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Ribotyping and assessment of toxigenic genes of clostridium difficile strains isolated from raw meat

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Abstract---*Background: Clostridium difficile* is an anaerobic, spore forming, rod shaped bacterium that can produce toxins and may transmit through some types of food samples. The present survey was done to assess the prevalence, toxigenic gene profile and ribotyping of *C. difficile* strains isolated from raw bovine and ovine meat samples. *Methods:* Two hundred and fifty raw meat samples were collected from Isfahan, Iran and analyzed for presence of *C. difficile* using the microbial culture. Genomic DNA was extracted from *C. difficile* isolates. Extracted DNA samples were subjected to PCR for determination of *tcdA*, *tcdB*, and *cdtA* toxigenic genes. Ribotyping was done using the *16S* and *23S rRNA* genes amplification in a separated PCR assay. *Results:* Fifteen out of 250 (6%) raw meat samples were contaminated with *C. difficile*. Prevalence of *C. difficile* strains amongst the raw bovine and ovine meat samples were 8% and 4%, respectively. The most commonly detected toxigenic genes were *tcdA* (33.33%) and *cdtA* (20%). Distribution of *tcdB* was 13.33%. Amongst the combined toxigenic genes, *tcdA+cdtA* had the highest distribution (13.33%). Among isolates of bovine meat samples, 027 ribotype was the most prevalent (40%). Among isolates of raw ovine meat samples, both 027 and 078 ribotypes had equal prevalence (20%). *Conclusion:* Role of raw meat as a source of toxigenic *C. difficile* strains, particularly 027 and 078 ribotypes was determined. Well-cooking of raw meat samples before consumption may diminish the risk of foodborne *C. difficile* infections.

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Introduction

Clostridium difficile is a gram-positive, obligate anaerobic, spore-forming and cytotoxin producing bacterium with optimum growth temperature at 35–40°C (1). It is recognized as a major cause of antimicrobial-associated and hospital-associated diarrhea, and the cause of almost all cases of pseudomembranous colitis (2). Foods maybe responsible in transmission of *C. difficile* into the human population (3). It is considered that *C. difficile* can be transmitted to human by foods since it is disseminated by oral-fecal route and isolated from food animals including poultry and in some lower cases raw bovine and ovine meat samples (4). Meat can be contaminated by *C. difficile* through infected animals or food handlers during slaughtering (5). Several studies determined that *C. difficile* spore contamination level is generally low (6). However, the spores of this pathogen, if present in meat or other foods, may not be killed by cooking and can survive at 71°C for two hours (7).

Some toxigenic genes are responsible for the pathogenesis of *C. difficile* infections (8). The two virulence factors associated with the *C. difficile* infection are toxin A (*tcdA*) and toxin B (*tcdB*), which are an enterotoxin and a cytotoxin, respectively (9). The binary toxin (*cdt*) is encoded by the genes *cdtA* and *cdtB* located outside PaLoc (10). *Cdt* is frequently observed in *C. difficile* strains associated with increased severity of *C. difficile* infection (CDI) (10). The epidemiology of CDI in humans have been changed as a consequence of the emergence and dissemination of new strains of *C. difficile* called ribotype 027 and 078 (11). Roles of these two ribotype in the epidemiology of *C. difficile* strains in food samples and the cases of foodborne diseases is not determined yet. According to the high importance of the *C. difficile*, the present survey was done to assess the ribotyping and toxigenic gene profiles of the *C. difficile* strains isolated from raw meat.

Materials and Methods

Samples

From march to August 2021, a total of 250 raw meat samples (100 g, tight muscle), including bovine (n= 125) and ovine (n= 125), were collected from butchers of the Isfahan province, Iran. Samples were collected separately using the sterile plastic bags. Samples were transferred to laboratory in cooler with ice packs at the day of sampling.

