Detection, Characterization and Antibiotic Susceptibility of Clostridioides (Clostridium) difficile in Meat Products

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Running Title: The Presence of *Clostridium difficile* in Meat Products

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Abstract

Clostridioides (Clostridium) difficile is a Gram (+), anaerobic, spore forming, rod shaped bacterium that can produce toxin. The objective of this study is to reveal the presence of *C. difficile* in meat products, to analyze the ribotype diversity by PCR and to evaluate the antibiotic susceptibility of isolated strains. The organism was isolated in 22 out of 319 (6.9%) examined meat product samples and 9 out of 22 (40.9%) isolates were identified as RT027 and all isolates had the ability of toxin production. In terms of antibiotic susceptibility, all isolates were susceptive to amoxicillin-clavulanic acid, tetracycline and vancomycin and 21 (95.4%) isolates to metronidazole. On the other hand, imipenem and cefotaxim resistance was observed in all. In conclusion, the results of this comprehensive study conducted in Turkey deduced the presence of *C. difficile* in different meat products. Therefore, these products can be evaluated as a potential contamination source of *C. difficile* from animals to humans especially for elders, youngsters, long terms wide spectrum antibiotic used and immuno-suppressed individuals.

Keywords: *C. difficile*, meat products, ribotype, antibiotic susceptibility, *C. difficile* toxin.

1. Introduction

Clostridioides (Clostridium) difficile is a Gram (+), anaerobic, spore forming, rod shaped and cytotoxin producing bacterium, which has an optimal growth temperature at 35 – 40℃. The organism can colonize throughout the intestinal tract of humans and various animal species (Pasquale et al., 2012; Pelaez et al., 2013; Troiano et al., 2015). The possibility of *C.difficile* presence in intestinal of healthy individuals and newborns are 2-3% and 40%, respectively (Libby and Bearman, 2009). The most frequent predisposing risk factor for *C. difficile* infection (CDI) in humans and animals is the destruction of regular intestinal microflora due to long-term antibiotic usage. CDI causes gastrointestinal symptoms such as diarrhea, pseudo-membranous colitis, toxic mega colon and even deaths can be seen in some serious cases (De Boer et al., 2011; Drudy et al., 2007; Rodriguez et al., 2012; Thitaram et al. 2016).

Some *C. difficile* strains produce Toxin A (enterotoxin) and Toxin B (cytotoxin) or both which were released from *tcdA* and *tcdB* genes, and some others can have *cdtA* and *cdtB* genes which produce binary toxin. The virulence of this bacterium is mainly related to the presence of these toxins. In terms of increased toxin production and enhanced sporulation attribute, some *C. difficile* hypervirulent ribotypes such as 027 (RT027) and 078 (RT078) are at the forefront and known as the main cause of human CDI that causes acute and recurring outbreaks with significant mortality in some critical cases (Jöbstl et al., 2010; Rahimi et al., 2015; Romano et al., 2012; Simango and Mwakurudza, 2008).

Generally, CDI is accepted as a nosocomial infection, however, the epidemiology of *C.difficile* has been changing according to researches reporting an increase in community-associated CDI that is not related with traditional risk factors (long-term antibiotic usage, age, hospitalization etc.) (Candel-Pérez et al., 2019). In this regard, *C. difficile* was isolated from different matrices such as soil, fresh and wastewater, butchery animals and meat products, poultry, sea food, vegetable and ready to eat food varieties by a number of researchers. All these data highlight

the importance of *C. difficile* transmission routes other than the hospital environment. Recently, the studies about the presence of *C. difficile* and its human pathogenic ribotypes in animal originated foods draw attention to butchery animals and therefore meat product varieties can be one of the possible transmission pathways for humans (Deng et al., 2015; Hampikyan et al., 2018; Metcalf et al., 2010; Metcalf et al., 2011; Rodriguez et al., 2013; Weese et al., 2010). The objective of this study is to reveal the presence of *C. difficile* in meat products, to analyze the ribotype diversity of isolates including RT027 and RT078 by PCR to designate the toxin production ability by ELISA and to determine the antibiotic susceptibility of the isolates against some antibiotics that are mostly used for the treatment of *C. difficile* infection.

