

Prevalence of *Clostridium perfringens* in the Chicken Meat Rendered at Retail Outlets of Namakkal, Tamilnadu

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Abstract

An investigation was conducted at retail chicken outlets to identify the meat contamination by *Clostridium perfringens*, which might result in food intoxication in humans. Out of 210 meat samples collected, 3.81% were positive by culture with a colonial count of $0.96 \pm 0.38 \log_{10}$ cfu/g. All cultures of positives were confirmed by biochemical and motility tests.

Keywords: Chicken meat; Contamination; *Clostridium perfringens*; Cfu; Biochemical tests

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 foodborne disease which occurs once in a while (Bhat and Nageswara Rao, 1987). So far no information exists on disease prevalence from India, even though it is well established in the west (Lovely Joshy *et al.*, 2006). *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 (Craven *et al.*, 2001). 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 (Susan Brewer, 1991). Hence the study aimed at the detection of degree of contamination of edible chicken meat by isolation of *Clostridium perfringens* and biochemical tests.

Materials and methods

A total of 210 chicken meat samples were collected under sterile conditions from the retail poultry meat processing plant in and around Namakkal and they were submitted to microbial assay on the day of collection itself.

Thioglycollate broth and Egg yolk agar (Hi media, Bombay) were used as media for cultivation of *Clostridium perfringens*. Five gram of chicken meat samples were taken aseptically and homogenized with 45 ml of normal saline, using sterile pestle and mortar to arrive an initial dilution of 10^{-1} . Serial ten fold dilutions were made up to 10^{-6} in pre-sterilized tubes containing nine ml of 0.85 per cent normal saline. Following incubation, the petridishes showing bacterial colonies were counted and the number of colonies in the original suspension was expressed as \log_{10} cfu/g of sample, by multiplying the counted colonies with the reciprocal of the dilution.

The inoculum of each dilution was heated at 80°C for 10 min and the processed samples were inoculated into Thioglycolate broth. Sterile liquid paraffin was poured to make a layer on the medium which was incubated at 37°C for 24h. The broth cultures were streaked on to Egg yolk agar (EYA) and the plates were incubated in the candle jar at 37°C for 48h. Opalescence colonies were counted and expressed as \log_{10} cfu/g of sample, by multi-

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plying the counted colonies with the reciprocal of the dilution. All the microbial cultures were identified by Gram's staining method.

To perform motility test, young broth cultures of the organism incubated at the optimum growth condition were examined in "hanging drop" preparations, using a high power dry objective and reduced illumination.

Biochemical tests were carried out for the confirmation of *Clostridium perfringens*. Biochemical tests like Triple sugar iron (TSI) agar was used for Oxidase test, Catalase test, Lecithinase test (Hi media, Bombay) were performed for confirmation.

Results

Opalescence colonies in EYA characteristic of *Clostridium* were observed in the culture of 8 samples out of 210 and the positivity was 3.81%. Microscopic examination revealed colonies of gram positive rods of *Cl. perfringens* revealed with rare spores. The colony count in EYA agar figured to $0.96 \pm 0.38 \log_{10}$ cfu/g.

All the colonies, by motility test, were non-motile and by biochemical tests, 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 (+).

Discussion

At present, the conventional means for diagnosing food-borne diarrhoea in the microbiology laboratory relies on the culture of bacteria from stool samples (Lovely Joshy *et al.*, 2006). The findings of opalescent colonies in EYA suggestive of *Clostridium* and oxidase test negative were in agreement with that of Barrow and Feltham (1998). The detection of lecithinase negative colonies was in accordance with the finding of Hall *et al.* (1963). Gram positive rods could be detected in all sample cultures as observed by Yamamoto *et al.* (1961). Sugar fermentation tests could confirm the presence of *Clostridium perfringens* (Quinn *et al.*, 1992).

A similar colony count of *Cl. perfringens* was also reported by Silva *et al.* (1997). However, no permissible level of *Cl. perfringens* was recorded by EU (2003) and hence, risk level was arbitrarily

established at $1.761 \log_{10}$ cfu/g. as anaerobic level was not routinely determined for establishing food limit.

In this study, it was found that in each of the retail chicken markets, about 30-35 chickens were slaughtered and chopped per day on a single wooden platform and no livestock meat was processed. Further, dressing and cleaning were performed with water from deep borewell as well as tap water at all the outlets and the meat were sold within an hour without any refrigeration. Hence, chopping of more number of chickens on the same wooden platform could be major risk factors in the establishment of contamination in all chicken meats cleaned therein.

Although vegetative cells play an important role in food intoxication by *C. perfringens*, spores should not be overlooked as spores may contaminate food as well and will survive not only cooking processes, but may germinate and establish during cooling and reheating. Hence, food should be cooled rapidly and refrigerated promptly at 4°C or below, or held above 60°C to prevent growth of surviving bacteria in cooked meat, gravies, and meat casseroles to be eaten later. Leftover foods should be reheated to 74°C to avoid food intoxication.

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