C. difficile isolation and identification

About 5 g of each sample was cultured in 25 mL of *C. difficile* moxalactam norfloxacin (CDMN) (Oxoid SR0048) and fortified with *C. difficile* selective supplement (Oxoid, SR0173) including 500 mg cysteine hydrochloride, 12 mg norfloxacin and 35 mg moxalactam per liter. The samples were incubated anaerobically for 7 days at 37 °C. Subsequently, 2 mL of enriched culture were

added to 2 mL of 98% ethanol (Merck, Germany) and kept for 2 h at room temperature. The tubes were centrifuged for 10 min at 10000 rpm. The sediment was streaked onto CDMN agar and then incubated under anaerobic conditions at 37 °C for 24-48 h. All *C. difficile* isolates were confirmed by morphology and L-proline- aminopeptidase test (prodisk, hardy diagnostics, Santa Maria, USA). Positive strains were recultured on blood agar and incubated at 37 °C for 36 h in anaerobic conditions (12).

DNA extraction

C. difficile isolates were sub-cultured on the CDMN broth and further incubated for 7 days at 37 ^{o}C. In addition, genomic DNA was extracted from bacterial colonies using the DNA extraction kit (Fermentas, Germany), according to the manufacturer's instructions (13-17). Furthermore, the purity (A260/A280) and concentration of the extracted DNA were checked (NanoDrop, Thermo Scientific, Waltham, MA, USA) (18-22). The truth of the DNA was assessed on a 2% agarose gel stained with ethidium bromide (0.5 µg/mL) (Thermo Fisher Scientific, St. Leon-Rot, Germany) (23-25).

Detection of toxigenic genes

Table 1 shows the PCR conditions used for detection of toxigenic genes (26). A programmable DNA thermal cycler (Eppendorf Mastercycler 5330, Eppendorf-Nethel-Hinz GmbH, Hamburg, Germany) was used for all PCR reactions. Next, 10 µL of the PCR product was exposed to electrophoresis in a 2% agarose gel in a 1X TBE buffer at 80 V for 30 min, which was stained with SYBR Green (27-30). Besides, the UVI doc gel documentation system (Grade GB004, Jencons PLC, London, UK) was utilized for the analysis of images (31-33).

Table 1
PCR conditions used for detection of toxigenic genes (26)

Target genes	Primer sequence (5'-3')	Product size (bp)	Thermal procedure	Volumes (50 µl)
tcdA	GCATGATAAGGCAACTTCAGTGGTA AGTTCCTCCTGCTCCATCAAATG	629	1 cycle: 94 ^{oC} ------------ 10 min. 35 cycles: 94 ^{oC} ------------ 50 s 54 ^{oC} ------------ 40 s 72 ^{oC} ------------ 50 s 1 cycle: 72 ^{oC} ------------ 3 min	5 µL PCR buffer 10X 2 mM Mgcl$_2$ 150 µM dNTP 0.75 µM of each primers F & R 1.5 U Taq DNA polymerase 3 µL DNA template
tcdB	CCAAARTGGAGTGTTACAAACAGGTG GCATTTCTCCATTCTCAGCAAAGTA	410		
cdtA	GGGAAGCACTATATTAAAGCAGAAGC CTGGGTTAGGATTATTTACTGGACCA	221		

Ribotyping of C. difficile isolates

For PCR ribotyping was done according to using two pairs of primers including 3'-GTGCGGCTGGATCACCTCCT-5' (*16S rRNA*) and 3'-CCCTGCACCCTTAATAACTTGACC-5' (*23S rRNA*) (34). The ribotype pattern was obtained by electrophoresis on 3% agarose gel at 80 v for 6 h and stained with ethidium bromide and then compared with ribotype patterns 027 and 078 recorded in reference laboratories.

Statistical analysis

Statistical analysis was performed using SPSS Statistics 21.0 (SPSS Inc., Chicago, IL, USA). Besides, chi-square and Fisher's exact two-tailed tests were employed to assess any significant relationships between the data obtained from the present study (35, 36). P-value < 0.05 was considered statistically significant as well.

