Clostridium difficile CONTAMINATION OF EUROPEAN POTATOES

Tkalec Valerija[1], Skok Barbara[1], Janezic Sandra[1, 2], Rupnik Maja[1, 2]

[1]National Laboratory for Health, Environment and Food, Centre for Microbiology, Maribor, Slovenia; [2]University of Maribor, Faculty of Medicine, Maribor, Slovenia

Contact: valerija.tkalec@nlzoh.si

INTRODUCTION

Foodborne transmissions have been considered as one of potential infection routes of *C. difficile* (Rodriguez *et al*., 2016; Rodriguez Diaz *et al*., 2018).

The bacterium has been detected in diverse range of foods, including meat, seafood and vegetables, however, studies on food, especially vegetables are limited (Al Saif and Brazier., 1996; Bakri *et al*., 2009; Metcalf et al., 2010; Pasquale *et al*., 2011; Pasquale *et al*., 2012; Eckert *et al*., 2013; Rodriguez *et al*., 2016; Rodriguez Diaz *et al*., 2018, Lim *et al*., 2018).

Here we present prevalence of *C. difficile* on potatoes, collected in 15 different countries across the Europe.

MATERIAL AND METHODS

A total of 242 potato samples from 15 European countries were collected between June 2015 and July 2018 (Table 1).

In general, three potatoes per batch were collected and swabbed as one sample. Swabs were incubated anaerobicaly in selective broth, followed by spore selection by alcohol shock and plating onto chromogenic plates (Figure 1).

