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Molecular Characterization of *Salmonella typhimurium* from meat samples in Multan, Pakistan

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Abstract

Global meat production has increased by 20% in the past 10 years only and has tripled over in the last 4 years. Most of the systemic intestinal infections are caused by gram-negative bacteria in humans, called *Salmonella typhimurium*. This research aimed to identify *Salmonella typhimurium* at molecular level through optimized PCR. The work was done by collecting samples from five different regions of Multan, Pakistan. The bacterial cells were grown on petri dishes and subsequently on broth media. DNA was extracted following protocol specified for bacterial cells. The quality of DNA was checked by agarose gel electrophoresis in 0.8% agarose gel for 45 minutes. The PCR was optimized by amplifying the DNA with species-specific primers. Further sensitivity of PCR was done by evaluating the identified DNA of *Salmonella typhimurium* by diluting the DNA up to 0.01 ng. 10% of the total collected samples for the identification of *Salmonella typhimurium* by PCR from the raw meat samples. This study revealed that raw meat was contaminated with *Salmonella typhimurium* at different meat retail shops in Multan. This study may help with the management of *Salmonella typhimurium* in Pakistan.

Keywords:

Salmonella typhimurium, Meat samples, Bacterial culture, DNA extraction, Molecular Characterization

1. Introduction

A serious crisis on human health caused by various foodborne infectious diseases leads to fast and precise detection of foodborne pathogens. Around the world, *Salmonella typhimurium* is considered to be the most common cause of foodborne diseases as compared to other pathogens(Kirk et al., 2015). The symptoms of diarrhea, abdominal pain and nausea are observed in people affected by *Salmonella typhimurium*. The illness is commonly associated with raw meat, milk, and eggs (GUPTA et al., 2016). *Salmonella typhimurium* is the primary cause of gastroenteritis, also called Salmonella flu. The diagnosis of gastroenteritis can usually be done by the culture of stool if suggested by the physician. Otherwise, there is no specific testing system for its diagnosis. Doctor may recognize the infection by looking at the physical conditions,symptoms or similar cases around the country.

The most interesting and highly pertinent question related to the inclusive pathogenicity of the organism is its metabolic adaptation needed to enable intracellular replication and survival within host cells. Initial perceptions of the intracellular metabolism of Salmonella were yielded by mouse infection models and cell culture involving Salmonella metabolic mutants. These include the catabolic pathways and means of ATP generation utilized by *Salmonella typhimurium*, major metabolites broken by and available to them. Some studies however succeeded in framing a comparatively good picture of metabolic pathways. These pathways are vigorously active in intracellular function including the availability of metabolites to *Salmonella typhimurium* within the host. It was found that in RAW macrophages, the most abundant carbon source needed for successful replication of *Salmonella typhimurium* is glucose (Dandekar, Fieselmann, Popp, & Hensel, 2012).

Approximately, 155,000-200,000 deaths are reported in 20-98.3 million non-typhoidal infection cases annually (Pui et al., 2011). Salmonella enters the small intestine invading epithelial cell lining after contaminated food or water is ingested. This invasion is mediated by a protein set encoded within SPI1 (Martínez et al., 2011).

Contaminated water is the main transmission route. Human fecal pollution is the major reason for water contamination. It is recorded in a survey that the bacterium survives for up to 9 days in seawater and weeks in sewage water. It survived better in groundwater than in a pond, stream or lake. The less grazing pressure by protozoa was attributed to being one of the reasons for its survival. The detection of *Salmonella typhimurium* is done by some traditional methods. These traditional methods include selective cultural techniques(Schönenbrücher, Mallinson, & Bülte, 2008). The detection process of Salmonella *typhimurium* needs 3-6 continuous working days in the laboratory (Wang et al., 2017). But for foodborne pathogens having zero tolerance, these traditional methods didn't prove to be effective as was expected (Settanni & Corsetti, 2007).

This study aimed to overcome the above mentioned drawbacks by following the optimized PCR detection method based on the amplification of targetted bacterial DNA from raw meat samples.