Results

C. difficile prevalence

Table 2 shows the prevalence of *C. difficile* strains isolated from raw meat samples. Fifteen out of 250 (6%) raw meat samples were contaminated with *C. difficile*. Prevalence of *C. difficile* strains amongst the raw bovine and ovine meat samples were 8% and 4%, respectively. Statistically significant differences were found between types of samples and *C. difficile* prevalence (*P* <0.05).

Table 2
Prevalence of *C. difficile* strains isolated from raw meat samples

Type of meat samples	N. collected	N. positive for C. *difficile* (%)
Bovine	125	10 (8)
Ovine	125	5 (4)
Total	250	15 (6)

C. difficile toxigenic genes

Table 3 shows the prevalence of toxigenic genes amongst the *C. difficile* strains isolated from raw meat samples. The most commonly detected toxigenic genes were *tcdA* (33.33%) and *cdtA* (20%). Distribution of *tcdB* was 13.33%. Amongst the combined toxigenic genes, *tcdA+cdtA* had the highest distribution (13.33%). Statistically significant differences were found between types of samples and distribution of *C. difficile* toxigenic genes (*P* <0.05).

Table 3
Prevalence of toxigenic genes amongst the *C. difficile* strains isolated from raw meat samples

Type of meat samples	N. positive	Distribution of toxigenic genes (%)					
		tcdA	*tcdB*	*cdtA*	*tcdA+tcdB*	*tcdA+cdtA*	*tcdB+cdtA*
Bovine	10	4 (40)	2 (20)	2 (20)	1 (10)	2 (20)	1 (10)
Ovine	5	1 (20)	-	1 (20)	-	-	-
Total	15	5 (33.33)	2 (13.33)	3 (20)	1 (6.66)	2 (13.33)	1 (6.66)

C. difficile ribotyping

Figure 1 shows the 027 and 078 ribotypes distribution amongst the *C. difficile* strains isolated from raw meat samples. Among isolates of bovine meat samples, 027 ribotype was the most prevalent (40%). Among isolates of raw ovine meat samples, both 027 and 078 ribotypes had equal prevalence (20%).

Figure 1. 027 and 078 ribotypes distribution amongst the *C. difficile* strains isolated from raw meat samples.

Discussion

Medical sciences have been developed in recent years (37-48). However, infections remain a big issue. In this regard, *C. difficile* is considered prevalent cause of gastrointestinal infections (1, 2). In the present survey, 6% of raw bovine and ovine meat samples were contaminated with *C. difficile* strains. Probable reasons for the prevalence of *C. difficile* strains in examined meat samples are meat contamination through hand manipulation by meat inspectors in slaughterhouses and also butchers. Additionally, transmission of *C. difficile* strains through the contaminated environment of the slaughterhouses should be considered. A relatively high incidence of *C. difficile* contamination was observed

in the United States, where 42% of different retailed meat products and raw meats were found to be contaminated with *C. difficile* (49). Another study in the United States showed 8% *C. difficile* prevalence in ground meat samples (50). *C. difficile* has also been detected in experiments run in Canada with an incidence of 20% meat samples analyzed by Rodriguez-Palacios et al. (51).

Previous survey showed a collection of 12 ribotypes from *C. difficile* isolates in meat samples through PCR-ribotyping. Overall, eight (67%) of the 12 isolates hadn't previously been identified and designated as belonging to the ribotype M31. The other identified ribotypes were 014, 077 and M26 (51). Furthermore, these authors cited the genetic diversity of *C. difficile* in retail raw meat in another study, including ribotypes 014, 077, M26, C, F, H, K and J (52). In contrast, some different studies showed a link between the presence of similar ribotypes in meat samples and clinical ones. For example, ribotype 012, 027, 078 identified in meat samples in Iran, USA and Europe (53-56).