2. Materials and Methods

2.1 Meat Product Samples

319 meat products (71 salami, 50 sausage, 52 sucuk, 50 pastrami, 36 uncooked meatball, 30 smoked meat and 30 cooked döner) were obtained from butcheries and supermarkets located in Istanbul, Turkey. 20 sucuk and 16 uncooked meatball samples were collected from 20 different butcheries and 71 salami, 50 sausage, 32 sucuk, 50 pastrami, 20 uncooked meatball and 30 cooked döner samples were obtained from 35 different supermarkets. An average of 15 samples were collected from one butchery and two supermarkets per month between February 2017 - November 2018 and were immediately taken to the Laboratories of Istanbul University-Cerrahpasa, Faculty of Veterinary Medicine Department of Food Hygiene and Technology in an insulated icebox and the analyses were started within the same day (less than 24 h).

2.2 *C. difficile* Isolation

The 25 g of each sample was mixed with 225 mL of C. difficile enrichment broth prepared according to Hampikyan et al., (2018). The mixture was incubated at 37 C° for 10 days under anaerobic conditions by using Anaerogen Kit (Oxoid, SR0173, UK), Anaerobic Jar (Oxoid, HP0011A, Basingstoke, Hampshire, UK.) and Anaerobic indicator (Oxoid, BR 0055B,

Basingstoke, Hampshire, UK.). After alcohol shocking, the sediment was spread on C. difficile selective agar (Oxoid CM0601+CDMN supplement SR 0173+5% defibrinated horse blood, UK) and then petri dishes were incubated for 48-72 hours at 37 ℃ under anaerobic conditions. Colonies with greyish ground glass appearance with horse manure odor were evaluated as suspected colonies and further analyses were carried out such as gram staining and latex agglutination test according to manufacturer's manual. (C. difficile test kit Oxoid DR1107A, UK). Before PCR analyses, the colonies were purified in tryptic soy agar (Oxoid CM0131, UK) including 5.0% defibrinated horse blood and incubated anaerobically at 37 ℃ for 48-72 hours. Before PCR analyses, the colonies were purified in tryptic soy agar (Oxoid CM0131, Basingstoke, Hampshire, UK) including 5.0% defibrinated horse blood and incubated under anaerobic conditions for 48-72 hours at 37 ℃.

2.3 DNA Preparation

For amplification process, a loopful of colony, which was cultivated in blood agar was diluted in 1 mL sterile saline solution (0.85%) and boiled for 10 minutes. Then extracted DNA was stored at - 20 ℃.

2.4 Confirmation of Isolates and Determination of Toxigenic Genes

C. difficile specific Triose phosphate isomerase (tpi) gene and toxin producing genes *tcdA* and *tcdB* were searched by PCR. For this purpose, the primers and protocols were used according to Lemee et al. (2004) with minor modification with simplex PCR on CG Palm-Cycler (CG 1-96 Genetix Biotech, Australia & Asia). Binary toxin genes (*cdtA* and *cdtB)* were determined by means of multiplex PCR explained by Stubbs et al. (2000). For electrophoresis process ethidium bromide, which contains 1.5% agarose gel, and for gel screening UV transilluminator were used and imaged with the Dolphin-DOC analysing system (Wealtec, Nevada, USA).

ATCC 9689 and BAA 1870 strains were used as positive control for *tpi, tcdA, tcdB and tpi, cdtA and cdtB* genes respectively.

2.5 PCR – Ribotyping

The 16S-23S intergenic spacer regions of *C. difficile* isolates were amplified according to Bidet, Barbut, Lalande, Burghoffer, and Petit, (1999) and ABI 310 was used for capillary electrophoresis. Genetic Analyser, a 36 cm array length, default fragment analysis, POP4 polymer and LIZ1200 as a size standard (Applied Biosystems). WEBRIBO database was used for ribotype determination after Gene Mapper® v4.9 (Applied Biosystems) software processing (Indra et al. 2008).

2.6 Toxin Detection Test

ELISA test kit (Ridascreen Art No: C0801, R-Biopharm AG, Germany) was used for the detection of toxin production. A loopfull of colonies cultured on blood agar and confirmed as *C. difficile* was diluted in 1 mL sample dilution buffer and centrifuged at 2500 x g for 5 minutes. After centrifuging step, supernatant was used for the detection of toxin presence according to the supplier's manual.