2. Materials and Methods

2.1 Sample collection

I collected raw meat samples from five different tehsils near/of Multan such as Bosan Road, Shujabad, Mumtazabad, Sher Shah Road, Shah Rukn-e-Alam. Two Samples of each chicken, mutton, and beef were taken from retail meat shops or slaughterhouses. Preferably took only 5, 6 samples from each area/location, So the lab work was done on approximately 30 meat samples. I collected each meat sample separately in polythene bags and stored them at -20C temperature in the lab. The bags were sealed airtight so that no other microbes could enter the meat. Each packet was labeled with the name and the sample No. of the area. Each piece of meat weighed almost 40-50 grams. The samples were taken from freshly slaughtered chicken, mutton and beef.

2.2 Bacterial Culture:

The bacterial culture was cultured and kept in the incubator overnight at 37C and the next day media solution was autoclaved for 20 minutes and brought down to room temperature before adding the bacterial colonies. The labeled flasks were incubated into the water bath at 37C overnight.

2.3 DNA extraction

The bacterial DNA was extracted followed by Maniatis et al. (Maniatis, Fritsch, & Sambrook, 1982) and the quality of DNA was checked 0.8% agarose gel by gel electrophoresis. The DNA samples were run at 160 volts, 400 mV for 45 minutes.

2.4 Primers design

The set of primers for *Salmonella typhimurium* was taken from literature and it was designed by (Rahn et al., 1992). This was verified by Primer-BLAST on the website of NCBI (https://www.ncbi.nlm.nih.gov/) in table 1.

Table 1. Primer sets used for the identification of *Salmonella typhimurium*.

No.	Target bacterial species	Oligonucleotide primer 5'–3'	Fragment Length (bp)	Reference
1	Salmonella *typhimurium*	F* GTGAAATTATCGCCACGTTCGGGCAA R* TCATCGCACCGTCAAAGGGAACC	284	**(Rahn et al., 1992)**

2.5 PCR Systems

A thermal cycler or PCR machine is the most commonly used amplifier machine used in the laboratory. This machine is used to amplify DNA fragments in a process called polymerase chain reaction (Dahal, Ellerbroek, & Poosaran, 2007). The extracted DNA samples were examined for the presence of *Salmonella typhimurium*. A 25 ul PCR mixture was prepared by mixing 2 ul of each primer, 1.5 ul of DNA template, 2.5 ul of the buffer, 1 ul of MgCl2, 1 ul of 10 mM dNTPs, 0.25 ul of Taq. polymerase and 16.75ul of dH2O. Amplification was performed on a PCR machine with settings of heat denaturation at 95C for 5 minutes, elongation step of 7 minutes at 72C, followed by 35 cycles.

2.6 Gel electrophoresis

The DNA samples were analyzed on agarose gel at 160 volts, 400 mV for 45 minutes. The results of DNA profiles or "fingerprints" were interpreted according to the size of the fragmented pattern.

3. Results

The specificity of primers was checked and there was no cross activity as shown in fig 1. Furthermore, the sensitivity of the primers was 0.01ng/ul of the targetted Salmonella (Fig. 2). Additionally, Salmonella isolates were identified from 3 of 30 samples of raw beef, mutton, and chicken. Salmonella was observed more in mutton as compared to any other meat. Samples were taken from 5 tehsils such as Mumtaz Abad, Sher-Shah road, Shujabad, Shah Rukn-e-Alam, and Bosan road. Samples from Mumtazabad, Shah Rukn-e-Alam, and Bosan road were found positive for *Salmonella typhimurium* (Table 1 & fig 3).

