Meat Science 139 (2018) 120–124

Contents lists available at ScienceDirect

Meat Science

journal homepage: www.elsevier.com/locate/meatsci

The prevalence of *Clostridium difficile* in cattle and sheep carcasses and the antibiotic susceptibility of isolates

Hamparsun Hampikyan[a], Enver Baris Bingol[b,*], Karlo Muratoglu[b], Esra Akkaya[b], Omer Cetin[b], Hilal Colak[b]

[a] Beykent University, The School of Applied Sciences, Department of Gastronomy and Culinary Arts, 34500 Istanbul, Turkey
[b] Istanbul University, Department of Food Hygiene and Technology, Faculty of Veterinary Medicine, 34320 Istanbul, Turkey

ARTICLE INFO

Keywords:
C. difficile
Ribotype
Butchery animal
Antibiotic susceptibility
C. difficile toxin
PCR

ABSTRACT

Clostridium difficile is an anaerobic, spore forming, rod shaped bacterium frequently isolated from butchery animals in recent years. The aim of this study is to evaluate the presence of *C. difficile* (especially ribotype 027 and 078) in cattle and sheep carcasses and to investigate the antibiotic susceptibility of isolates. The bacterium was isolated in 83 out of 247 (33.6%) cattle and 78 out of 308 (25.3%) sheep carcass samples. 15/83 (18.1%) cattle and 6/78 (7.7%) sheep isolates were identified as ribotype 027, whereas the other hypervirulent isolate ribotype 078 could not be detected among the analysed samples. Almost all isolates were susceptible to amoxicillin-clavulanic acid (98.8%), vancomycin (96.9%) and tetracycline (97.5%), whereas resistant to cefotaxim (97.5%) and imipenem (87.0%). In conclusion, the results demonstrate the presence of toxigenic *C. difficile* isolates in cattle and sheep carcasses on the slaughter line. As a result, the results of this study demonstrate the presence of toxigenic *C. difficile* isolates in cattle and sheep carcasses on the slaughter line.

1. Introduction

Clostridium difficile is a Gram positive, anaerobic, spore forming, rod shaped bacterium, isolated by Hall and O'Toole in 1935 for the first time from the healthy new-born infant feces (Pasquale et al., 2012; Troiano et al., 2015). The microorganism can colonize through the intestinal tract of humans and various animals and *C. difficile* is excreted in young animals (Pelaez et al., 2013). This nosocomial pathogen causes infectious diarrhea that usually develops in patients after hospitalization and antibiotic treatments. Beside these, the use of proton inhibitor drugs increases the risk of infection. (De Boer, Zwartkruis-Nahuis, Heuvelink, Hurmanus, & Kuijper, 2011; Rodriguez et al., 2012). The use of antibiotics for a long time in humans and animals is the most frequently predisposing risk factor because the antibiotics can damage the regular micro flora of the bowel and under this circumstances, *C. difficile* can be located and colonized through the intestinal tract and cause gastrointestinal symptoms. The symptoms of *C. difficile* infection (CDI) can show alteration from uncomplicated mild diarrhea to serious, life threatening pseudo-membranous colitis and toxic mega colon (Drudy, Fanning, & Kyne, 2007; Thitaram et al., 2016).

The virulence of *C. difficile* is mainly related to the presence of Toxin A (enterotoxin) and Toxin B (cytotoxin) or both encoded by the *tcdA* and *tcdB* genes from pathogenity locus (PaLoc) on the *C. difficile* chromosome. In addition to these, some strains have binary toxin (ADP-ribosyltransferase) encoded by *cdtA* and *cdtB* genes other than PaLoc. In this context, some human pathogenic ribotypes such as 027 (R027) and 078 (R078) have importance in terms of human CDI. Both R027 and R078 have been named 'hypervirulent' on account of their increased toxin production and enhanced sporulation attribute (especially R027). They have both been linked with more severe outcomes or complications, and R027, a common endemic strain in many regions, cause outbreaks all around the world. Contrary to this, some *C. difficile* strains do not have the ability of toxin producing and as a result, they do not cause any CDI symptoms (Curry, 2010; Jöbstl et al., 2010; Nelly, Lambert, Van Broeck, & Delmee, 2017; Rahimi, Afzali, & Baghbadorani, 2015; Romano et al., 2012; Simango & Mwakurudza, 2008; Weese, Avery, Rousseau, & Reid-Smith, 2009).