2.7 Antibiotic Susceptibility Test

The antibiotic susceptibility of *C. difficile* isolates was examined by Minimum Inhibitor Concentration (MIC) Evaluator strips (Oxoid, UK) according to the supplier's manual. According to this, the colonies were passaged to tryptic soy agar (Oxoid CM0131, UK) with 5% defibrinated horse blood and incubated for 12 hours under anaerobic conditions. The colonies confirmed by PCR were spread on Brucella Agar (Oxoid CM0169) containing 5 µg/mL Hemin, 1 µg/mL Vitamin K1 and sheep blood (5.0%) and two MIC Evaluator strips were placed on agar. The breakpoint values of tested antibiotics were gained from Clinical and Laboratory Standards Institute (CLSI, 2018) and from The European Committee on Antimicrobial Susceptibility Testing (EUCAST, 2019).

3. Results and Discussion

The present study investigated the presence of *C. difficile* in various meat products in Turkey. A total of 319 different meat products were analyzed for the presence of tpi gene which is specific for *C. difficile* by PCR and the organism isolated in 17 (23.9%) salami, 3 (5.8%) sucuk, 1 (2.8%) uncooked meatball and 1 (2.0%) sausage samples. On the other hand, the organism could not be detected in pastrami, smoked meat and cooked döner samples (Table 1).

Also, a number of studies from many countries were conducted for the determination of *C. difficile* from various meat products. In a research, Esfandiari, Weese, Ezzatpanah, Jalali, and Chamani (2014) detected *C. difficile* in 4 out of 56 (7.1%) beef hamburger samples. In another study conducted in Texas USA by Harvey et al. (2011), the organism was isolated from pork chorizo in a rate of 9.5% (23/243). In 2007, Songer et al. (2009) declared that 17 out of 46 (37.0%) different meat products obtained from grocery stores in Arizona, USA were contaminated with *C. difficile*. In a study performed by Rodriguez et al. (2014) in Belgium, *C. difficile* was found in 5 out of 107 (4.7 %) pork sausage and 3 out of 133 (2.3%) beef burger samples. Our results were found to be similar to those of Esfandiari et al. (2014), whereas lower than Harvey et al. (2011) and Songer et al. (2009), but higher than Rodriguez et al. (2014). In our country, in a similar study conducted on a limited number of beef meat products by Ersöz and Coşansu (2018), *C. difficile* was detected in one of each 18 uncooked meatball and 12 cooked meat doner samples (in a rate of 5.5% and 8.3%, respectively) whereas, the bacterium could not be isolated in four salami, one frankfurter and one bacon samples. Contrary to this, in France Bouttier et al. (2010) reported that they could not detect any *C. difficile* strain from 59 pork sausage samples. Similar result was found by Pires et al. (2018) who could not determine the bacterium from 80 meat products (beef, pork, hamburger).

The presence of *C. difficile* in various animal carcasses has been reported by a number of researchers due to some important factors such as, unhygienic slaughterhouse conditions,

193 removing the animal remains and extraneous matter improperly, contamination of carcasses
194 with faeces, improper chilling processes, unhygienic storage conditions, lack of personnel and
195 equipment hygiene (Hampikyan et al. 2018; Rodriguez et al. 2013; Susick et al., 2012; Harvey
196 et al. 2011; Songer et al. 2009). In the light of these data, it can be understood that the meat
197 used in manufacturing of meat products may be contaminated with *C. difficile* during the
198 slaughtering and post-slaughtering processes. In addition to this, lack of microbiological quality
199 of ingredients and personnel-equipment hygiene along the meat products production line,
200 unhygienic production processes, insufficient heat and time treatments for those heat processed
201 meat products have an important role in *C. difficile* contamination for these foods.
202 According to our results, high prevalence of *C.difficile* in salami samples are quite remarkable.
203 This situation can be explained by as follows; because salami is thicker, voluminous and more
204 sizable than the other examined samples, it constitutes better suitable and anaerobic conditions
205 for the bacteria. The heat treatments used in salami production can be applied in a shorter time
206 and lower temperature than required accidentally or intentionally (due to economic reasons),and
207 as a result, the inhibition effect of temperature on bacteria remains insufficient. Moreover,
208 having higher water content and pH levels compared to other analyzed samples are some other
209 factors that can help the bacteria survive in salami.
210 According to PCR ribotyping, 9/22 (40.9%) strains were characterized as RT027, while RT078
211 could not be isolated in any examined meat product samples. However, four out of 22 isolates
212 were identified as most likely (ML) RT027, two of them ML-R241 and one ML-R686 whereas,
213 seven of them were defined as new ribotype according to WEBRIBO database (Table 2). Lately,
214 the isolated *C. difficile* strains from various meat and meat products show similarities with some
215 certain strains such as RT027 and RT078 responsible for CDI outbreaks in humans. In this
216 context, Curry et al. (2012) examined 102 pork sausage and found RT078 in 2 (1.96%) samples.
217 In another study, Rodriguez et al. (2014) detected *C. difficile* in 3 out of 133 (2.3%) burger beef

