

Isolation and identification of *Clostridium perfringens* from food in Basrah city and study its characterization and detection of responsible gene of food poisoning

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Summary

It was obtained 55 local isolates of *Clostridium perfringens* out of 153 samples taken from different food sources included (meat , chicken , fish and shrimp and miscellaneous foods).

All of the samples were taken from six locally markets in Basrah city included (Old Basrah , Al-Ashaar market , Al-Assmai market ,Five miles market ,Karmat Ali market and Al-Hartha market). Isolation , identification and the studying characteristics tested were carried out after on growing on TSC Agar . All isolates were selected and subjected for studying cultural and morphological in addition to biochemical test were done . All isolates were gave black colonies on the TSC Agar , from all these tests , its indicate that the isolate were belong to *Clostridium perfringens*. Microscopic examination showed that bacteria were bacilli shape , Gram positive , obligately anaerobic , capsule forming , spores forming , moreover the shapes of spores was oval (subterminal) internal spores and non-motile .

Bacteria were grown on Blood agar medium (5% Sheep blood) , Egg yolk medium , Crossley milk medium , and Reinforced clostridial broth , the results appeared double zone of haemolysis , produced Lecithinase enzyme with clear zone hydrolysis , Clear stormy fermentation , produce hydrogen sulphite (H_2S) with black colour for Blood agar , Egg yolk agar , Crossley milk medium and Reinforced clostridial broth , respectively . The isolates bacteria had the ability to reduce nitrate to nitrite , in addition to gelatin liquefaction (liquefaction gelatin after 48 hours) . The isolates bacteria were negative for catalase , oxidase , starch hydrolysis , lipolytic , negative for indole , positive for methyl red , ferment glucose , sucrose , lactose , maltose , galactose and trehalose , however it was non ferment xylose , melibiose , arabinose , salicin, mannitol, and raffinose .

Tolerance tests were applied to study the some environmental conditions such as pH (3-10) , temperature (8-55) C° and NaCl % (0-10). Results showed that optimum conditions were (6-7) , (37-40)C° and (0-1) for pH , temperature and NaCl , respectively . The frequently prevalence of these were 48 , 46 , 24 , 23 and 10% for chicken meat , red meat , fish and shrimp , dairy products and miscellaneous foods ,

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respectively . While to the local markets the frequently prevalence were 46 , 44 , 37 , 30 , 32 , 24 % for Karmat Ali , Old Basrah , Hartha , , Five miles ,AL-Ashaar and Al-Assmai , respectively .

On the other hand , in this study and the first time new selective medium prepared instead of TSC Agar which used Neomycin antibiotic instead of Cycloserine . The new medium showed good results compared with TSC Agar because it was cheap , efficient , precise in isolation and identification tests and shortly of the isolation time .

Susceptibility antibiotics tests toward 30 antibiotics was assayed. The isolates of *Clostridium perfringens* were resistance (100%) for 3 antibiotics Neomycin , Gentamycin , Streptomycin and susceptibility (100%) for 7 antibiotics Cloxacillin , Chloramphenicol , Amoxicillin , Nitrofurantion , Nalidixic acid , Cefotaxime and Vancomycin .

The PCR Technique was used to detect the toxins genes that are responsible for food poisoning . The DNA was isolated and identified by using 16S rDNA and **cp** toxins .The results showed that the selected isolated contained toxin thus confirmed this bacteria *Clostridium perfringens* certainly . The PCR results showed that there were three types in tested isolates . Type A (71.43%) which contain toxin ,this was responsible of food poisoning . Type B (7.14%) which contain , and toxins . Type C (21.43%) which contain and toxins , however the results of PCR did not show any type for both D (which contain , toxins) and E (which contain , *i* toxins) .