Isolation of *C. difficile*

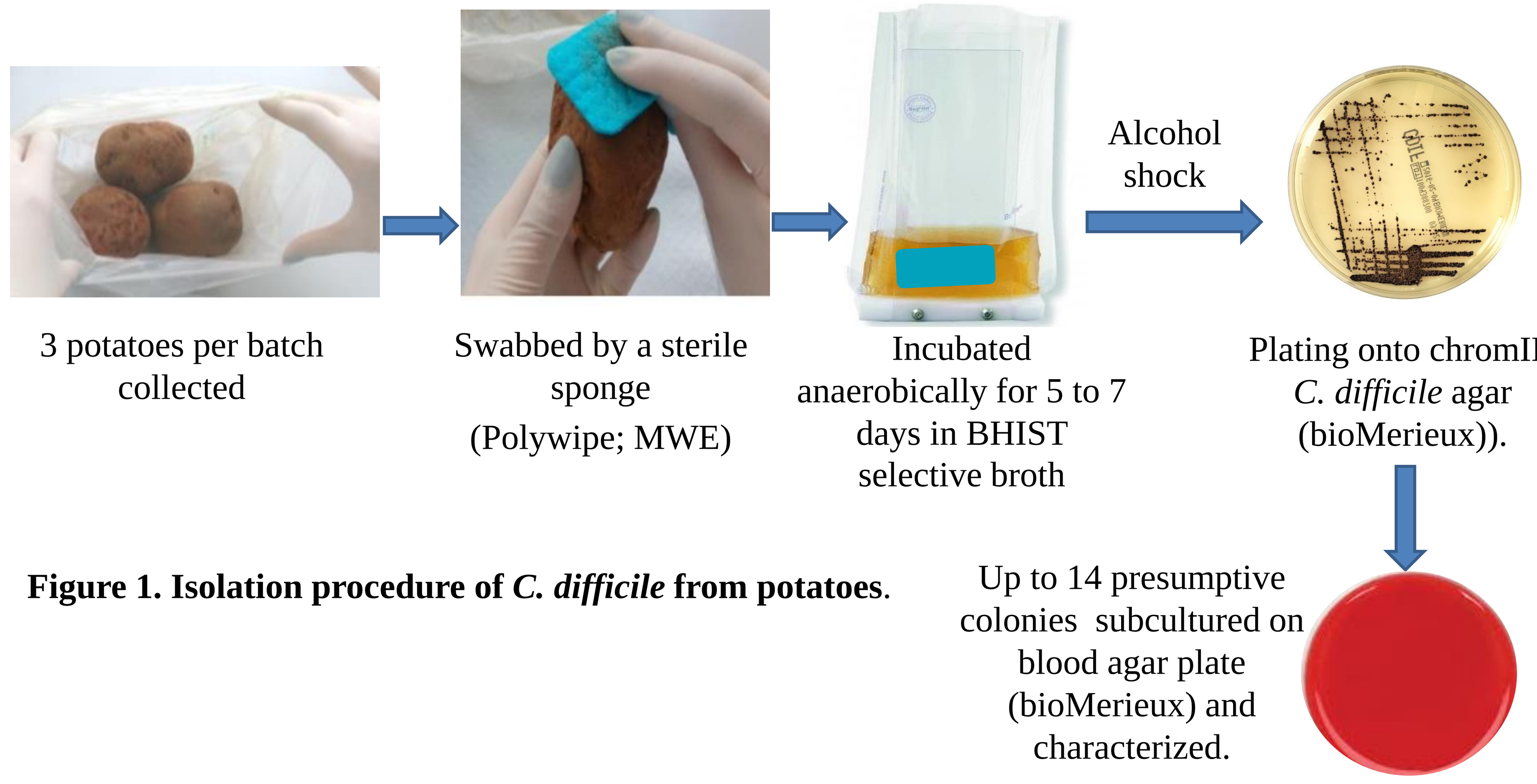

Figure 1. Isolation procedure of *C. difficile* from potatoes.

Identification and characterization

Identification was confirmed by - detection of molecular marker *cdd*3 or
- mass spectrometry (MALDI-TOF Biotyper System; Bruker).

All isolates were characterized by PCR ribotyping (Janezic and Rupnik, 2010).

One representative PCR ribotype from each sample was toxinotyped and binary toxin gene was detected by partial amplification of *cdt*B as described by Janezic and Rupnik (2010) and Rupnik and Janezic (2016).

RESULTS AND DISCUSSION

Table 1. Prevalence of *C. difficile* in European potatoes between years 2015 and 2018 with overview of detected genotypes and origin countries for *C. difficile* positive samples.

Country of sample collection	Sampling period	Number of collected samples	Number of *C. difficile* positive samples (%)	PCR ribotypes	Toxinotypes	Countries of origin for *C. difficile* positive samples
Albania	July, 2018	5	4/5 (80,0 %)	014/020, 002 SLO 110, 3 new PCR ribotypes	0 (BTb +), ND	Albania
Austria	From January, 2018 to June, 2018	12	1/12 (8,3 %)	014/020, 027, 106, 126, SLO 110	0 (BTb -), III (BTb +), V (BTb +)	Austria
Bosnia and Herzegovina (BiH)	May, 2018	2	2/2 (100,0 %)	014/020, 032 (CE), 001/072, 1 new PCR ribotype	0 (BTb -), I (BTb -), Tox – (BTb-)	BiH
France	May and June, 2018	14	2/14 (14,3 %)	010, 015, 029, 126, 128, SLO 259	0 (BTb-), V (BTb+), Tox- (BTb-)	France
Greece	June and July, 2018	15	4/15 (26,7 %)	014/020, 3 new PCR ribotypes	0 (BTb-), Tox- (BTb-)	Cyprus, Greece
Ireland	July, 2018	12	5/12 (41,7 %)	005 , 014/020, 081, 127	0 (BTb-), V (BTb+), VII (BTb+) Tox- (BTb)	Ireland
Italy	From April to July ,2018	19	5/19 (26,3 %)	001/072, 023, 078, 258, 764, 2 new PCR ribotypes	0 (BTb-), IV (BTb+), V (BTb+), XII (BTb-) Tox- (BTb-)	Italy
Netherlands	June and July, 2018	10	2/5 (40,0 %)	014/020, 1 new PCR ribotype	0 (BTb-), Tox- (BTb-)	Egypt, Netherlands
Poland	June, 2018	10	5/10 (50,0 %)	002, 003, 005, 010, 018, 027 3 new PCR ribotypes	0 (BTb-), IV (BTb+), IV (BTb+), Tox- (BTb-)	Poland, Greece
Romania	June and July, 2018	7	7/7 (100,0 %)	002 , 014/020, 011/049, 059, 126, SLO 015, 2 new PCR ribotypes	0 (BTb-), V (BTb+), XII (BTb-), Tox- (BTb-)	Romania
Slovakia	January and July, 2018	9	0/9 (0,0 %)	na	na	na
Slovenia	June, 2015 February and March, 2016 From January to September, 2017 February and April, 2018	77	22/77 (28,6 %)	014/020, 053, 126, 150 and 14 other PCR ribotypes	0 (BTB-), III (BTb+), V (BTb+), XII (BTB-), Tox- (BTb-)	Slovenia, France, Austria, Africa, USA and other 8 countries
Spain	June and July, 2018	10	6/10 (60,0 %)	014/020, 126, 258, SLO 116, SLO 196	0 (BTb-), 0/v (BTb+), V (BTb+), XII (BTb-), Tox- (BTB-)	No data
Sweden	June, 2018	10	2/10 (20,0 %)	002, 029, SLO 110, 2 new PCR ribotypes	0 (BTb-), IV (BTb+), Tox- (BTb-)	Sweden
UK	From January to July, 2018	30	2/31 (6,5 %)	010, 1 new PCR ribotype	Tox- (BTb-)	UK, Egypt, no data
Total	**From June 2015 to September 2018**	**242**	**69/242 (28,5 %)**	**60 different PCR ribotypes**	**8 different toxinotypes and Tox-**	**na**

Tox- -nontoxigenic strain, BTb+ -binary toxin positive; BTb- -binary toxin negative; na-not applicable.