218 samples and one isolate was RT078. In a similar study, Songer et al. (2009) reported that *C.*
219 *difficile* was found in 1 out of 7 (RT027) summer sausage, 10 out of 16 (two isolates RT027
220 and seven RT078) braunschweiger, 3 out of 10 (one isolate RT027 and two RT078) chorizo
221 and 3 out of 13 (one isolate RT027 and two RT078) pork sausage samples. In contrary to this,
222 in our study RT078 could not be detected in any analyzed samples, however our results for
223 RT027 were correlated well with above-mentioned findings.
224 In various studies, *C. difficile* and its hypervirulent ribotypes were found in some meat products
225 with the different rates of prevalence. The reason of this difference can be explained by the
226 efficiency of good hygiene practices in establishments, different heat-time treatment in
227 production process, animal characteristics (age, breed etc.), geographical and seasonal
228 differences, sampling amount and the isolation methods.
229 In terms of antibiotic susceptibility, MIC values of *C. difficile* strains isolated from meat
230 products were shown in Table 3. All isolates were susceptive to amoxicillin-clavulanic acid,
231 tetracycline and vancomycin and 21 (95.4%) to metronidazole. On the other hand, imipenem
232 and cefotaxim resistance was observed in all detected isolates (Table 4). Concerns about the
233 use of antibiotics for to promote growth, to treat sick animals and to prevent diseases in animal
234 husbandry have gradually increased in recent years. Some certain antibiotics such as
235 vancomycin, amoxicillin-clavulanic acid, metronidazole are used to treat various infections in
236 butchery animals and CDI/CDI related diarrhea in humans. Some important factors such as host
237 susceptibility, patient age and the unconscious antibiotic usage in food animals has deduced the
238 significance of *C. difficile*, which is responsible for 15–30% of cases of antibiotic associated
239 diarrhea around the world (Thitaram et al., 2016, Hampikyan et al., 2018).
240 Within this scope, the researches demonstrate that most of the isolated *C. difficile* strains from
241 various foods are resistant to imipenem and cefotaxim whereas, susceptible to amoxicillin,
242 ampicillin, tetracycline, metronidazole and vancomycin (Troiano et al., 2015; Thitaram et al.,

2016; Jöbstl et al., 2010; Rahimi et al., 2015; Hampikyan et al., 2018; Simango and Mwakurudza 2008; Varshney et al. 2014). As it is shown in Table 4, our results are similar to above-mentioned findings. The results of our study demonstrate that all isolates recovered from different meat products were susceptible to amoxicillin, tetracycline, vancomycin, ampicilline and clindamycin in a rate of 100.0%, except metronidazole (94.1%). On the other hand, all isolates have shown resistance to cefotaxim and imipenem. Interestingly, Ersöz and Cosansu (2018) reported that two isolates recovered from uncooked meatball and cooked meat döner showed resistance to tetracycline-vancomycin and metronidazole-vancomycin, respectively. These different results situated in the various literatures can be explained by the genetic characteristic of isolated *C. difficile* strains or the exposure of food animals to antibiotics during farm rearing.

The toxin genes (*tcdA, tcdB* and *cdtA/B*) of *C. difficile* strains were determined by PCR. *tcdA, tcdB* and *cdtA/B* genes were detected in 22 (100%), 22 (100%) and 19 (86.4%) out of 22 different meat products, respectively. The evaluation of the toxin genes of isolates and the number of ribotypes detected from various meat product samples were shown in Table 2. Three (100%) sucuk, 1 (100%) sausage, 1 (100%) meatball and 14 salami sample isolates have all three toxin genes whereas, 3 salami samples did not enclose any *cdtA/B* genes. ELISA was used for the detection of *C. difficile* Toxin A and B. As it can be seen from Table 2 all detected isolates had the toxin producing ability. Toxin production by *tcdA, tcdB, cdtA* and *cdtB* genes is one of the main virulence factor of *C. difficile*. In our study, all detected isolates from different meat product samples were toxigenic (Table 2). Likely, in a research performed by Songer et al. (2009), it was reported that all isolated (37 out of 88) *C. difficile* strains from various meat products (summer sausage, braunschweiger, chorizo and pork sausage) were toxigenic. In similar studies about the presence of *C.difficile* in hamburgers, two and three isolates were

detected by Von Abercron et al. (2009) and Rodriguez et al. (2014), respectively and all isolates were found to be toxigenic. These findings show parallelism to our results.