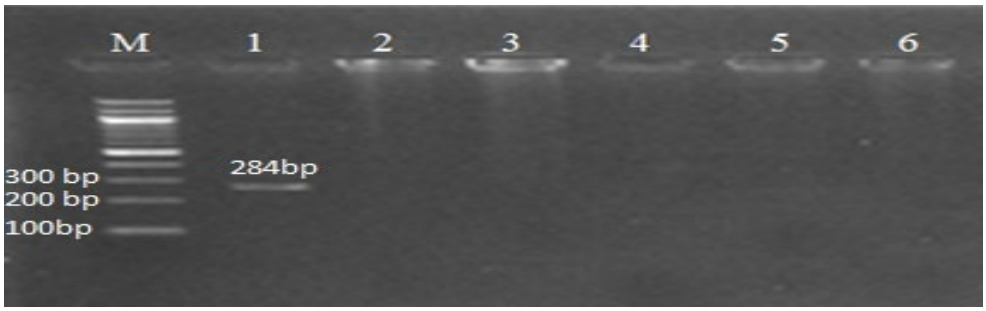

Fig 1. Specificity of PCR assay of DNA from *Salmonella typhimurium* sample: M: marker, 100 bp, (1) *Salmonella typhimurium*, (2) *Bacillus anthracis*, (3) *Escherichia coli*, (4) *Staphylococcus aureus*, (5) *Streptococcus agalactiae*,(6) *Staphylococcus epidermidis*.

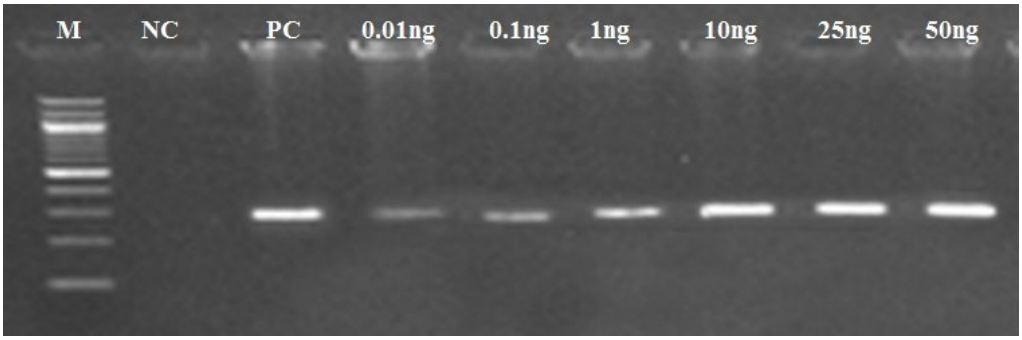

Fig 2. Evaluation of PCR assay sensitivity for *Salmonella typhimurium* DNA sample; M: marker 100 bp. NC: negative control (reagents with primers without DNAs) PC; *Salmonella typhimurium*DNA, (1) 0.01ng (2) 0.1ng, (3) 1ng, (4) 10ng, (5) 25ng, (6) 50ng

Table 1. Results of PCR for identification of *Salmonella typhimurium* from the Raw meat samples collected from various areas of District Multan

	Results of PCR for identification of *Salmonella typhimurium*				
Sr#	***Mumtaz abad***	***Sher shah***	***Shujabad road***	***Shah rukn- e-alam***	***Boson road***
1	-	-	-	+	-
2	-	-	-	-	-
3	-	-	-	-	-
4	-	+	-	-	+
5	-	-	-	-	-
6	-	-	-	-	-