C. difficile was isolated from 69 out of 242 (28,5 %) samples (Table 1).

Percentage of *C. difficile* positive samples ranged from 0,0 % to 100,0 % per studied country (Table 1).

The positivity rate correlates to already published results on root vegetables (Al Saif and Brazier, 1996; Lim *et al*., 2018), which is somewhat higher than reported for other types of vegetables (Bakri *et al*., 2009; Metcalf *et al*., 2010; Eckert *et al*., 2013).

Altogether 662 of isolates were obtained and distributed into 60 different PCR ribotypes.

Two thirds of all ribotypes were toxigenic and PCR ribotype 014/020 was the most numerous.

Altogether 15 out of 60 (25,0 %) of detected PCR ribotypes were new to our PCR ribotype library.

One third of *C. difficile* positive potato samples was imported from foreign countries of three different continents.

Forty-two of determined PCR ribotypes (70,0 %) have previously been reported in humans, animals, soil or water.

CONCLUSIONS

Our results show that **root** vegetables are often **contaminated** with highly **diverse** *C. difficile* PCR ribotypes.

We suggest that potatoes may represent a **transnational and transcontinental** way of *C. difficile* **spread**.

REFERENCES

Al Saif N., Brazier J.S., 1996. The distribution of *Clostridium difficile* in the environment of South Wales. J. Med. Microbiol. 45(2), 133-137.

Bakri M.M., Brown D.J., Butcher J.P., Sutherland A.D., 2009. *Clostridium difficile* in ready-to-eat salads, Scotland. Emerg. Infect. Dis. 15(5), 817-818.

Eckert C., Burghoffer B., Barbut F., 2013. Contamination of ready-to-eat raw vegetables with *Clostridium difficile* in France. J. Med. Microbiol. 62(Pt 9), 1435-1438.

Janezic S., Rupnik M., 2010. Molecular typing methods for *Costridium difficile*: pulsed-field gel electrophoresis and PCR ribotyping. Methods. Mol. Biol. 646, 55-65.

Lim S.C., Foster N.F., Elliott B., Riley T.V., 2018. High prevalence of *Clostridium difficile* on retail root vegetables, Western Australia. J. Appl. Microbiol. 124(2), 585-590.

Metcalf D.S., Costa M.C., Dew W.M., Weese J.S., 2010. *Clostridium difficile* in vegetables, Canada. Lett. Appl. Microbiol. 51(5), 600-602.

Pasquale V., Romano V.J., Rupnik M., Dumontet S., Ciznar I., Aliberti F., Mauri F., Saggiomo V., Krovacek K., 2011. Isolation and characterization of *Clostridium difficile* from shellfish and marine environments. Folia Microbiol. 56, 431–437.

Pasquale V., Romano V., Rupnik M., Capuano F., Bove D., Aliberti F., Krovacek K., Dumontet S., 2012. Occurrence of toxigenic *Clostridium difficile* in edible bivalve molluscs. Food Microbiol. 31, 309–312.

Rodriguez C., Taminiau B., Van Broeck J., Delmée M., Daube G., 2016. *Clostridium difficile* in food and animals: A comprehensive review. Adv. Exp. Med. Biol. 932, 65-92.

Rodriguez Diaz C., Seyboldt C., Rupnik M., 2018. Non-human *C. difficile* reservoirs and sources: animals, food, environment. Adv. Exp. Med. Biol. 1050, 227-243.

Rupnik M., Janezic S., 2016. An Update on *Clostridium difficile* toxinotyping. J. Clin. Microbiol. 54(1), 13-18.

Acknowledgements

The authors would like to thank Axel Hartke, James I. Prosser, Beata Walter, Sarah Kuehne, Adam P. Roberts, Edit Szekely, Patrizia Spigaglia, Frederic Barbut, Cecile Gateau, David Eyre, Bauke Oudega, Luka Jernejsek, Jana Jass, Luka Safaric, Tina Bedekovic, Alenka Zeme, Vincenzo Pasquale, Oltiana Petri, Uros Cerkvenik, Hercules Sakkas, Christina Chatedaki, Hanna Pituch, Michal Piotrowski, Athanasios Tsakris, Miroslava Horniackova, Elena Reigadas Ramirez, Eduard Torrents, Fidelma Fitzpatrick, Catherine Stanton and Miran Lovrencic for their contributions to sample collection.

Part of the work was suported by EU grant IMI 2 COMBACTE-CDI.