4. Conclusion

In conclusion, the results of this comprehensive study conducted in Turkey reveals the presence of *C. difficile* in different meat products. The main cause of this presence can be explained by the contamination of carcasses during slaughterhouse, transport, cold storage processes, also contamination of the products during meat production processes in facilities or in retail markets during selling and presenting. Although, there is no certain proof indicating that *C. difficile* is a food-borne pathogen, it should be considered that the presence of this bacterium in meat and meat products may be a potential risk for consumers. Therefore, these products can be evaluated as a potential contamination source of *C. difficile* from animals to humans especially for elders, youngsters, long terms wide spectrum antibiotic used and immuno-suppressed individuals.

Conflicts of interest

The authors declare that they have no potential conflict of interest.

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Author Contributions

Conceptualization: Hampikyan H. Data curation: Bingol EB. Formal analysis: Muratoglu K, Akkaya E. Methodology: Muratoglu K, Akkaya E. Software: Bingol EB, Muratoglu K. Validation: Colak H, Hampikyan H, Cetin O. Investigation: Akkaya E, Bingol EB, Cetin O. Writing - original draft: Colak H, Hampikyan H, Akkaya E. Writing - review & editing: Colak H, Hampikyan H, Akkaya E, Muratoglu K.

Ethics Approval

This article does not require IRB/IACUC approval because there are no human and animal participants.

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TABLES

Table 1. Number of *C. difficile* and RT027 Positive Samples

Samples	N	n* (%)	RT027 (%)
Salami	71	17 (23.9)	6 (35.9)
Sausage	50	1 (2.0)	1 (100.0)
Sucuk	52	3 (5.8)	1 (33.3)
Pastrami	50	ND	-
Uncooked Meatball	36	1 (2.8)	1 (100.0)
Smoked Meat	30	ND	-
Cooked Döner	30	ND	-
TOTAL	319	22 (6.9)	9 (40.9)

n*: Number of positive samples
ND: Not Detected

Table 2. The distribution of the virulence genes and the toxin producing ability of *C. difficile* isolates

Samples	N	*n (%)	tcdA+ (%)	tcdB+ (%)	cdtA/B+ (%)	Ribotypes	Toxin (+) (%)
Salami	71	17 (23.9)	17 (100)	17 (100)	14 (82.4)	027(6), *ML-027(3), *ML-241(2), ML*-686(1), *NR (5)	17 (100)
Sucuk	52	3 (5.8)	3 (100)	3 (100)	3 (100)	027(1), *NR(2)	3 (100)
Sausage	50	1 (2.0)	1 (100)	1 (100)	1 (100)	027(1)	1 (100)
Uncooked Meatball	36	1 (2.8)	1 (100)	1 (100)	1 (100)	027(1)	1 (100)
TOTAL	209	22 (10.5)	22 (100)	22 (100)	19 (86.4)		22 (100)

***n:** Number of positive sample *__ML:__ Most Likely *__NR:__ New Ribotype

438 **Table 3.** Minimum Inhibitor Concentration (MIC) values of *C. difficile* strains isolated from meat
439 products