Toxigenic genes had the high distribution among isolates. We found the distribution of *tcdA*, *tcdB* and *cdtA* as 33.33%, 13.33%, and 20%, respectively. Razmyar et al. (2017) (57) reported that out of 65 packed chicken samples, 10 (15.30%) samples were positive for *C. difficile* and 7 (10.70%) samples were *tcdA* and *tcdB* positive, one sample had only shown harboring *tcdA*, and two (3%) of them were binary toxin positive. Muratoglu et al. (2020) (58) stated that the distribution of *tcdA*, *tcdB*, and *cdtA* toxigenic genes amongst the *C. difficile* strains isolated from meat products samples were 100%, 100%, and 86.40%, respectively, which was much higher than our findings. The impact of veterinary and food hygiene in medical sciences has been determined before (59-67).

Conclusion

As shown, toxigenic *C. difficile* strains were isolated from raw meat samples of ovine and bovine species. This study may show the role of raw meat samples of animal species in transmission of toxigenic *C. difficile* strains, particularly 027 and 078 ribotypes. As these two ribotypes are mainly associated with severe gastrointestinal diseases, the consumption of raw meat samples contained these ribotypes may cause several foodborne infections. However, supplementary studies should be done to assess the main risk of these ribotypes in the *C. difficile* strains isolated from raw meat samples.

References

Abdolmaleki Z, Mashak Z, Dehkordi FS. Molecular and virulence characteristics of methicillin-resistant Staphylococcus aureus bacteria recovered from hospital cockroaches. Jundishapur Journal of Microbiology. 2019 Dec 31; 12(12).

Abolhasani M, Ghasemi E, Fathi AH, Hayatizadeh MJ. Color Change of Ceramill Zolid FX Following Abrasion with/without Toothpaste. Journal of Iranian Dental Association. 2021; 33(3):51-7.

Abolhasani M, Givehchian P, Fathi A, Goudarzi S. Relationship of Life Satisfaction and Satisfaction with Fixed Implant-Supported Prostheses in the Elderly. Journal of Iranian Dental Association. 2021; 33(1):17-21.

Ashtiani AH, Mardasi N, Fathi A. Effect of multiple firings on the shear bond strength of presintered cobalt-chromium alloy and veneering ceramic. The Journal of Prosthetic Dentistry. 2021; 126(6):803-e1.

Barakati B, Khodadadi R, Asadi P, Fathi A. Evaluate The Effect Of Various Titanium Abutment Modifications On The Behavior Of Peri-Implant Soft Tissue Healing, Inflammation, And Maintenance: A Systematic Review And Meta-Analysis. Turkish Onilne Journal of Qualitative Inquiry. 2021; 12:11401-11410.

Barbut F. How to eradicate Clostridium difficile from the environment. Journal of Hospital Infection. 2015 Apr 1; 89(4):287-95.

Bidet P, Lalande V, Salauze B, Burghoffer B, Avesani V, Delmée M, Rossier A, Barbut F, Petit JC. Comparison of PCR-ribotyping, arbitrarily primed PCR, and pulsed-field gel electrophoresis for typing Clostridium difficile. Journal of clinical microbiology. 2000 Jul 1; 38(7):2484-7.

Bouttier S, Barc MC, Felix B, Lambert S, Collignon A, Barbut F. Clostridium difficile in ground meat, France. *Emerg Infect Dis.* 2010; 16(4):733–5.

Curry SR, Marsh JW, Schlackman JL, Harrison LH. Prevalence of Clostridium difficile in uncooked ground meat products from Pittsburgh, Pennsylvania. *Appl Environ Microbiol.* 2012; 78(12):4183–6.

Dehkordi FS, Barati S, Momtaz H, Ahari SN, Dehkordi SN. Comparison of shedding, and antibiotic resistance properties of Listeria monocytogenes isolated from milk, feces, urine, and vaginal secretion of bovine, ovine, caprine, buffalo, and camel species in Iran. Jundishapur Journal of Microbiology. 2013a; 6(3):284.

