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Isolation of Clostridium difficile from chicken meat sold in meat stores of Isfahan City

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ABSTRACT

Clostridium difficile is a hot-positive, anaerobic spore regarded as important pathogens in human pathogenesis. Clostridium difficile is a nosocomial pathogen associated with diarrhea and colitis (inflammation of the colon) in patients admitted to hospital and its infections obtained in hospital but the disease through the consumption of poultry have been reported. Recent studies have reported out breaks of Clostridium difficile in animal-based food sthatactas a source of in fection for humans. The purpose of this study was the incidence of Clostridium difficile in chicken meats old in Isfahan-Iran. In this study, a total of 120 raw meat samples of chicken meats old by the city of Isfahan-Iran were purchased randomly then transferred to the laboratory of Infectious and Tropical Diseases Research Center of Isfahan University, were evaluated of Clostridium difficile by the enrichment method and culture on CDMN medium in anaerobic conditions and biochemical tests and observed with hot staining. In this study, total of120 cases of Clostridium difficile contamination of chicken meat was studied. Among 120 meat samples examined, 19 samples (83/15%) were carriers of Clostridium difficile. Clostridium difficile in chicken meats old in Isfahan-Iran is isolated and thus there is a potential risk to humans through microorganisms especially meat consumption.

Key words: Clostridium difficile, chicken, Isfahan, chicken meats

Introduction

Clostridium difficil is a positive warm anaerobic spore bacterium and considered as important pathogenic in human infection [1]. In 1970 it was recognized as pathogen agent associated with diarrhea.

And now is one of the most common bacterial cause of diarrhea and is associated with pseudo membranous colitis [2], and in almost all cases C. difficil cause intestinal disease in other species such as pigs [3] dogs [4] and horses [5]. Target organ of Clostridium difficil in humans and various animal species is intestine. Most strains of Clostridium difficil create toxin and bring about wide range of clinical symptoms, including mild self-limiting diarrhea, to life-threatening pseudo membranous colic, mega colon and bowel perforation caused to [2,6].

It is assumed that the disturbances in the normal micro flora of the gastrointestinal are important factor in overgrowth of Clostridium difficil and the disease finally [7]. Virulence of main factors in diseases that are currently known has two types of clostridia toxins including toxin A (TcdA, an enter toxin) and toxin B (TcdB, a cytotoxin) [8]. Another factor in the virulence of this pathogen is called binary toxin (CDT) which can be produced by some strains of Clostridium difficil [9,10]. The role of binary toxin in disease is unknown [11,12], but the data show that this toxin may have subclinical symptoms [13]. Most pathogenic strains produce both toxins of A and B and only a small percentage of strains with low virulence produce toxin B produced and they can' t produce toxin A [14]. The rate of binary strain toxin-producing is low (less than 10%) [9,15]. However, these strains have increased in recent years and binary toxin-producing strains in some studies have also been isolated of 30% of human subjects [10,12,16]. It should be noted that C. difficil cause diarrhea in patients with HIV / AIDS [17].

The three main factors which are concerned about this pathogen are: the increased infection diagnosis of Clostridium difficil in the general population, recent studies that have identified Clostridium difficil in foods of animal origin, and Clostridium difficil isolation from food patients who have the history of 5 previous month's hospitalization. Although it is currently unclear whether eating contaminated food can cause illness or infection. Many questions about the role of clostridium difficil in community about its relationship with diarrhea. Remains unanswered: its

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source can be contaminated foods, infecting dose and relationship between contaminated foods and disease. The critical role of this pathogen in human disease and potential demonstration as infecting pathogen in society reveals that accurate assessment of different sources in exposures to infection is food, but to determine potential infection role of Clostridium difficil in the pathogenesis of food can be difficult. Clostridium difficil causes critical disease in humans, especially for people who are hospitalized. So this study designed and implemented to assess possibility of contamination of poultry to this pathogen.

The purpose of this study was the incidence of Clostridium difficile in chicken meats old in Isfahan-Iran.

Materials and Methods

Samples collection:

A total of 120 samples of chicken meat store in the city of Isfahan collected randomly. Each sample transferred to research center of tropical infection disease of Isfahan University in exposure to ice and studied promptly.

Isolation: Approximately 5 g of meat sample was homogenized in 25 ml of enriched broth medium of C. difficil. The cultivation include 40 gr/l Protease peptone, 50 g/l disodium hydrogen phosphate, 1/0 g/l Epsom, 0/2 g/l sodium chloride, 6 g/l fructose and 1/0g/l sodium Tarcolat with cysteine, Norfeloxacin and Muxaltam hydrochloride supplements. This combination put for 7-5 days at 37 ° C under anaerobic conditions. Then, 2 ml of each culture was added to an equal volume of ethanol and the mixture was placed at room temperature for 1 h. Alcohol cultures were then centrifuged (10 Minutes /4000 rpm) and mass inserted on Agar Norfeloxacin Muxaltam C.difficil (CDMN) and for 24-48 hours at 37 ° C under anaerobic conditions were warm, home setting. Sensitive colonies were grown and identified on the basis of colony morphology (irregular, gray), flavor (horse/elephant manure), warm morphology and Amino Peptides L-praline test.

Results:

In this study, a total of 120 cases of chicken meat were studied regarding contamination to Clostridium difficil. Then of 120 meat samples examined, 19 samples ((15/83%)) containing C. difficil.

Discussion:

Poultry meat as a source of protein by high quality known in the world and contains essential amino acids along with vitamins and minerals. Poultry meat contains more similar proteins with the same level in beef or lamb, as well as poultry, especially chickens due to its low price, is widely used. Poultry meat consumption can be a source of pathogen infection of Clostridium difficil for human.

Clostridium difficil outbreaks in poultry samples was relatively high (83/15%) and comparable to that reported by rise and colleagues in 2010 (12/8 percent) [18] and Indra in (5%) [11] And Weese *et al* (15 percent) [19]. However, a higher incidence of c.difficil in chicken carcasses (29 percent) reported by Simango and Mwakurudza in [20]. These differences may result from differences in health status during the breeding or cross-contamination, it can be made during filling, discharging offal or cutting carcasses. Given that the studies about isolation of the pathogen from poultry meat are limited therefore several research groups have worked on other cases: including: In 2007, a study was conducted in Canada on minced meat for human consumption. Using cultivation, C.difficil isolated of 20 percent of miced meat and 14 percent of beef samples.

In a similar study in America, Isolation of Clostridium difficil from miced meat reported 50%, Summer Sausage 14 percent, pork sausage 43%, pork meat 63%, corizo 30%, pork sausages 23% and Turkish meat44% [21].

Roudari and colleagues in Canada showed that out of 149 samples of lamb, 10 samples and out of 65 samples of beef, 3 samples of C.difficil isolated. [22]. In July 2010, Roger *et al* collected 32 samples of 5 meat stores in Bryan and Station College of Texas and the rate of 12/5 percent reported [23].

Boroven, et al in 2009 reported isolation of Clostridium difficil from 3 of 40 samples (5/7 percent) of prepared salad [24]. This study like our research confirms food contamination to C. difficil pathogen so there is the potential risk to humans through food consumption and particularly microorganisms.

Conclusion:

C. difficil isolated of chicken meat sold in Isfahan-Iran, so there is a potential risk to human through microorganism particularly meat consumption.

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