Antibiotic	AMP	AMC	DA	IPM	MTZ	TE	VA	CTX
Concentration (µg/mL)	256-0.015	256-0.015	256-0.015	32-0.002	256-0.015	256-0.015	256-0.015	256-0.015
MIC Breakpoints (µg/mL)	≤0.5-1-≥2	≤4/2-8/4-16/8	≤2-4-≥8	≤4-8-≥16	≤8-16-≥32	≤4-8-≥16	≤2->2	≤16-32-≥64
References / Samples	CLSI 2018	CLSI 2018	CLSI 2018	CLSI 2018	CLSI 2018	CLSI 2018	EUCAST 2019	CLSI 2018
UM 5	0.5 (S)	0.5 (S)	4 (I)	≥ 16 (R)	0.25 (S)	0.015 (S)	1 (S)	≥ 64 (R)
SA 20	0.5 (S)	0.5 (S)	2 (S)	≥ 16 (R)	0.06 (S)	0.015 (S)	1 (S)	≥ 64 (R)
SA 21	1 (I)	0.12 (S)	4 (I)	≥ 16 (R)	≥32 (R)	0.06 (S)	1 (S)	≥ 64 (R)
SA 22	0.5 (S)	0.5 (S)	1 (S)	≥ 16 (R)	0.12 (S)	0.015 (S)	2 (S)	≥ 64 (R)
SA 25	0.03 (S)	0.03 (S)	0.25 (S)	≥ 16 (R)	0.015 (S)	0.015 (S)	1 (S)	≥ 64 (R)
SA 26	1 (I)	0.5 (S)	4 (I)	≥ 16 (R)	0.06 (S)	0.015 (S)	2 (S)	≥ 64 (R)
SA 27	1 (I)	0.5 (S)	2 (S)	≥ 16 (R)	0.25 (S)	0.015 (S)	1 (S)	≥ 64 (R)
SA 29	1 (I)	0.5 (S)	1 (S)	≥ 16 (R)	0.12 (S)	0.015 (S)	1 (S)	≥ 64 (R)
SA 31	2 (R)	1 (S)	4 (I)	≥ 16 (R)	0.25 (S)	0.03 (S)	1 (S)	≥ 64 (R)
SA 32	0.5 (S)	0.25 (S)	1 (S)	≥ 16 (R)	0.12 (S)	0.015 (S)	1 (S)	≥ 64 (R)
SA 33	0.5 (S)	0.06 (S)	2 (S)	≥ 16 (R)	0.12 (S)	0.015 (S)	1 (S)	≥ 64 (R)
SA 38	0.5 (S)	0.5 (S)	2 (S)	≥ 16 (R)	0.12 (S)	0.03 (S)	1 (S)	≥ 64 (R)
SA 39	0.25 (S)	0.06 (S)	2 (S)	≥ 16 (R)	0.03 (S)	0.06 (S)	0.5 (S)	≥ 64 (R)
SA 40	0.12 (S)	0.12 (S)	2 (S)	≥ 16 (R)	0.06 (S)	0.03 (S)	0.5 (S)	≥ 64 (R)
SA 41	1 (I)	0.25 (S)	2 (S)	≥ 16 (R)	0.25 (S)	0.03 (S)	1 (S)	≥ 64 (R)
SA 42	2 (R)	0.25 (S)	4 (I)	≥ 16 (R)	0.12 (S)	0.015 (S)	1 (S)	≥ 64 (R)
SA 43	1 (I)	0.25 (S)	4 (I)	≥ 16 (R)	0.03 (S)	0.03 (S)	1 (S)	≥ 64 (R)
SA 47	1 (I)	0.5 (S)	1 (S)	≥ 16 (R)	0.12 (S)	0.015 (S)	1 (S)	≥ 64 (R)
SAU 25	1 (I)	0.25 (S)	2 (S)	≥ 16 (R)	0.25 (S)	0.015 (S)	0.5 (S)	≥ 64 (R)
SU 18	1 (I)	0.5 (S)	0.5 (S)	≥ 16 (R)	0.03 (S)	0.015 (S)	1 (S)	≥ 64 (R)
SU 22	1 (I)	1 (S)	2 (S)	≥ 16 (R)	0.03 (S)	0.03 (S)	1 (S)	≥ 64 (R)
SU 23	2 (R)	0.12 (S)	1 (S)	≥ 16 (R)	0.06 (S)	0.015 (S)	1 (S)	≥ 64 (R)

440 UM: Uncooked Meatball, SA: Salami, SAU: Sausage, SU: Sucuk, (S): Sensitive, (I): Intermediate, (R): Resistance
441 AMP: Ampicillin, AMC: Amoxycillin/clavulanic acid, DA: Clindamycin, IPM: Imipenem, TE: Tetracycline,
442 VA: Vancomycin, CTX: Cefotaxime
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454 **Table 4.** Susceptibility profiles of 22 *C. difficile* isolates from meat products.