Dehkordi FS, Gandomi H, Basti AA, Misaghi A, Rahimi E. Phenotypic and genotypic characterization of antibiotic resistance of methicillin-resistant Staphylococcus aureus isolated from hospital food. Antimicrobial Resistance & Infection Control. 2017; 6(1):1-1.

Dehkordi FS, Haghighi Borujeni MR, Rahimi E, Abdizadeh R. Detection of Toxoplasma gondii in raw caprine, ovine, buffalo, bovine, and camel milk using cell cultivation, cat bioassay, capture ELISA, and PCR methods in Iran. Foodborne Pathogens and Disease. 2013; 10(2):120-5.

Dehkordi FS, Haghighi N, Momtaz H, Rafsanjani MS, Momeni M. Conventional vs real-time PCR for detection of bovine herpes virus type 1 in aborted bovine, buffalo and camel foetuses. Bulgarian Journal of Veterinary Medicine. 2013; 16(2):102-12.

Dehkordi FS, Khamesipour F, Momeni M. Brucella abortus and Brucella melitensis in Iranian bovine and buffalo semen samples: The first clinical trial on seasonal, Senile and geographical distribution using culture, conventional and real-time polymerase chain reaction assays. Kafkas Univ Vet Fak Dergisi. 2014; 20(6):821-8.

Dehkordi FS, Momtaz H, Doosti A. Application of Real-Time PCR for detection of Aspergillus species in aborted ruminant foetuses. Bulgarian Journal of Veterinary Medicine. 2012; 15(1):30-6.

Dehkordi FS, Parsaei P, Saberian S, Moshkelani S, Hajshafiei P, Hoseini SR, Babaei M, Ghorbani MN. Prevalence study of Theileria annulata by comparison of four diagnostic Techniques in shouthwest Iran. Bulgarian Journal of Veterinary Medicine. 2012; 15(2): 123-130.

Dehkordi FS, Saberian S, Momtaz H. Detection and segregation of Brucella abortus and Brucella melitensis in aborted bovine, ovine, caprine, buffaloes

and camelid fetuses by application of conventional and real-time polymerase chain reaction. The Thai Journal of Veterinary Medicine. 2012; 42(1):13.

Dehkordi FS, Taghizadeh F. Prevalence and some risk factors associated with brucellosis and leptospirosis in aborted fetuses of ruminant species. Res Opin Anim Vet Sci. 2012; 2:275-81.

Dehkordi FS, Tavakoli-Far B, Jafariaskari S, Momtaz H, Esmaeilzadeh S, Ranjbar R, Rabiei M. Uropathogenic Escherichia coli in the high vaginal swab samples of fertile and infertile women: virulence factors, O-serogroups, and phenotyping and genotyping characterization of antibiotic resistance. New Microbes and New Infections. 2020; 38:100824.

Dehkordi FS, Tirgir F, Valizadeh Y. Effects of Guajol® ointment synthesized from medicinal smoke condensate of jennet feces on burn wound healing on Wistar rat. Veterinary Research Forum. 2017; 8(3):215.

Dehkordi FS, Valizadeh Y, Birgani TA, Dehkordi KG. Prevalence study of Brucella melitensis and Brucella abortus in cow's milk using dot enzyme linked immuno sorbent assay and duplex polymerase chain reaction. Journal of Pure and Applied Microbiology. 2014b; 8(2):1065-9.

Dehkordi FS, Yazdani F, Mozafari J, Valizadeh Y. Virulence factors, serogroups and antimicrobial resistance properties of Escherichia coli strains in fermented dairy products. BMC Research Notes. 2014c; 7(1):1-8.

Dehkordi FS. Prevalence study of Bovine viral diarrhea virus by evaluation of antigen capture ELISA and RT-PCR assay in Bovine, Ovine, Caprine, Buffalo and Camel aborted fetuses in Iran. AMB Express. 2011; 1(1):1-6.

