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MOLECULAR DETECTION OF PATHOGENIC BACTERIA *PROTEUS MIRABILIS* CONTAMINATION IN CHICKEN MEAT IN SELECTED DISTRICTS OF KERALA, INDIA

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ABSTRACT *Proteus mirabilis* has been suggested as a possible causative agent of outbreaks of gastroenteritis, resulting from the consumption of contaminated food. In such a situation the study on *P. mirabilis* in chicken meat in selected districts of Kerala, India, leads to know the extent of *P. mirabilis* contamination of chicken meat in the area. 50 Samples of raw chicken meat were collected from slaughterhouses and meat shops in the study area by simple random sampling method. Bacteriological analysis was carried out on samples and further confirmed by PCR analysis. 50 samples were studied and 1 sample was contaminated by *Proteus mirabilis*. The health of individuals is at risk so maintaining a proper hygienic environment is an important step to avoid *P. mirabilis* related health hazards in the consumers or spreading to other birds. Microbial control in each and every stage of chicken production can control an outbreak of food poisoning and reduce the pathogenicity of *P. mirabilis.*

KEYWORDS : *Proteus mirabilis*, chicken meat, human pathogen, molecular analysis.

INTRODUCTION

Food hygiene is very important while storing, preparing and cooking meat. By proper hygienic measures, we can avoid contamination by bacteria like *Salmonella, Campylobacter, Staphylococcus aureus, Clostridium perfringens, Proteus mirabilis* etc. Without proper cooking, storing and handling of food materials it may cause food poisoning, and it is toxic to the individual who consumes it. The condition of the person becomes severe when it is contaminated by pathogenic bacteria. The contamination of bacteria in raw meat is from the infected parts of chicken like intestine, skin etc. This will cross-contaminate others, from knife, slaughter board or from the person who handles it.

Proteus bacteria are one of the causes of serious infections in humans, along with *Escherichia, Klebsiella, Enterobacter* and *Serratia* species. These bacteria are generally known as human opportunistic pathogens, isolated from urine, wounds and other clinical samples. *Proteus mirabilis* has been suggested as a possible causative agent of outbreaks of gastroenteritis, resulting from the consumption of contaminated food [1].

Chicken meat is one of the most popular food products worldwide. Various nutritional factors such as high protein, low fat and also favorable content of unsaturated fatty acids contribute to the acceptance of chicken meat. For understanding the hygiene of meat and contamination level by *P. mirabilis*, it is pertinent to examine the chicken meat in selected districts of Kerala, India.

MATERIALS AND METHODS

The present study was carried out in different areas in selected districts of Kerala (Malappuram, Palakkad, Calicut and Kannur), India. 50 Samples of raw chicken meat were collected from slaughter houses and meat shops in the study area by simple random sampling method. Bacteriological analysis were carried out and the black colonies appeared on the plates were selected and drew out for further confirmation by PCR analysis. The amplified DNA products from PCR were resolved on 2% TAE agarose gel stained with ethidium bromide [2] and visualized by UV illumination. It was successfully amplified using PCR and the product was sequenced using Sanger's method. Then the trimmed forward and reverse sequences were assembled by using Clustal Omega and consensus sequence was taken for analysis by nucleotide BLAST programme.

RESULTS

By examining 50 samples, it was confirmed that sample from Thalappara (Malappuram district) was contaminated by *Proteus mirabilis* based on the conventional bacteriological analysis and further confirmed by DNA sequence analysis. Molecular level analysis is an excellent way to study the species that contaminate chicken meat. The techniques in molecular biology helped for the identification of bacteria that isolated from collected samples. The sequenced PCR product of bacteria is identical to *P. mirabilis* species.

The PCR amplification of the 16S rRNA gene of the sample yielded a product of 920 bp long fragment. The BLASTn program clearly states that this species is having 100% sequence similarity to the *Proteus mirabilis* species reported from West Bengal, India with GenBank accession number MK209629.1.

DISCUSSION

The PCR assay targeting 16S rRNA gene of bacteria was used for rapid detection and confirmation of *Proteus mirabilis.* This study shows valuable information about the *P. mirabilis* contamination in chicken meat. The health of individual is at risk so maintaining a proper hygienic environment is an important step to avoid *P. mirabilis* related health hazards in the consumers or spreading to other birds. Microbial control in each and every stage of chicken production can control an outbreak of food poisoning and reduce the pathogenicity of *P. mirabilis.* It is important from an environmental and occupational health perspective considering the risk involved to those people associated with meat-trade and other related activities.

In spite of indispensable mandate to follow strict guidelines of biosecurity to limit and avoid transmission of potential pathogens by poultry products [3, 4], the benchmark rules of biosecurity are often not practiced in majority of poultry farms in India as the latest information revealed [5, 6, 7]. This factor plausibly contribute in transmitting potential zoonotic pathogens to poultry farmers workers directly (while handing fecal contaminated meat/eggs) or indirectly (while processing/dressing infected poultry). [8, 9, 10]

The extent of the contamination not only depends on the quantity of microbes but also on the physical factors such as handling of the meat products. To keep up a trust on the food that we use, making sure there is no spoilage by bacterial cross contamination in meat and meat products. For that we need to have strict handling methods. To avoid contamination of meat products there should be strict quality control checks at slaughterhouses, markets, and butchers should be aware of cleanliness and techniques behind it and the sterilization methods. [11]

Taking into account that most of the birds are brought into Kerala from neighboring states where chicken farms are numerous. Further study on the bacteria in its micro-environment will help to take strategic steps in maintaining the public and environmental health and to avoid the exotic serovars (with potential to infect other domestic avian species) entering and spreading in the state. The healthy practice in handling chicken meat is an important step to avoid potential *P. mirabilis* contamination manually.

CONCLUSION

The molecular level analysis is an excellent way to study the pathogens that contaminate the food materials. The techniques in molecular

biology helped for the identification of the bacteria isolated from the collected chicken meat. The products from PCR amplification of bacterial gene with 16S rRNA primer were further confirmed by DNA sequencing. The sequenced PCR product results of bacteria were identified as *Proteus mirabilis* species.

In the present study, the species *Proteus mirabilis* from sample (920bp) were showed 100% similarity to the same species reported from West Bengal, India.

Fig No.1 *P. mirabilis* growth in agar slant

Fig No.2 Chromatogram showing forward sequence of partial 16S rRNA of *P. mirabilis* in sample

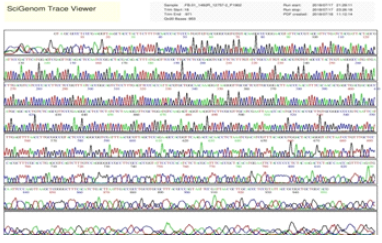

Fig No.3 Chromatogram showing reverse sequence of partial 16S rRNA of *P. mirabilis* in sample

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