THE VARIABILITY OF *Clostridium difficile* RIBOTYPES ISOLATED FROM A SINGLE WASTEWATER TREATMENT PLANT OVER A SIX MONTH PERIOD

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INTRODUCTION

The wastewater treatment plants (WWTP) are an environment where *C. difficile* strains from different environments such as hospitals, farms, households etc. are collected. WWTPs could therefore be a very suitable sampling site to assess the overall diversity of *C. difficile* in a given region. The aim of this study was to detect *Clostridium difficile* in wastewater effluent and to determine variability of PCR ribotypes over a 6-month screening period.

MATERIALS AND METHODS

Study was conducted between January and June 2012 on a single wastewater treatment plant. Altogether 6 samples (one per month) were collected at effluent.
After heat shock at 70^{0}C for 20 minutes water samples were filtered through a 0.2-µm pore size cellulose nitrate membrane filter (Whatman).
Filters were placed on commercial selective chromID™ *C. difficile* agar (BioMérieux) and incubated anaerobically at 37^{0}C for up to 3 days (Fig. 1).
After incubation, 20 black colonies were picked from each filter and subcultured on fresh medium.
C. difficile was confirmed by detection of molecular marker *cdd3* and characterized by toxinotyping (Rupnik *et al*., 1998), and PCR ribotyping (Bidet *et al*., 1999).

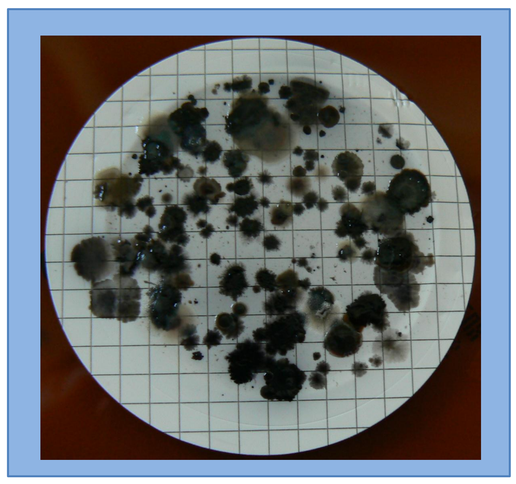

Figure1: Filter placed on commercial selective chromID™ *C. difficile* agar (BioMérieux) after 3 days of incubation

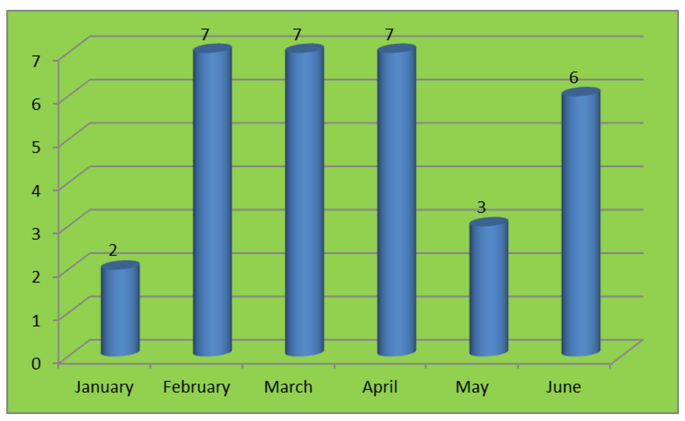

Figure 2: The PCR ribotypes diversity of *C.difficile* in effluent of WWTP during sample period

RESULTS AND DISCUSSION

- All tested samples were *C. difficile* positive and altogether 49 strains were isolated. From each sample, 2 to 13 isolates were isolated and 79.6% of all isolates were toxigenic (Table 1).
- In samples collected in March and April most strains (13 strains each sample) and most PCR ribotypes (7 types each sample) were found (Fig. 2).
- Overall, 18 different PCR ribotypes were identified.
- PCR ribotype 014/020 was detected in all tested samples, 010 and 046 in 66,7% of tested samples and 002 in half of all samples.
- All four PCR ribotypes that were predominant among isolated strains, encounting for from 8,2% to up to 38,8% of all isolates.
- Other 14 PCR ribotypes were rare (2% - 4% of all isolates) and were detected in only one to two out of all 6 tested samples.
- Interestingly, PCR ribotypes 014/020 and 002 are also the most prevalent PCR ribotypes in hospitalized patients in Slovenia and also frequently found in animals and environment in Slovenia (Avbersek *et al*., 2009; Zidaric *at al*, 2010; Janezic *et al*., 2012).

Table1: *C.difficile* genotypes isolated from effluent of wastewater treatment plant. Water was examined once a monthly for 6 consecutive months

PCR Ribotypes/Sampling	Wastewater treatment plant						Presence of PCR ribotype in various samples (%)	Proportion of PCR ribotype (% of all isolates)
	January	February	March	April	May	June		
002		2	1			1	50%	4 isolates (8,2%)
003				1			16,7%	1 isolate (2,0%)
005(SLO 016)						1	16,7%	1 isolate (2,0%)
005 (SLO 027)			1				16,7%	1 isolate (2,0%)
010	1	1		3		1	66,7%	6 isolates (12,2%)
011/049 (SLO 070)			1				16,7%	1 isolate (2,0%)
014/020	1	1	7	4	1	5	100%	19 isolates (38,8%)
015				1			16,7%	1 isolate (2,0%)
045		1					16,7%	1 isolate (2,0%)
046		1	1	1		1	66,7%	4 isolates (8,2%)
070					1		16,7%	1 isolate (2,0%)
SLO 063		1					16,7%	1 isolate (2,0%)
SLO 064		1			1		33,3%	2 isolates(4,1%)
SLO 097						1	16,7%	1 isolate (2,0%)
SLO 142			1				16,7%	1 isolate (2,0%)
SLO 153				1			16,7%	1 isolate (2,0%)
SLO 154				2			16,7%	2 isolates (4,1%)
SLO 155			1				16,7%	1 isolate (2,0%)
Number of isolates	2	8	13	13	3	10		49
Number of different ribotypes	2	7	7	7	3	6		18

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