Dehkordi FS. Prevalence study of Coxiella burnetii in aborted ovine and caprine fetuses by evaluation of nested and real-time PCR assays. American Journal of Animal and Veterinary Sciences. 2011; 6(4):180-6.

Dupuy B, Govind R, Antunes A, Matamouros S. Clostridium difficile toxin synthesis is negatively regulated by TcdC. Journal of medical microbiology. 2008 Jun 1; 57(6):685-9.

Dwijayanti, N., Mufdlilah, M., & Suryaningsih, E. K. (2022). The role of midwives in the application of classroom services for pregnant women during the COVID-19 pandemic period. International Journal of Health & Medical Sciences, 5(3), 228-239. https://doi.org/10.21744/ijhms.v5n3.1918

Ebadian B, Fathi A, Khodadad S. Comparison of the effect of four different abutment screw torques on screw loosening in single implant-supported prosthesis after the application of mechanical loading. International Journal of Dentistry. 2021; 2021:1-6.

Ebadian B, Fathi A, Savoj M. In Vitro Evaluation of the Effect of Different Luting Cements and Tooth Preparation Angle on the Microleakage of Zirconia Crowns. International Journal of Dentistry. 2021; 2021:1-7.

Farhad SD, Mojtaba SR. Prevalence study of Coxiella burnetii in aborted fetuses of small ruminants in various partum and seasons in Iran. African Journal of Microbiology Research. 2012 Jul 19; 6(27):5594-600.

Fathi A, Salehi A. Antimicrobial resistance properties of Helicobacter pylori strains isolated from dental plaque and saliva samples. Academic Journal of Health Sciences. 2021; 36 (4):51-55.

Fathi AH, Fardi E, Zamani Ahari U, Naghdipour Mirsadeghi M, Oraie F. The relationship between periodontitis and preeclampsia: A systematic review. The Iranian Journal of Obstetrics, Gynecology and Infertility. 2021; 24(11):79-86.

Gerding DN, Johnson S, Rupnik M, Aktories K. Clostridium difficile binary toxin CDT: mechanism, epidemiology, and potential clinical importance. Gut microbes. 2014 Jan 1; 5(1):15-27.

Ghorbani F, Gheisari E, Dehkordi FS. Genotyping of vacA alleles of Helicobacter pylori strains recovered from some Iranian food items. Tropical Journal of Pharmaceutical Research. 2016; 15(8):1631-6.

Harvey RB, Norman KN, Andrews K, Norby B, Hume ME, Scanlan CM, Hardin MD, Scott HM. Clostridium difficile in retail meat and processing plants in Texas. Journal of Veterinary Diagnostic Investigation. 2011 Jul; 23(4):807-11.

Houser BA, Soehnlen MK, Wolfgang DR, Lysczek HR, Burns CM, Jayarao BM. Prevalence of Clostridium difficile toxin genes in the feces of veal calves and incidence of ground veal contamination. *Foodborne Pathog Dis.* 2012; 9(1):32–6.

Hung YP, Huang IH, Lin HJ, Tsai BY, Liu HC, Liu HC, Lee JC, Wu YH, Tsai PJ, Ko WC. Predominance of Clostridium difficile ribotypes 017 and 078 among toxigenic clinical isolates in southern Taiwan. PloS one. 2016 Nov 18; 11(11):e0166159.

Khamisi N, Fathi A, Yari A. Antimicrobial resistance of Staphylococcus aureus isolated from dental plaques. Academic Journal of Health Sciences. 2022; 37(1):136-140.

Maalekipour M, Safari M, Barekatain M, Fathi A. Effect of Adhesive Resin as a Modeling Liquid on Elution of Resin Composite Restorations. International Journal of Dentistry. 2021; 2021:1-9.

Mashak Z, Banisharif F, Banisharif G, Reza Pourian M, Eskandari S, Seif A, Safarpoor Dehkordi F, Alavi I. Prevalence of listeria species and serotyping of Listeria monocytogenes bacteria isolated from seafood samples. Egyptian Journal of Veterinary Sciences. 2021 Apr 1; 52(1):1-9.