The bacterium has also been detected in the environment other than hospitals such as soil, water, animals (butchery animals, poultry, sea foods etc.) and a diverse range of foods such as vegetables, various meat products. In recent years, the studies conducted by several researchers demonstrated that, *C. difficile* has been isolated from food animals such as cattle, sheep and pig. Especially, some human pathogenic ribotypes (R027 and R078) of this bacterium have been isolated from butchery animals and animal originated retail food. This situation draws attention to animals which are potential contamination source of *C. difficile*

* Corresponding author.
E-mail address: bingolb@istanbul.edu.tr (E.B. Bingol).

https://doi.org/10.1016/j.meatsci.2018.01.020
Received 15 June 2017; Received in revised form 19 January 2018; Accepted 23 January 2018
Available online 03 February 2018
0309-1740/

Table 1
Primer sequence list used in this study.

Gene	Primers	Amplicon size	Reference
tpi	F: 5′-AAAGAAGCTACTAAGGGTACAAA-3′ R: 5′-CATAATATTGGGTCTATTCCTAC-3′	230 bp	Lemee et al., 2004
*tcd*A	F: 5′-AGATTCCTATATTTACATGACAATAT-3′ R: 5′-GTATCAGGCATAAAGTAATATACTTT-3	369 bp	Lemee et al., 2004
*tcd*B	F: 5′-GGAAAAGAGAATGGTTTTATTAA-3′ R: 5′-ATCTTTAGTTATAACTTTGACATCTTT-3′	160 bp	Lemee et al., 2004
*cdt*A	F: 5′-TGAACCTGGAAAAGGTGATG-3′ R: 5′-AGGATTATTTACTGGACCATTTG-3′	353 bp	Stubbs et al., 2000
*cdt*B	F: 5′-CTTATTGCAAGTAAATACTGAGAGTACTATATC-3′ R: 5′-ACCGGATCTCTTGCTTCAGTC-3′	490 bp	Stubbs et al., 2000

for human and therefore, animal originated food varieties can be one of the possible transmission routes from animals to humans (Deng, Plaza-Garrido, Torres, & Paredes-Sabja, 2015; Metcalf et al., 2011; Rodriguez et al., 2013; Weese, Reid-Smith, Avery, & Rousseau, 2010).

The aim of this comprehensive study is to investigate the presence of *C. difficile* in cattle and sheep carcasses at the slaughterhouses, to identify human pathogenic ribotypes R027 and R078, to determine the distribution of toxinogenic genes of isolated *C. difficile* strains by PCR, to detect the toxin producing ability of the organism by ELISA and additionally, to evaluate the antibiotic susceptibility of *C. difficile* strains against ampicillin, cefotaxime, clindamycin, amoxicillin-clavulanic acid, imipenem, metronidazole, tetracycline and vancomycin.

2. Material and method

2.1. Samples

A total of 247 cattle (12–24 months) and 308 sheep (8–12 months) carcass swabs were obtained from 24 slaughterhouses with capacities of 0.5–5 t from 9 different cities located in Marmara Region were visited within 1 year (two slaughterhouses each month).

The samples were taken from final carcasses which are destined both for meat production and retail outlets (butchers, markets etc.) and immediately taken to the Laboratories of Istanbul University Faculty of Veterinary Medicine Department of Food Hygiene and Technology, under cold conditions for further analysis.

2.2. Sampling technique

The sampling method was carried out using swabbing technique. For this purpose, sterile cotton swabs were moistened in sterile 0.1% peptone and 0.85% NaCl for 5 s and then swabbed (Cultiplast, Italy) in horizontal and vertical directions using a template and the same swabbing procedure was repeated on the same sampling surface with dry swabs. Thus, a total of 8 swabs taken from 4 different sampling areas of a carcass were incubated in the same enrichment broth (Cycloserin Cefoxitin Fructose Broth (CCFB)) together (EU Commission Decision, 2001).

