**PREVALENCE AND MOLECULAR CHARACTERIZATION OF CLOSTRIDIUM PERFRINGENS IN CHICKEN MEAT IN RETAIL OUTLETS IN HASSAN DISTRICT, KARNATAKA**

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A study on the prevalence of *Clostridium perfringens* in chicken meat obtained from retail outlets in and around Hassan, Karnataka was carried out and 75 Chicken meat samples were collected from various retail outlets and the isolation and identification of *C. perfringens* was carried out. Isolation was done following standard protocols and the presumptive isolates were subjected to biochemical characterization for presumptive detection of isolated bacteria. Bacteriological investigation revealed the presence of *C. perfringens* in 51 samples (68.00 %) based on biochemical characterization. All these isolates were subjected to Polymerase chain reaction (PCR) targeting 16S rRNA. The PCR product was run on 1.5 % agarose gel and visualized under UV, which revealed a product size of 120 bp. All the 51 isolates were positive for PCR.

**MATERIALS AND METHODS**

Isolation and identification of *C. perfringens* from poultry meat: A total of 75 meat samples were collected from retail outlets in and around Hassan, Karnataka, India. Meat samples were inoculated directly into cooked meat broth medium (Himedia) and were incubated anaerobically in an anaerobic candle jar for 24 h at 37°C. 100 µL of growth were then streaked onto 5% sheep blood agar, supplemented with neomycin sulphate. The plates were incubated anaerobically for 24 h at 37°C. Colonies producing clear zone of haemolysis were suspected for *C. perfringens*. The colonies were subjected to macroscopic examination, including morphotyping (shape, size and texture of the colonies on blood agar plates). The presumptive detection of isolated bacteria was carried out by various biochemical methods⁵. Isolates were stored at -20°C in cooked meat broth (HiMedia) containing 40% (v/v) glycerol in cryovials until further use. The 51 isolates confirmed by biochemical characterization were subjected to 16S rRNA gene of *C. perfringens* by Polymerase chain reaction.

**DNA Extraction:** Genomic DNA of *C. perfringens* was extracted by using an extraction kit (QIAamp®DNA Mini Kit,
Qiagen) as per manufacturers instruction.

Oligonucleotide primers: Primers used in this study were synthesized commercially from M/s. Bioserve Biotechnologies (India) Pvt. LTD, Hyderabad. The Oligonucleotide primers targeting 16S rRNA as per Yoo et. al.\(^7\) Forward: 5’-ATGCAAGTCGAGCGA(G/T)G-3’ and reverse: 5’-TATGCGGTATTAATCT(C/T)CCCTT-3’, yielding a product of 120 bp size.

PCR Amplification: The PCR reaction mixture (25 µL) contained 5 µL of bacterial lysate as template DNA, 2.5 µL of 2 mM dNTP’s, 2 µL 10 × PCR buffer, 0.25 µL of 5 U/µL Taq DNA polymerase (Bangalore Genie), 1 µL of each of the primers (10 pmol/µL) and volume were made up by distilled water. The PCR reaction mixtures were placed in a PCR thermal cycler. Following initial denaturation for 5 min at 94°C, the samples were subjected to 30 cycles of denaturation at 94°C for 1 min, annealing at 55°C for 1 min and extension at 72°C for 1 min. After the last cycle, a final extension for 10 min at 72°C was performed. The PCR reaction mixtures (10 µL) were analyzed by electrophoresis on a 1% (w/v) agarose gel in the presence of 100-bp DNA ladder (Bangalore Genie). The agarose gel was added with ethidium bromide in order to visualize the DNA on an UV transilluminator.

Figure-1. PCR of DNA extracted from isolates of \textit{C. perfringens} obtained from chicken meat using primers targeting 16S rRNA gene (120bp) Lane M, 100-bp DNA molecular marker; Lanes 1 through 8: \textit{C. perfringens} isolates, lane 9: negative control

RESULT AND DISCUSSION

In the present study, 75 chicken meat samples from retail outlets were analyzed and \textit{C. perfringens} was isolated from 51 samples (68.00 %). All bacterial isolates exhibited the characteristic features of \textit{C. perfringens}. Similarly, Singh \textit{et. al.}\(^8\), has reported incidence of 70.4% in chicken meat collected from markets and slaughter houses in India and Nowell \textit{et. al.}\(^9\), who observed prevalence of 66% in wing and chicken leg quarter samples in Canada. However, slightly higher incidences as compared to the present study have been reported by Guang-Hua and Xiao-Ling\(^10\) in chicken carcasses consumed in Beijing (88 %) and Miwa \textit{et. al.}\(^11\) in chicken samples in Japan (84%). The higher rate of prevalence in the chicken meat can be due to the ubiquitous nature of the bacteria, cross-contamination during slaughter process and lack of sanitation and hygiene conditions\(^12\). All the isolates showed typical colony characteristics on blood agar (dew drops smooth greyish convex colonies with a double zone of haemolysis). Microscopic characters revealed gram positive non motile rods. All the colonies were subjected to various biochemical tests, and they were oxidase and catalase negative, and lecithinase positive (an opalescence around the colony in the EYA). The sugar fermentation reactions proved
glucose (+), lactose (+), maltose (+) and sucrose (+).

All presumptive isolates were subjected to PCR targeting 16S rRNA and all the isolates were positive and revealed a PCR product of 120 bp (Fig.-1) confirming that all isolates were *C. perfringens*.

In conclusion, the present study revealed that a significant proportion of chicken meat sold in the wet market in Hassan District of Karnataka was contaminated with *C. perfringens* and that PCR can be used as an easy and reliable method for confirmation and typing of *C. perfringens* isolates. Hence, to eliminate or to minimize the risk of *C. perfringens* contamination, implementation of strict hygiene and methodologies to prevent cross contamination should be considered.

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**REFERENCES**