



Isolation of *Clostridium difficile* from turkey and ostrich meat Sold in meat stores of Isfahan city

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ABSTRACT

Clostridium difficile is a binding warm-positive rod and anaerobic organism, which was first described in 1935 as *Bacillus difficile*. Epidemiology of CDI has changed considerably on 2000. Occurrence and severity of the disease including ulcerative colitis (inflammation of the colon), colectomy (colon section) and mortality had increased. Recent studies have reported *Clostridium difficile* prevalence in animal foods, act as a source of infection for humans, its source can be regarded as a contaminated food, infecting dose and the relationship between food contamination and disease. *Clostridium difficile* causes critical disease in humans. So, this study by the aim of evaluation of possible contamination of turkey and ostrich meat to the pathogen was designed and implemented. To conduct this study, a total of 240 samples of raw ostrich meat (n=120) and Turkey (n=120) purchased randomly from meat stores of Isfahan - Iran and then transferred to the Laboratory of Infectious and Tropical Diseases Research Center, Isfahan University, evaluated regarding existence of *Clostridium difficile* by enrichment method and then culture on CDMN medium under anaerobic conditions and biochemical tests and observations of warm staining. In this study, a total of 240 samples, 25 samples (10/41%) were carriers of *C. difficile*. Of 11 of 120 ostrich meat samples (9/16%) and 14 samples out of 120 samples of turkey meat (11/66%) *Clostridium difficile* was isolated.

Clostridium difficile in turkey and ostrich meat sold in stores in Isfahan - Iran is isolated and thus a potential risk to humans especially through meat consumption are microorganisms.

Key words: *Clostridium difficile*, Ostrich-Turkey, Isfahan

INTRODUCTION

Clostridium difficile is a necessary anaerobic and warm positive rod organism, which was first described in 1935 as *Bacillus difficile* (Hall and Toole, 1935). *C. difficile* was named by Hall and O'Toole and whose name suggests that its difficult separation is attributed to the relative growth. Its growth compared with most other members of the *Clostridium* genus is slow (Jump *et al*, 2007). Diarrhea and inflammation of the colon that is seen in CDI are associated with the effects of the two big toxins A (Tcd A) and toxin B (Tcd B) which both toxins are encoded as the locus pathogens (Paloc) on the chromosome of *Clostridium difficile* (Soehn *et al*, 1998; Braun *et al*, 1996). Also, *C. difficile* has pathogenic factors outside of Paloc such as a binary toxin that is encoded by *cdtA* and *cdtB* (Mc Ellistrem *et al*, 2005; Rupnik *et al*, 2003). Binary toxin-producing strain rate is low (less than 10%) (Pituch *et al*, 2005; Goncalves *et al*, 2004). Yet these strains have increased in recent years. Binary toxin-producing strains in some studies isolated of

30% of human subjects (Loo *et al*, 2005; Martin *et al*, 2008; Paltansing *et al*, 2007). It should be noted that *C. difficile* cause diarrhea in patients with HIV / AIDS (Barbut *et al*, 1997). In 2000, Epidemiology of CDI has changed considerably. Occurrence and severity of the disease, including ulcerative colitis (inflammation of the colon), colectomy (colon section) and mortality have increased. (Loo *et al*, 2005; Muto *et al*, 2005). This event has been reported worldwide and incidence of *C. difficile* in the United States doubled from 1996 to 2003 (Mc Donald *et al*, 2006). Also, *C. difficile* cause intestinal disease in other species, including pigs (Songer *et al*, 2000), dogs (Marks *et al*, 2002) and horses (Arroyo *et al*, 2004). *C. difficile* is an important pathogen in animals and found in retail meat and even salad and lead to CDI infections in the community acquired through food (Bakri *et al*, 2009; Indra *et al*, 2009). Three main factors are concerned about this pathogen, the increased virulence of *Clostridium difficile* infection in the general population, recent studies that have identified *Clostridium difficile* in foods of animal origin and Isolation of *Clostridium difficile* from patients food having history of hospitalization in over the past 5 months. Although it is currently unclear whether eating contaminated food can cause illness or infection. Many questions about the role of *Clostridium difficile* in diarrhea in community relationship remain unanswered. Its source can be contaminated food and a contaminated and relationship between pollution and disease. Its significant role in the in human disease and potential emergence as a pathogen causing infection indicated that exact assessment of exposure to different sources of contamination is food but determination of *C. difficile* potential role in infection in foods is difficult. *Clostridium difficile* causes critical disease in humans, so the present study designed and implemented to evaluate the possible contamination of turkey and ostrich meat to the pathogen and implemented. The aim of this study is isolation of *Clostridium difficile* and turkey and ostrich meat Sold in meat stores of Isfahan city.