10 × 10 cm aluminium template was used to swab 100 cm^2 areas on cattle (neck, brisket, flank and rump) and on sheep carcasses (flank, thorax lateral, brisket, breast), yielding a total of 400 cm^2 for each carcass samples. For template sterilization, first it was dipped into 70% ethanol and then inflamed before each sampling process.

2.3. Isolation of C. difficile from samples

The samples were enriched in 50 mL Cycloserin Cefoxitin Fructose Broth (CCFB) with adding 2 vial/L CDMN selective supplement (Oxoid SR0173, Basingstoke, Hampshire, UK) and incubated anaerobically by using Anaerogen Kit (Oxoid, AN0035 Anaerogen Atmosphere Generating Systems, Basingstoke, Hampshire, UK.), Anaerobic Jar (Oxoid, HP0011A, Basingstoke, Hampshire, UK.) and Anaerobic indicator (Oxoid, BR 0055B, Basingstoke, Hampshire, UK.) at 37 °C for 10 days (Rodriguez et al., 2013). After alcohol shocking step, the sediment was spread on *C. difficile* selective agar (Oxoid CM0601 + CDMN supplement SR 0173 + 5% defibrinated horse blood, Basingstoke, Hampshire, UK) and incubated anaerobically at 37 °C for 48–72 h. Up to 2 suspected colonies (with greyish, ground glass appearance and characteristic horse manure odour) confirmed with Gram staining and latex agglutination kit (*C. difficile* test kit Oxoid DR1107A, Basingstoke, Hampshire, UK).

2.4. Genomic DNA preparation

In order to prepare DNA sample for PCR amplification, a loopful of colony was taken from blood agar and suspended in 1 mL 0.85% sterile saline solution, then boiled for 10 min and the genomic DNA was kept at − 20 °C for further PCR analysis.

2.5. Molecular confirmation of isolates and detection of toxin producing genes

Molecular confirmation of *C. difficile* isolates was performed by PCR, with the detection of triose phosphate isomerase (*tpi*) gene which is specific for *C. difficile*. For this purpose, with minor modifications (instead of multiplex, monoplex was used) the primers and protocols were used described by Lemee et al. (2004) (Table 1) with monoplex PCR on CG Palm-Cycler (CG 1-96 Genetix Biotech, Australia & Asia).

The same method described by Lemee et al. (2004) was also used in detection of toxin producing genes *tcdA* and *tcdB*.

Binary toxin producing genes, *cdtA* and *cdtB* were amplified according to the primers and protocols described by Stubbs et al. (2000) with multiplex PCR (Table 1). In this study, ATCC 9689 *C. difficile* strain was used as positive control for *tpi, tcdA* and *tcdB* genes, BAA 1870 strain for *tpi*, *cdtA and cdtB* genes and mili q water as negative control.

All PCR products were resolved by electrophoresis on a 1.5% agarose gel containing ethidium bromide and visualization of the gels were performed with a UV transilluminator and the gels were captured with the Dolphin-DOC system and Dolphin 1D Gel analysing software (Wealtec, Nevada, USA).

2.6. PCR – ribotyping

Amplification of 16S-23S intergenic spacer regions was performed using the primers (5P-CTGGGGTGAAGTCGTAACAAGG-3P and 5P-GGTACCTTAGATGTTTCAGTTC- 3P) described by Bidet, Barbut, Lalande, Burghoffer, and Petit (1999) and capillary electrophoresis was performed using an ABI 310 Genetic Analyser (Applied Biosystems), a 36 cm array length, default fragment analysis, POP4 polymer and LIZ1200 (Applied Biosystems) as a size standard. The ribotypes were determined using the freely available WEBRIBO database (https://webribo.ages.at/) after Gene Mapper® v4.9 (Applied Biosystems)