only provide a qualitative result. Nevertheless, PCR techniques have expedited the process of pathogen detection and in some cases, replaced traditional methods for bacterial identification, characterization, and enumeration in foods .[31] Conventional PCR detected more positive results than bacteriological culture method, as expected from previous studies. [32],[33],[34],[35]. This simple method is expected to enable a rapid risk assessment of pathogen contamination of foods at a low cost. The *invA* gene primer pair specific for *Salmonella* was used in PCR reaction for the genomic DNA isolated from different food samples which produced a band of 284 bp. Two (GI 2 & PI4) out of twenty samples were detected to contain *Salmonella* and revealed the presence of the amplified product of the size 284 bp. Previous study have reported the specificity of PCR compared to the conventional culturing and serological method. *Salmonella* carry the *invA* gene, which is not carried by any other bacterial species. Therefore if 284 bp amplified product appeared in the PCR it would indicate that the sample contains an *invA gene* of Salmonella [9].

Traditional approaches for analysis of *Salmonella* has relied on cultural techniques and several selective differential media have used for differentiation. However, biochemical analysis for an enzyme associated with the particular pathogenic trait could be cross reactive with other enteric bacteria. In addition, the cultural methods also require multiple sub-culturing stages followed by biochemical and serological confirmatory tests with can take long time to get a confirmed positive result. In contrast to the long time culture method, in this study, by PCR assay using *invA* primer, offers a rapid and good diagnostic tool for the routine monitoring for detection of *Salmonella* in different food samples. The presence of *Salmonella* in food samples could be due to several reasons such as contamination of raw material, poor hygienic conditions, and contamination of different sources.

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