MATERIALS AND METHODS

Sample collection: A total of 240 samples of turkey and ostrich meat including 120 samples of turkey and ostrich meat (n=120) and 120 turkey meat samples (n=120) collected of meat stores of Isfahan city randomly. Each sample transferred to laboratory of tropical infectious disease research center of Isfahan University and examined promptly.

Isolation

Isolation: Approximately 5 g of meat sample was homogenized in 25 ml of enriched broth medium of *C. difficile*. The cultivation of 40 gr / l, proteose peptone 40 g/l, 50 g / l disodium hydrogen phosphate, 1 g / l Magnesium Sulfate, Epsom, 2 g / l 10 Sodium Chloride, 6 g / l Fructose, 1 g / l Tarokolat sodium with supplements of Cysteine, Nurfeloxacin and Muxasaktam hydrochloride. This combination kept 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 / 4000rpm) and mass on Agar nurfeloxacin muxasaktam (CDMN) of *C. difficile* were homed for 48-24 hours at 37 ° C under warm anaerobic conditions. Sensitive colonies were grown and on the basis of colony morphology (irregular gray), flavor (horse/elephant manure), warm morphology and Aminopeptidas L-proline test were identified.

RESULTS AND DISCUSSION

In this study, a total of 240 samples of turkey and ostrich meat examined regarding *Clostridium difficile* contamination. From 240 samples, 25 samples (10/41%) are carrier of *C. difficile*. From 11 samples of 120 ostrich meat (9/16%) and 14 of 120 samples of turkey meat (11/66%), *Clostridium difficile* was isolated. The results of the study showed that the highest prevalence of infection between the two types of turkey and ostrich meat related to turkey meat with 11/66 percent and then ostrich with 9/16.

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 difficile* for human. *Clostridium difficile* outbreak in turkey meat samples (11/66) was lower in comparison with research done by Songer in 2007 in U.S.A (44 percent). *C. difficile* outbreak in turkey and ostrich meat samples of this research was higher in comparison with chicken outbreak which reported by Indra in 2009 (5 percent) (Indra *et al*, 2009). However, regarding Weese, et al research about chicken meat in 2009 (15 percent) (Weese *et al*, 2009) and Simango and Mwakurudza in 2008 (29 percent) there was lower incidence of *C. difficile* in turkey and ostrich carcasses in comparison with chicken. 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. difficile* isolated of 20 percent of minced meat and 14 percent of beef samples. In a similar study in America, Isolation of *Clostridium difficile* from minced meat reported 50%, Summer Sausage 14 percent, pork sausage 43%, pork meat 63%, corizo 30%, pork sausages 23% and Turkish meat 44% (Rodriguez-Palacios *et al*, 2007). 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. difficile* isolated (Rodriguez-Palacios *et al*, 2009). 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 (Harvey *et al*, 2011). Boroven et al (2009) reported isolation of *Clostridium difficile* from 3 of 40 samples (5/7 percent) of prepared salad (Bakri *et al*, 2009). These studies like our research confirms food contamination to *C. difficile* pathogen so there is the potential risk to humans through food consumption and particularly microorganisms.

Conclusions

Clostridium difficile in turkey and ostrich meat sold in stores in Isfahan - Iran is isolated and thus there is a potential risk to humans through microorganisms especially meat consumption.

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