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Identification of bacteria *Salmonella typhimurium, Escherichia coli, Streptococcus pneumonia, and Staphylococcus aureus* from meat samples through multiplex PCR

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Abstract

Food-borne microbes e.g. bacteria are causing serious diseases and even leading death in many developed and under developing countries by spending billions of dollars in medical treatment and socio-economic cost. Polluted and defiled raw meats are the most common reasons which lead to food-borne diseases. The aim of this research was to identify the most common food- borne bacteria such as *Salmonella typhimurium, Escherichia coli, Streptococcus pneumonia,* and *Staphylococcus aureus* by the development of a multiplex PCR. The bacterial cells were cultured on Petri dishes taken from meat samples. The DNA was extracted by following an optimized bacterial DNA extraction protocol. The multiplex PCR was optimized by amplifying the DNA with species-specific designed primers. Further sensitivity of PCR was done by evaluating the identified DNA of all bacterial species by diluting the DNA up to 0.01 ng. The results showed that the developed PCR worked successfully. This study was a preliminary work and further studies will be needed to investigate the level of contamination.

Keywords: Bacterial culture, DNA extraction, Multiplex PCR, Identification.

Introduction

According to 2003 survey report, Pakistanis eat red meat three times more than Indians. Meat consists of 20% of protein, 75% of water, carbohydrates, fats and many other bioactive components that are essential for regulation of human body mechanisms.

The systematic classification of bacteria was first time started in end of the 19th century. The bacterial groups were separated on the basis of their size, shape, locomotion and morphology. Ferdinand Cohn first time launched this investigation, which led the idea of assortment of microorganisms basis of their diversity and transmission of characteristics to upcoming generation (Rosselló-Mora & Amann, 2001). Now-a-days, molecular detection methods are used to detection of bacterial pathogens because they are more specific and sensitive than classical micro detection methods.

It has been noticed that when an animal is slaughtered, most of microorganism are added from intestinal tract but many of other bacteria come from exterior of the animal like air, carts, workers and knives etc. Food-brone microbes e.g. bacterial are causing serious diseases and even leading death in many developed and under developing countries by spending billions of dollars in medical treatment and socio- economic cost (Fratamico,

Bhunia, & Smith, 2005). Polluted and defiled raw meat is most common reason which leads food-brone diseases (Bhandare, Sherikar, Paturkar, Waskar, & Zende, 2007).

The aim of this study was to develop a new technique of bacterial identification through multiplex PCR and examine the microbiological (bacterial) effect in the food industries especially in raw meat that sold commonly in the market and butcher shops in different places of Multan.

Materials and Methods Sample collection

The raw meat samples (beef, chicken, mutton) were collected from five different tehsils of Multan such as Sher Shah Road, Bosan Road, Mumtazabad, Shujabad and Shah rukn-e-Alam. The collected samples were from chicken; mutton and beef (3:1:1) taken from retail meat shops or slaughter houses of each tehsil. Preferably took only 5, 6 samples from each area/location. So, the lab work was done on approximately 25 meat samples and stored them at -20C temperature in laboratory.

Bacterial isolation from sample and DNA isolation

Sample solution was made by mixing 1g of ground meat in 9 ml of dH2O. Mixing was continued until the water turned reddish. Mixture was made by crushing the meat with blade and mixing it thoroughly in water in autoclaved test tubes separately. The place was clean with 70% ethanol during all process and was done with gloves so that to avoid any type of contamination. Finally DNA was extracted from the isolated bacteria according to the manufactured protocol.

Primers design

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

Simplex and multiplex PCR Protocol

PCR amplification was performed in a final volume of 25 µl containing master mix, 0.1 mM of each primers and 70-90ng/µl of DNA template. Amplification was performed in a Thermocycler Techne with the following cycling conditions; after an initial heat denaturation step at 94ºC for 10 min, 35 cycles were programmed as follows: 94ºC for 30 s, 60ºC for 1 min, 72ºC for 1 min and final extension at 72ºC for 5 min.

Gel electrophoresis

Gel electrophoresis was done at Genetics laboratory. DNA samples were analyzed on agarose gel at 160 volts, 400 mV for 45 minutes. The results of DNA profiles or "finger prints" were interpreted according to the size of fragmented pattern.

Results

Initially simplex PCR was employed by the DNA extracted *Escherichia coli, Streptococcus pneumoniae, Salmonella typhimurium* and *Staphylococcus aureus* species to check specificity of the primers with reference bacterial samples taken from BZU,

Multan. Afterword simplex and multiplex PCR were employed by the DNA extracted from targeted bacteria in the reference samples for the development of technique. The sensitivity of multiplex PCR targeting species DNAs were verified until the minimum amount of 0.1% tested in DNA mixtures. The results of multiplex PCR assays showed that the local market samples were contained with different bacterial species.

Discussion

There are many diseases that can be caused by contaminated meat that sold out by local retailers on daily basis. The bacteria *Escherichia coli*, *Streptococcus pneumoniae*, *Salmonella typhimurium*, *Staphylococcus aureus* are, well known meat borne bacteria, responsible to cause many diseases including scarpie (a fatal disease). Food brone bacteria commonly present in retail butcher shops are directly sources of contamination of meat in Pakistan. The prevalence of pathogenic *Salmonella* and *E.coli* in beef and its products vary from product to product and country to country. This study was mainly undertaken to determine the contamination of raw meat with four most common pathogens such as *Escherichia coli*, *Streptococcus pneumoniae*, *Salmonella typhimurium*, *Staphylococcus aureus.* The developed technique was identified the targeted bacteria successfully. Five samples out of twenty five samples were positive, but further analysis needed.

Additionally, proper slaughter conditions and hygienic rules should be followed to lower the prevalence of food borne pathogens. Meat retailers and butchers do not maintain essential hygienic and slaughter conditions that provide a favorable environment for microbial growth, so there should be proper check on them by higher authorities.

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