Mashak Z, Jafariaskari S, Alavi I, Shahreza MS, Dehkordi FS. Phenotypic and genotypic assessment of antibiotic resistance and genotyping of vacA, cagA, iceA, oipA, cagE, and babA2 alleles of Helicobacter pylori bacteria isolated from raw meat. Infection and drug resistance. 2020; 13: 257.

Monajem Zade SM, Elyaskhil M, Fathi A, Asadinejad SM. Evaluate Risk Markers For Periodontal Disease In Children With Type 1 Diabetes: A Systematic Review And Meta-Analysis. 2021; 12:5715-5722.

Mosharraf R, Molaei P, Fathi A, Isler S. Investigating the Effect of Nonrigid Connectors on the Success of Tooth-and-Implant-Supported Fixed Partial Prostheses in Maxillary Anterior Region: A Finite Element Analysis (FEA). International Journal of Dentistry. 2021; 2021:1-12.

Mousavi S, Safarpoor Dehkordi F, Valizadeh Y. Genotyping of Helicobacter pylori strains isolated from raw milk and dairy products. Journal of Food Microbiology. 2017 Nov 22; 4(3):41-53.

Muratoglu K, Akkaya E, Hampikyan H, Bingol EB, Cetin O, Colak H. Detection, characterization and antibiotic susceptibility of clostridioides (Clostridium) difficile in meat products. Food Science of Animal Resources. 2020 Jul; 40(4):578.

Nayebpoor F, Momeni M, Dehkordi FS. Incidence of ochratoxin A in raw and salted dried fruits using high performance liquid chromatography. Am Eur J Toxicol Sci. 2013; 5:1-6 .

Nejat S, Momtaz H, Yadegari M, Nejat S, Safarpour Dehkordi F, Khamesipour F. Seasonal, geographical, age and breed distributions of equine viral arteritis in Iran. Kafkas Univ Vet Fak Derg. 2015; 21(1):111-6.

Persson S, Torpdahl M, Olsen KE. New multiplex PCR method for the detection of Clostridium difficile toxin A (tcdA) and toxin B (tcdB) and the binary toxin (cdtA/cdtB) genes applied to a Danish strain collection. Clinical microbiology and infection. 2008 Nov 1; 14(11):1057-64.

Persson S, Torpdahl M, Olsen KE. New multiplex PCR method for the detection of Clostridium difficile toxin A (tcdA) and toxin B (tcdB) and the binary toxin (cdtA/cdtB) genes applied to a Danish strain collection. Clinical microbiology and infection. 2008 Nov 1; 14(11):1057-64.

Rahi A, Kazemeini H, Jafariaskari S, Seif A, Hosseini S, Dehkordi FS. Genotypic and phenotypic-based assessment of antibiotic resistance and profile of staphylococcal cassette chromosome mec in the methicillin-resistant Staphylococcus aureus recovered from raw milk. Infection Drug Res. 2020; 13:273.

Rahimi E, Jalali M, Weese JS. Prevalence of Clostridium difficile in raw beef, cow, sheep, goat, camel and buffalo meat in Iran. *BMC Public Health.* 2014; 14:119.

Ranjbar R, Dehkordi FS, Heiat M. The frequency of resistance genes in Salmonella enteritidis strains isolated from cattle. Iranian Journal of Public Health. 2020 May; 49(5):968.

Ranjbar R, Seif A, Dehkordi FS. Prevalence of antibiotic resistance and distribution of virulence factors in the shiga toxigenic Escherichia coli recovered from hospital food. Jundishapur J Microbiol. 2019; 12(5):8.

Ranjbar R, Yadollahi Farsani F, Safarpoor Dehkordi F. Antimicrobial resistance and genotyping of vacA, cagA, and iceA alleles of the Helicobacter pylori strains isolated from traditional dairy products. Journal of Food Safety. 2019 Apr; 39(2):e12594.