Samples	n	Susceptibility	AMP (%)	AMC (%)	DA (%)	IMP (%)	MTZ (%)	TE (%)	VA (%)	CTX (%)
Salami	17	Susceptible	8 (47)	17 (100)	12 (70.6)	0 (0)	16 (94.1)	17 (100)	17 (100)	0 (0)
		Intermediate	7 (41.2)	0 (0)	5 (29.4)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
		Resistant	2 (11.8)	0 (0)	0 (0)	17 (100)	1 (5.9)	0 (0)	0 (0)	17 (100)
Sucuk	3	Susceptible	0 (0)	3 (100)	3 (100)	0 (0)	3 (100)	3 (100)	3 (100)	0 (0)
		Intermediate	2 (66.7)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
		Resistant	1 (33.3)	0 (0)	0 (0)	3 (100)	0 (0)	0 (0)	0 (0)	3 (100)
Sausage	1	Susceptible	0 (0)	1 (100)	1 (100)	0 (0)	1 (100)	1 (100)	1 (100)	0 (0)
		Intermediate	1 (100)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
		Resistant	0 (0)	0 (0)	0 (0)	1 (100)	0 (0)	0 (0)	0 (0)	1 (100)
Uncooked Meatball	1	Susceptible	1 (100)	1 (100)	0 (0)	0 (0)	1 (100)	1 (100)	1 (100)	0 (0)
		Intermediate	0 (0)	0 (0)	1 (100)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
		Resistant	0 (0)	0 (0)	0 (0)	1 (100)	0 (0)	0 (0)	0 (0)	1 (100)
TOTAL	22	Susceptible	9 (40.9)	22 (100)	16 (72.7)	0 (0)	21 (95.5)	22 (100)	22 (100)	0 (0)
		Intermediate	10 (45.5)	0 (0)	6 (27.3)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
		Resistant	3 (13.6)	0 (0)	0 (0)	22 (100)	1 (4.5)	0 (0)	0 (0)	22 (100)

455 AMP: Ampicillin; CTX: Cefotaxim; DA: Clindamycin; AMC: Amoxicillin-clavulanic Acid;
456 IMP: Imipenem; MTZ: Metronidazole; TE: Tetracycline; VA: Vancomycin; n: Sample Number
457

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459 TABLES

460 **Table 1.** Number of *C. difficile* and RT027 Positive Samples

Samples	N	n* (%)	RT027 (%)
Salami	71	17 (23.9)	6 (35.9)
Sausage	50	1 (2.0)	1 (100.0)
Sucuk	52	3 (5.8)	1 (33.3)
Pastrami	50	ND	-
Uncooked Meatball	36	1 (2.8)	1 (100.0)
Smoked Meat	30	ND	-
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TOTAL	319	22 (6.9)	9 (40.9)

461 **n*:** Number of positive samples
462 **ND**: Not Detected
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471 **Table 2.** The distribution of the virulence genes and the toxin producing ability of *C. difficile*
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Sucuk	52	3 (5.8)	3 (100)	3 (100)	3 (100)	027(1), *NR(2)	3 (100)
Sausage	50	1 (2.0)	1 (100)	1 (100)	1 (100)	027(1)	1 (100)
Uncooked Meatball	36	1 (2.8)	1 (100)	1 (100)	1 (100)	027(1)	1 (100)
TOTAL	209	22 (10.5)	22 (100)	22 (100)	19 (86.4)		22 (100)

473 ***n:** Number of positive sample *ML: Most Likely *NR: New Ribotype
474

Table 3. Minimum Inhibitor Concentration (MIC) values of *C. difficile* strains isolated from meat products

