Clostridium difficile IN MEAT PRODUCTS, EGGS AND VEGETABLES IN SLOVENIA

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Introduction

Clostridium difficile is an important nosocomial enteropathogen. Recently, an increase in community acquired *Clostridium difficile* infection (CDI) has been observed and was followed by raised interest on community sources of CDI that could include animals and food (Gould and Limbago, 2010; Rupnik *et al*., 2009).

The proportion of *C. difficile* positive samples in different European countries (Austria, Sweden, the Netherlands, Switzerland, Scotland, France, Italy) is low (0, 0% - 7, 5%). PCR ribotypes of the detected food isolates partially overlap with PCR ribotypes found in animals or in humans in a given country (Bakri *et al*., 2009; Bouttier *et al*.,2010; de Boer *et al*., 2011; Gould and Limbago, 2010; Hoffer *et al*., 2010; Joebstl *et al*., 2010; von Abercron *et al*., 2009).

The objective of our study was to determine the presence of *C. difficile* in meat, meat products, vegetables and eggs in Slovenia.

Table 1. Detection of *Clostrdium difficile* in eggs and vegetable samples in Slovenia and molecular characteristics of isolated strains.

Food product			Number of cultured samples	Number of *C. difficile* positive samples (%)	*C. difficile* isolates: toxinotype/PCR ribotype (number of isolates/number of all detected isolates)
Type of food product		Origin/Ingredients			
Eggs	egg surface	na	49	1 (2,0%)	0/014/020 (2/4) 0/002 (2/4)
Vegetables	ready-to-eat salats	green letice, carrots, cabbage, sprouts, beans,…	3	0	na
	fresh sprouts		5	0	na

na- not applicable

Materials and methods

Sampling and *C. difficile* isolation:

Altogether 59 retail meat samples and ready-to-eat meat products, 8 samples of ready-to-eat salads and fresh sprouts and 49 chicken eggs were tested. Egg surface was sampled with moistened sponge swab (Polywipe; MWE) which was included in further testing.
Samples were collected in 2008, 2011 and 2012 on several different occasions and originated from 27 different production facilities and farms. Of them 26 (96,3%) were located in Slovenia and one in Austria.

Different culturing methods were used for *C. dificile* isolation. Enrichment cultures were prepared either in selective CDA broth (*C. difficile* agar base prepared without agar (Oxoid) supplemented with *C.difficile* selective supplement (Oxoid)), selective CDALT broth (*C. difficile* agar base prepared without agar (Oxoid) supplemented with *C.difficile* selective supplement (Oxoid), sodium choleate and lysozyme) or in BHI (Biolife) supplemented with sodium choleate, yeast extract and cysteine. For BHI enrichment samples were subjected to heat shock. After 5 days of incubation under anaerobic conditions, spores were selected by alcohol shock and plated onto selective CDALT agar (*C. difficile* agar base (Oxoid) supplemented with *C.difficile* selective supplement (Oxoid), sodium choleate and lysozyme) or on different commercial selective agar plates: CLO (bioMerieux) or chromID *C. difficile* (bioMerieux).

Identification and characterization:

Recovered *C. difficile* colonies were identified by morphology. Identification of persumptive colonies was confirmed by detection of molecular marker *cdd*3 (Zidaric *et al*., 2008).

Isolates were characterized by toxinotyping and PCR ribotyping. Toxinotyping was done by PCR amplification and restriction analysis of A3 and B1 fragments of *tcd*A and *tcd*B genes as previously described (Rupnik *et al*., 1998; www.mf.uni-mb.si/mikro/tox). Binary toxin gene was detected by partial amplification of *cdt*B (Stubbs *et al*., 2000).
PCR ribotyping was performed by primers and conditions described by Bidet *et al*. (1999). PCR ribotype results were analyzed by BioNumerics software (version 5.10 Applied Maths).

Results and discussion

C. difficile was not detected in any of the tested meat and vegetable samples (Table 1., Table 2.).

Low prevalence of *C. difficile* meat and other food products in our study correlates to other European reports on food contamination and low occurence of *C. difficile* in food animals at slaughter (Bakri *et al*., 2009; Bouttier *et al*.,2010; de Boer *et al*., 2011; Gould and Limbago, 2010; Hoffer *et al*., 2010; Joebstl *et al*., 2010; von Abercron *et al*., 2009).

A single chicken egg out of 49 (2, 0%) was positive and four colonies were obtained (Table 1).

All 4 egg isolates belonged to toxinotype 0 and to two distinct PCR ribotypes: 014/020 and 002. Both PCR ribotypes are currently most prevalent in hospitalized patients in Slovenia and are also among most prevalent European human isolates. They are frequently isolated from poultry and reported in other food animals in Slovenia (Zidaric *et al*., 2008; Janezic *et al*., 2012).

Table 2. Occurence of *Clostrdium difficile* in meat and meat products in Slovenia.

Food product		Number of cultured samples	Number of *C. difficile* positive samples
Type of meat product	Origin		
ground meat	pork and beef	13	0
	beef	2	0
	chicken	9	0
	turkey	14	0
ready-to-eat meat products (sausages, meat cheese, hot-dogs,…)	pork	1	0
	chicken and turkey	3	0
	chicken	9	0
	turkey	1	0
	pork and beef	5	0
	beef	2	0
Total number of meat samples		59	0

Conclusions

The *C. difficile* zero recovery rates from meat and meat products in our study is in correlation to data from previous reports from EU.

This is the first report of contamination of chicken eggs with *C. difficile* and together with reports on relatively high prevalence in poultry highlights the importance of poultry as *C. difficile* reservoir and adds to its potential as a source of infection for humans.

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