Razmyar J, Jamshidi A, Khanzadi S, Kalidari G. Toxigenic Clostridium difficile in retail packed chicken meat and broiler flocks in northeastern Iran. Iranian Journal of Veterinary Research. 2017; 18(4):271.

Rodriguez C, Taminiau B, Broeck JV, Delmée M, Daube G. Clostridium difficile in food and animals: a comprehensive review. Advances in Microbiology, infectious diseases and public health. 2016:65-92.

Rodriguez-Palacios A, Reid-Smith RJ, Staempfli HR, Daignault D, Janecko N, Avery BP, et al. Possible seasonality of Clostridium difficile in retail meat, Canada. *Emerg Infect Dis.* 2009; 15(5):802–5.

Rodriguez-Palacios A, Staempfli HR, Duffield T, Weese JS. Clostridium difficile in retail ground meat, Canada. Emerging infectious diseases. 2007 Mar; 13(3):485.

Rodriguez-Palacios A, Staempfli HR, Duffield T, Weese JS. Clostridium difficile in retail ground meat, Canada. Emerg Infect Dis. 2007; 13(3):485–7.

Rodriguez-Palacios A, Staempfli HR, Duffield T, Weese JS. Clostridium difficile in retail ground meat, Canada. *Emerg Infect Dis.* 2007; 13(3):485–7.

Rostami F, Rahimi E, Yahaghi E, Khodaverdi Darian E, Bagheri Moghadam M. Isolation and evaluation virulence factors of Salmonella typhimurium and Salmonella enteritidis in milk and dairy products. Iranian Journal of Medical Microbiology. 2014 Jun 10; 8(1):54-61.

Safarpordehkordi F, Yahaghi E, Khodaverdi Darian E. Prevalence of antibiotic resistance in Escherichia coli isolated from poultry meat supply in Isfahan. Iranian Journal of Medical Microbiology. 2014; 8(2):41-7.

Safarpour Dehkordi F, Hosseini S, Rahimi E, Momeni M, Yahaghi E, Khodaverdi Darian E. Investigate the frequency of virulence genes Vibrio parahaemolyticus isolated from fish, lobsters and crabs caught from Persian Gulf. Iranian Journal of Medical Microbiology. 2014; 8(2):1-7.

Safarpour Dehkourdi F, Momtaz H, Esmailzade S, Khayyat Khameneie M, Yahaghi E. Detection of virulence factors of Uropathoigenic Escherichia coli isolates from infertile women high vaginal swabs. Iranian Journal of Medical Microbiology. 2014; 7(4):1-8.

Shakerian A, Jahangiri A, Rahimi E, Dehkordi FS., Enterotoxins from Clostridium perfringens in meat products, Onl J Vet Res., 20(3):158-166, 2016.

Smits WK, Lyras D, Lacy DB, Wilcox MH, Kuijper EJ. Clostridium difficile infection. Nature reviews Disease primers. 2016 Apr 7; 2(1):1-20.

Songer JG, Trinh HT, Killgore GE, Thompson AD, McDonald LC, Limbago BM. Clostridium difficile in retail meat products, USA, 2007. *Emerg Infect Dis.* 2009; 15(5):819–21.

Suryasa, I. W., Rodríguez-Gámez, M., & Koldoris, T. (2021). The COVID-19 pandemic. *International Journal of Health Sciences*, *5*(2), vi-ix. https://doi.org/10.53730/ijhs.v5n2.2937

Warriner K, Xu C, Habash M, Sultan S, Weese SJ. Dissemination of Clostridium difficile in food and the environment: Significant sources of C. difficile community-acquired infection? Journal of applied microbiology. 2017 Mar; 122(3):542-53.

Weese JS, Avery BP, Rousseau J, Reid-Smith RJ. Detection and enumeration of Clostridium difficile spores in retail beef and pork. *Appl Environ Microbiol.* 2009; 75(15):5009–11.