MOLECULAR CHARACTERIZATION OF ALPHA TOXIN GENE OF CLOSTRIDIUM PERFRINGENS FROM CHICKEN MEAT

NITHIN PRABHU, K.,1 WILFRED RUBAN, S.,2 NAVEEN, B. R.3 AND RAGHUNATH, B. V.2

1Department of Veterinary Microbiology, Veterinary College, KVAFSU, Hebbal, Bangalore 560 024, Karnataka; 2Department of Livestock Products Technology, Veterinary College, Hassan 573 201; 3Department of Pathology, Veterinary College, Bangalore 560 024.

Email: nithinprabhuk@yahoo.com Mobile: +91 9844995078

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Abstract: Present research contribution aims to investigate the prevalence of Clostridium perfringens in chicken meat obtained from retail outlets in and around Bangalore, Karnataka. In total 71 Chicken meat samples were collected and were processed for the isolation and identification of C. perfringens. Bacteriological investigation revealed the presence of C. perfringens in 58 samples (81.69 %) based on biochemical characterization. All these isolates were subjected to virulence determination for the presence of alpha (α) toxin gene by Polymerase chain reaction [PCR]. This PCR determination of virulence genes suggested that chicken meat sample may be considered a significant source C. perfringens. The high incidence of this bacterium in poultry meat may indicate insanitary conditions and improper handling at processing area.

Key words: Chicken meat, Clostridium perfringens, alpha toxin.

INTRODUCTION

Currently, an increase in food borne infections has been attributed to animals used for human consumption, changing food habits, increased international trade of food and feeds, and increased environmental pollution. In India, attention is not paid to any outbreak of food borne disease which occurs once in a while [1]. So far no information exists on disease prevalence from India, even though it is well established in the west [2].

Among the food borne pathogens that thrives in high protein and canned food items, Clostridium perfringens always comes foremost. C. perfringens is ubiquitous, anaerobic, gram positive rod shaped bacteria which causes numerous enteric diseases caused by food infections to human, wildlife and domestic animals [3]. C. perfringens thrives in high-protein foods of animal origin such as meat and meat products, meat dishes, stews, soups, gravies, and milk. On the basis of production of four major toxins [alpha, beta, iota and epsilon], isolates of C. perfringens are classified into five genotypes A to E [4].

Clostridium perfringens, isolated from the intestinal tract of poultry and from the processed carcass is a major cause of human disease due to the consumption of contaminated poultry and other meat products [5]. Meat and poultry are frequently contaminated with these spores from one or more sources during processing. The signs of this food poisoning in man are rapid onset (8-24 hours), severe diarrhea and acute abdominal cramps, usually without vomiting [6]. Hence the study aimed at the detection of degree of contamination of edible chicken meat by isolation of Clostridium perfringens and to detect virulence.
factor based on alpha (α) toxin gene by Polymerase Chain Reaction.

**MATERIALS AND METHODS**

**Isolation and identification of *Clostridium perfringens* from poultry meat:** A total of 71 meat samples were collected from retail shops and slaughterhouses in and around Bangalore, Karnataka, India. The samples were collected aseptically in a sterile bag (Hi-media, India) and immediately transported to the laboratory under chilled conditions for microbiological analysis and analyzed within 3–4 h of collection. 200g of the market meat samples were minced in phosphate buffered saline and centrifuged at 2000 rpm for 5 min to separate the solid particle and the supernatant was inoculated directly into cooked meat broth medium (Hi-media) and a small amount of liquid paraffin was layered on the medium. The tubes 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). In total, 58 no of isolations were obtained, all of them were also gram-stained and examined by light microscopy.

**Biochemical characterization:** The 58 bacterial isolates were subjected to biochemical identification patterns, including Catalase test, lecithinase activity on egg yolk salt agar (EYSA), haemolytic activity on sheep blood agar and sugar fermentation (glucose, maltose, lactose, inulin, sucrose, mannitol, inositol and salicin). The inoculated sugar media were incubated anaerobically at 37°C for 24 h and examined for acid and gas production [7,8].

**Oligonucleotide primers:** Primers used in this study were designed according to Yoo et al. [9] and were synthesized commercially from M/s. Bioserve Biotechnologies (India) Pvt. LTD, Hyderabad. The sequence of oligonucleotide primers used in the present study for alpha (α) toxin gene (402 bp): Forward primer: 5’-GTGATAGCGCAGGACATGTTAAG-3’; Reverse primer: 5’-CATGTAGTCATCTGTCCAGCATC-3’.

**PCR:** 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 the primers (10 pmol/ µL) and volume were made up by distilled water. The PCR reaction mixtures were placed in a BioRad 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 supplemented with ethidium bromide in order to visualize the DNA on an UV transilluminator.

**Fig 1:** PCR of DNA extracted from isolates of *C. perfringens* obtained from chicken meat using primers targeting alpha toxin (402bp) Lane M, 100-bp DNA molecular marker; Lanes 1 through 9, *C. perfringens* isolates.
RESULTS

In this study, 71 chicken meat samples from retail outlets were analyzed and C. perfringens was isolated from 58 (81.69%) samples. All bacterial isolates exhibited the characteristic features of C. perfringens. The colonial characters on blood agar showed 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 (+).

In PCR, all the 58 [100%] suspected isolates of C. perfringens irrespective of source and place of isolation amplified the primers specific for alpha toxin gene [cpa] of products approximately 402 bp (Fig 1).

DISCUSSION

C. perfringens is an important food poisoning organism and is ubiquitous in nature, however, there is limited understanding for such obligate and facultative anaerobes in poultry meat. In this study, the Robertson’s cooked meat media broth and haemin and the nutrient agar media enriched with 5% sheep blood agar, supplemented with neomycin sulphate under anaerobic atmosphere at 37°C for 24-48 hr were found suitable for the isolation of C. perfringens [10,11]. In recent decades many surveys have been conducted on the incidence of C. perfringens in raw and processed meat and poultry. These reports indicate widespread occurrence of the organism in meat and poultry [12]. Our results are in agreement with those published by Singh et al. [13], who reported the incidence of C. perfringens in poultry meat samples as 71%. Similarly Miwa et al. [14] detected C. perfringens in 42 (84%) of 50 chicken samples in Japan. In the presence of enteropathogens in the intestines of broilers, the carcass can become contaminated with the microorganisms during the slaughter process, which results in the contamination of the end products.

In this study it was found that all the isolates were C. perfringens type A. All gave only one band very close to 400 bp as mentioned by Yoo et al. [9] to be 402 bp. These findings agreed with the results reported by several authors which mentioned that C. perfringens type A was the most predominant type isolated from broiler chickens [15].

CONCLUSION

It could be concluded from the present study that C. perfringens is commonly prevalent in poultry meat and harboring toxin genes are prevalent in Poultry. Based on the molecular characterization the most common circulating C. perfringens strain in poultry meat is C. perfringens type A. Moreover, the obtained data may be important for understanding C. perfringens epidemiology and a control strategy plan. Further research is needed to study the molecular characterization of other toxin genes in C. perfringens from poultry meat and their source of infection.

REFERENCES