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Isolation of Pathogenic *Clostridium perfringens* from Meat Samples Collected from Kolkata and Adjacent Areas

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Abstract

A total of 354 specimens of chicken meat and meat dishes were examined for the presence of *Clostridium perfrigens.* Of this, 221 were raw chicken, 133 were processed meats and meat dishes. *Clostridium perfringens* was isolated from 143 (40.3%) of these specimens. The highest percentage of contamination (136 = 61.6%) was found in raw meats and the lowest 7 (5.2%) in cooked meat dishes. Studies of the effects of various holding temperatures on the growth of *C. perfringens* indicated that extremely rapid growth occurred at temperatures around 45 C but the growth was accomplished between 37 and 47 C. Tentative *Clostridium perfringens* isolates detected by the biochemical tests, were confirmed by sandwich ELISA. Then the positive samples were tested for their pathogenicity in Swiss albino mice showing high (80—85%) percentage of mortality.

Key words : *Clostridium perfringens*, Pathogen, Isolation, Meat samples.

Food poisoning caused by *Clostridium perfringens* may occur when foods such as meat or poultry are improperly cooked without maintaining adequate temperature. The presence of small numbers of *C. perfringens* is common in raw chicken meats causing enterotoxaemic infections in human beings. It can produce a variety of toxins leading to mild to severe infection. The spores of some strains are resistant to temperatures as high as 100 C for more than 1 h, therefore their presence in foods may be unavoidable. Spores that survive cooking may germinate and grow rapidly in foods that are inadequately refrigerated after cooking. Thus, when clinical and epidemiological evidence suggests that *C. perfringens* is the cause of a food poisoning outbreak, the presence of hundreds of thousands or more of these organisms per gram of food substantiates the diagnosis. Considering the importance and pathogenic effects, the present study was aimed at detection of *Clostridium perfringens* from different chicken meat samples collected from different parts of Kolkata and its adjacent areas and to study their pathogenicity for identifying the depth of the problem.

Methods

A total of 354 chicken meat samples were collected for this study among which 221 were raw chicken collected from roadside stalls and rests were processed/cooked chicken meats and meat dishes collected from different hotels of Kolkata and adjacent areas for isolating *Clostridium perfringens.* Around 25—30 g of meat samples from the freshly cut chicken from the stalls and cold chicken dishes from the hotels and then packed in the serile polysterene packets. Then these packets were transported to the laboratory under ice cover and then these were processed on the same day within 3 hours.

The samples were firstly enriched on freshly deaerated and cooled cooked meat broth (Himedia) at 37 C for 12—18 hours. Then the enriched culture medium was inoculated onto *Clostridium perfringens* agar base (Himedia) and then incubated anaerobically at 37 C for 48 hours. Then the tentative isolates were collected and again streaked onto TSC agar plate and incubated anaerobically at 37 C for 24 hours. Then the obtained black colonies of *Clostridium perfringens* were examined by Gram's staining. Biochemical characterization of the isolates were performed by different tests like sugar fermentation tests (glucose, lactose, maltose, salicin, dulcitol and mannitol), iron-milk presumptive

test (Stormy clot reaction), H_2S gas production, IMViC tests and catalase test.

Then the tentative isolates were confirmed by applying sandwich ELISA (using Bio-X *Clostridium perfringens* Elisa Kit (Bio K 086). First, the cell free toxin/culture supernatants are used as antigen to the specific *Clostridium perfringens* monoclonal antibody coated the multi-well plates. Around 100 µl of each test samples was added to the wells (double well for each sample) and then incubated at 37 C for 1 hour. In second step, the test wells along with positive and negative controls were washed by the washing buffer at least for 3 times. In a further step, the diluted (1 : 50 with the dilution buffer) conjugate was added to the well at 100 µl / well and incubated for 1 h. Then the plate was washed as before. The plates were kept for 10 minutes at room temperature after adding the indicator solution. Immediately after adding the stopping solution positive reaction was detected by reading the OD values (using 450 nm filter).

The identified specimens were tested for their pathogenicity in two groups (each group comprising 20 test mice and a control mouse) of Swiss albino mice. The isolates were inoculated on cooked meat medium (Himedia) tubes and incubated at 37 C for 24 hours. Then these tubes centrifuged at 6,000 rpm for 45 minutes. The supernatant containing the cell free toxin, was separated and preserved for testing. The young broth culture of the organism was also used in the pathogenicity test.

The cell free toxin was injected (at 0.2 ml) via intravenous route to the test mice of group I. Again 0.2 ml of young broth culture (anaerobically grown on cooked meat medium) was injected intraperitoneally in the test mice while 0.2 ml of sterile broth was injected in the control ones in both the groups. Then the mice were observed for at least four days for pathogenicity changes.

Results and Discussion

ELISA analysis of the samples showed the presence of 143 (40.3%) *Clostridium perfringens* in this study. From the raw chicken meat samples, around 136 (61.6%) samples were detected to be positive for *Clostridium perfringens* (1). But a least no. (7 i.e. 5.2%) of positive isolates were detected from the cooked meat samples which were also supported by Sutton and Hobbs (2). These findings are in acordance with Vanderzant and Splittstoesser (3) and Willis (4). The tentative black color colonies of *Clostridium perfringens* were separated and then tested biochemically. The isolates were indole negative, methyle red negative, Voges-Proskauer negative, catalase negative (5). The isolates produced acid and gas from glucose, lactose and maltose but not from salicin, dulcitol and mannitol (6). In litmus milk test, there is acid and huge gas production which breaks the clot forming stormy clot formation in positive cases (7). These results tentatively detect the presence of *Clostridium perfringens* in the test samples. Tentative 143 *Clostridium* spp. isolates were confirmed by using Bio-X *Clostridium perfringens* ELISA kit (Bio K 086). The positive samples showed difference in optical density greater than or equal to 0150. The samples showed the difference in OD value lower than 0150 were declared as negative (8). The detection of *Clostridium perfringens* by sandwich ELISA was also followed by McClane and Strouse (9).

Then identified specimens were detected to be pathogenic to Swiss albino mice. Around 80—85% of the test mice of group I died within 24 hours of inoculation with cell free toxin. Rests died with 48 hours. But in group II, around 72% mice died within 24 hours and rests died within 72 hours post inoculation leaving the control ones unaffected in both the cases. Post-mortem examination of the dead mice revealed congestion of lung, heart, liver and intestine. The reculture of the infected intestines, viscera confirmed the presence of the bacterium in these samples (10).

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