Antibiotic	AMP	AMC	DA	IPM	MTZ	TE	VA	CTX
Concentration (µg/mL)	256-0.015	256-0.015	256-0.015	32-0.002	256-0.015	256-0.015	256-0.015	256-0.015
MIC Breakpoints (µg/mL)	≤0.5-1-≥2	≤4/2-8/4-16/8	≤2-4-≥8	≤4-8-≥16	≤8-16-≥32	≤4-8-≥16	≤2->2	≤16-32-≥64
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SA 20	0.5 (S)	0.5 (S)	2 (S)	≥ 16 (R)	0.06 (S)	0.015 (S)	1 (S)	≥ 64 (R)
SA 21	1 (I)	0.12 (S)	4 (I)	≥ 16 (R)	≥32 (R)	0.06 (S)	1 (S)	≥ 64 (R)
SA 22	0.5 (S)	0.5 (S)	1 (S)	≥ 16 (R)	0.12 (S)	0.015 (S)	2 (S)	≥ 64 (R)
SA 25	0.03 (S)	0.03 (S)	0.25 (S)	≥ 16 (R)	0.015 (S)	0.015 (S)	1 (S)	≥ 64 (R)
SA 26	1 (I)	0.5 (S)	4 (I)	≥ 16 (R)	0.06 (S)	0.015 (S)	2 (S)	≥ 64 (R)
SA 27	1 (I)	0.5 (S)	2 (S)	≥ 16 (R)	0.25 (S)	0.015 (S)	1 (S)	≥ 64 (R)
SA 29	1 (I)	0.5 (S)	1 (S)	≥ 16 (R)	0.12 (S)	0.015 (S)	1 (S)	≥ 64 (R)
SA 31	2 (R)	1 (S)	4 (I)	≥ 16 (R)	0.25 (S)	0.03 (S)	1 (S)	≥ 64 (R)
SA 32	0.5 (S)	0.25 (S)	1 (S)	≥ 16 (R)	0.12 (S)	0.015 (S)	1 (S)	≥ 64 (R)
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SA 41	1 (I)	0.25 (S)	2 (S)	≥ 16 (R)	0.25 (S)	0.03 (S)	1 (S)	≥ 64 (R)
SA 42	2 (R)	0.25 (S)	4 (I)	≥ 16 (R)	0.12 (S)	0.015 (S)	1 (S)	≥ 64 (R)
SA 43	1 (I)	0.25 (S)	4 (I)	≥ 16 (R)	0.03 (S)	0.03 (S)	1 (S)	≥ 64 (R)
SA 47	1 (I)	0.5 (S)	1 (S)	≥ 16 (R)	0.12 (S)	0.015 (S)	1 (S)	≥ 64 (R)
SAU 25	1 (I)	0.25 (S)	2 (S)	≥ 16 (R)	0.25 (S)	0.015 (S)	0.5 (S)	≥ 64 (R)
SU 18	1 (I)	0.5 (S)	0.5 (S)	≥ 16 (R)	0.03 (S)	0.015 (S)	1 (S)	≥ 64 (R)
SU 22	1 (I)	1 (S)	2 (S)	≥ 16 (R)	0.03 (S)	0.03 (S)	1 (S)	≥ 64 (R)
SU 23	2 (R)	0.12 (S)	1 (S)	≥ 16 (R)	0.06 (S)	0.015 (S)	1 (S)	≥ 64 (R)

UM: Uncooked Meatball, SA: Salami, SAU: Sausage, SU: Sucuk, (S): Sensitive, (I): Intermediate, (R): Resistance
AMP: Ampicillin, AMC: Amoxycillin/clavulanic acid, DA: Clindamycin, IPM: Imipenem, TE: Tetracycline, VA: Vancomycin, CTX: Cefotaxime

Table 4. Susceptibility profiles of 22 *C. difficile* isolates from meat products.

Samples	n	Susceptibility	AMP (%)	AMC (%)	DA (%)	IMP (%)	MTZ (%)	TE (%)	VA (%)	CTX (%)
Salami	17	Susceptible	8 (47)	17 (100)	12 (70.6)	0 (0)	16 (94.1)	17 (100)	17 (100)	0 (0)
		Intermediate	7 (41.2)	0 (0)	5 (29.4)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
		Resistant	2 (11.8)	0 (0)	0 (0)	17 (100)	1 (5.9)	0 (0)	0 (0)	17 (100)
Sucuk	3	Susceptible	0 (0)	3 (100)	3 (100)	0 (0)	3 (100)	3 (100)	3 (100)	0 (0)
		Intermediate	2 (66.7)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
		Resistant	1 (33.3)	0 (0)	0 (0)	3 (100)	0 (0)	0 (0)	0 (0)	3 (100)
Sausage	1	Susceptible	0 (0)	1 (100)	1 (100)	0 (0)	1 (100)	1 (100)	1 (100)	0 (0)
		Intermediate	1 (100)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
		Resistant	0 (0)	0 (0)	0 (0)	1 (100)	0 (0)	0 (0)	0 (0)	1 (100)
Uncooked Meatball	1	Susceptible	1 (100)	1 (100)	0 (0)	0 (0)	1 (100)	1 (100)	1 (100)	0 (0)
		Intermediate	0 (0)	0 (0)	1 (100)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
		Resistant	0 (0)	0 (0)	0 (0)	1 (100)	0 (0)	0 (0)	0 (0)	1 (100)
TOTAL	22	Susceptible	9 (40.9)	22 (100)	16 (72.7)	0 (0)	21 (95.5)	22 (100)	22 (100)	0 (0)
		Intermediate	10 (45.5)	0 (0)	6 (27.3)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
		Resistant	3 (13.6)	0 (0)	0 (0)	22 (100)	1 (4.5)	0 (0)	0 (0)	22 (100)

AMP: Ampicillin; CTX: Cefotaxim; DA: Clindamycin; AMC: Amoxicillin-clavulanic Acid; IMP: Imipenem; MTZ: Metronidazole; TE: Tetracycline; VA: Vancomycin; n: Sample Number