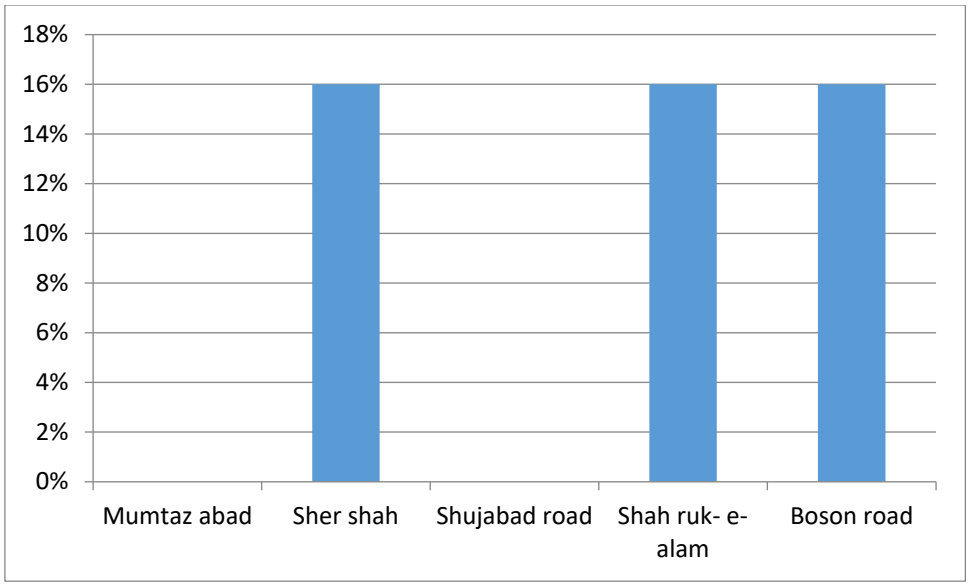

Fig 3. Percentages of contaminated samples of identification of *Salmonella typhimurium* by PCR from the raw meat samples collected from various areas of District Multan

4. Discussion

The understanding of some aspects of cell biology e.g., signal transduction and endocytosis may be carried out more easily by a better understanding of the influx of several pathogenic micro-organisms into host eukaryotic cells. Salmonella is one of such invasive bacteria which makes its entry into the host cells via Peyer's patches as well as enterocytes of intestinal epithelium (Finiay & Falkow, 1989). Peyer's patches are epithelial cells that are specialized for taking large particles up as they are considered to be significant in the sampling of antigens.

Frequent outbreaks are caused by frequently increasing food-borne Salmonella, along with fatal infections often. Intake of animal origin food is the main cause of the infectious attack of salmonella species such as shellfish, eggs, beef, chicken, and milk (D'Aoust & Maurer, 2007). To avoid the health problems caused by Salmonella, public health programs make routine detection of salmonella in foods.

The study was mainly undertaken to determine the contamination of raw meat by *Salmonella typhimurium*. The sample collection was done by collecting meat samples from five different regions of the Multan district. The PCR was optimized by amplifying the DNA with species-specific primers. Further sensitivity of PCR was done by evaluating the identified DNA of *Salmonella typhimurium* by diluting the DNA up to 0.01 ng. 10% of the total collected samples for the identification of *Salmonella typhimurium* by PCR from the raw meat samples. Different researchers worked on the gel with different concentrations i.e. Rahn et al. loaded his samples in agarose gel with a concentration of 2%. The DNA fragments were amplified by using an already resigned specific primer of 284-bp length with 90% specificity (Rahn et al., 1992). Another study also testified the better and more sensitive working of PCR for detecting the presence of salmonella in food especially meat and meat products (Eyigor, Carli, & Unal, 2002). The detection of bacterial pathogens by PCR in a variety of clinical specimens with cultural methods has been widely dependent on DNA extraction methods before the amplification process. There has been a successful demonstration of direct detection of Salmonella *typhimurium* in water by PCR amplification as well as ground meat as there are low

concentrations of inhibitory factors in such kinds of samples (Starnbach, Falkow, & Tompkins, 1989).

So we concluded that salmonella could be the primary cause of intestinal infection around the globe. A huge number of people are reported to die due to this bacterial infection. This study was mainly undertaken to determine the contamination of raw meat by *Salmonella typhimurium*. The identification and isolation of bacteria were done by following general bacterial culture preparation, DNA extraction and subsequent amplification of DNA fragments on PCR. 10% of the total collected samples for the identification of *Salmonella typhimurium* by PCR from the raw meat samples. This study revealed that raw meat was contaminated with *Salmonella typhimurium* at different meat retail shops in Multan. This study may help with the management of *Salmonella typhimurium* in Pakistan.

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