



Prevalence of Clostridium difficile PCR ribotypes in Bulgaria during and after the pan European surveillance study 2008-2010

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ABSTRACT

Objectives: To analyze the prevalence of *Clostridium difficile* ribotypes in Bulgaria determined during the pan European Surveillance Study and the next 16 months.

Methods: For the period November 2008 - March 2010, sixty five fecal samples from patients with mild to severe enterocolitis and previous antibiotic treatment have been investigated using conventional microbiological and genetic methods. The stool samples derived from nine hospitals situated in Sofia and Plovdiv regions. Strains were typed and further characterized for the presence of toxins A (TcdA), B (TcdB) and binary toxins (CdtA and CdtB).

Results: The incidence of *C. difficile* infections estimated recently for Bulgaria was 3.12 per 10,000 patient admissions (0.7 per 10,000 patient-days). Fifteen patients with different clinical forms of CDI were identified during the present study. Six of the isolates (40%) belonged to PCR ribotype 017 (TcdA-; TcdB+; CdtA/B-), followed by 002 and 014. The remaining *C. difficile* isolates were grouped as 012, 046 and 078 ribotypes. All of the identified ribotypes have been reported to cause outbreaks worldwide.

Conclusion: During the pan European surveillance study Bulgaria initiated a laboratory-based investigation of CDI. The most prevalent *C. difficile* ribotype in our country is 017 (40%). All determined ribotypes were found to be associated with severe CDI and high percentage of lethal outcome.

Introduction

C. difficile NAP1/BI/027 is an important pathogen causing severe forms of diarrhoeal infections worldwide. In 2008, 16 European countries reported outbreaks and a number of sporadic cases due to *C. difficile* 027. This prompted ECDC to perform a surveillance study in 34 European countries. Sixty-five different PCR ribotypes were found, among which 014/020 (16%), 001 (9%), 078 (8%) and 027 (5%) were prevailing. The aim of the present work is to analyze the prevalence of *Clostridium difficile* ribotypes in Bulgaria determined during the pan European Surveillance Study, 2008-2010.



MATERIALS AND METHODS

Patients

For the period November 2008 - March 2010, sixty five fecal samples have been investigated at the National Reference Laboratory for Enteric Pathogens, National Center of Infectious and Parasitic Diseases (NCIPD), Sofia, Bulgaria. The stool samples derived from nine hospitals situated in Sofia and Plovdiv regions. Patients with enterocolitis were selected according to the criteria accepted by ECDC. Ribotyping was performed according to Bidett et al. 1999 and fragments were separated either by QiAxcel capillary electrophoresis system (Qiagen) (NCIPD, Bulgaria) or by regular agarose electrophoresis (CDRL, Leiden). Patterns were compared to reference ECDC *C. difficile* collection.



RESULTS

The incidence of *C. difficile* infections estimated recently for Bulgaria was 3.12 per 10,000 patient admissions (0.7 per 10,000 patient-days). In total, 65 stool samples were tested for *C. difficile* toxins, fourteen of them were toxin positive and fifteen *C. difficile* strains were isolated. Table 1 summarizes the patients's clinical records, laboratory results and the antimicrobial therapeutic courses.

Table1. <i>C. difficile</i> isolates, laboratory characteristics and clinical records of studied patients									
No	Laboratory number	Age, sex	Hospital	Clinical diagnosis	Previous antimicrobial therapy	gluD	Ribotype / toxin type of <i>C. difficile</i>	Presence of toxins	Outcome
1.	09	66, female	University Hospital, "Saint Anna", Sofia	Hepatic carcinoma	Ciprofloxacin, Ceftriaxon	+	078/V	(A ⁺ /B ⁻) Binary toxin (+)	lethal
2.	15	3, male	University Hospital, "Saint Anna", Sofia	Enterocolitis	Amoxicillin Cefalexine	+	046/0	(A ⁺ /B ⁻)	recovered
3.	36	66, female	Hospital of Infectious Diseases "Prof. Kirov", Sofia	Neurological operation, Enterocolitis	Lincomycin	+	017/VIII	(A ⁺ /B ⁻) Binary toxin (-)	recovered
4.	181	75, female	Hospital of Infectious Diseases "Prof. Kirov", Sofia	Acute bronchitis, Pseudomembranous colitis with peritonitis	Ampicillin/ Sulbactam	+	017/VIII	(A ⁺ /B ⁻) Binary toxin (-)	lethal
5.	217	76, male	University Hospital of Pulmonary Infections, "Saint Sofia", Sofia	Empyema, chemodalytisis	Ciprofloxacin Amoxicillin/clavulanic acid	+	017/VIII	(A ⁺ /B ⁻) Binary toxin (-)	lethal
6.	223	19, male	Hospital of Infectious Diseases, Plovdiv	Orthopaedic operation	Cefalosporines	+	ND*		recovered
7.	225	25, female	University Hospital, "Queen Joanna", Sofia	Chronic enterocolitis	Ciprofloxacin	+	012		recovered
8.	237	75, female	University Hospital of Pulmonary Infections, "Saint Sofia", Sofia	Tuberculosis	Tuberculostatics	+	017/VIII	(A ⁺ /B ⁻) Binary toxin (-)	lethal
9.	238	61, female	University Hospital of Pulmonary Infections, "Saint Sofia", Sofia	Tuberculosis	Tuberculostatics	+	017/VIII	(A ⁺ /B ⁻) Binary toxin (-)	recovered
10.	239	77, female	University Hospital, "Queen Joanna", Sofia	Acute bronchitis, Enterocolitis	Ciprofloxacin, Cefalexine	+	ND*	(A ⁺ /B ⁻)	lethal
11.	240	56, female	University Hospital, "Queen Joanna", Sofia	Enterocolitis	Ciprofloxacin	+	002		recovered
12.	250	44, male	University Hospital of Pulmonary Infections, "Saint Sofia", Sofia	Tuberculosis	Tuberculostatics	+	017/VIII	(A ⁺ /B ⁻) Binary toxin (-)	recovered
13.	253	27, male	University Hospital, "Saint Anna", Sofia	Enterocolitis, Pseudomembranous colitis	Ciprofloxacin	+	014**		recovered
14.	256	28, male	University Hospital, "Queen Joanna", Sofia	Enterocolitis	Ciprofloxacin	+	014**		recovered
15.	262	80, female	University Hospital of Infectious Diseases, Plovdiv	Orthopaedic operation, Enterocolitis	Ciprofloxacin Cefalosporines	+	002**		recovered

*ND - type not available in the reference database

** Type not confirmed at CDRL, Leiden

Six of the isolates (40%) belonged to PCR ribotypes 017 (TcdA-; TcdB+; CdtA/B-). The second most prevalent ribotypes were 002 and 014 (13%). The remaining *C. difficile* isolates belonged to 012, 046 and 078 ribotypes (Table 2, Fig. 1). All of these ribotypes have been reported to cause outbreaks worldwide. Figure 1 represents the determined PCR ribotypes of the Bulgarian *C. difficile* isolates.

Table 2. Prevalent ribotypes among Bulgarian <i>C. difficile</i> isolates.	
Ribotype (number of isolates)	Percent out of all determined ribotypes (%)
017 (6)	40
002 (2)	13
014 (2)	13
012 (1)	7
046 (1)	7
078 (1)	7
Non typable (2)	13

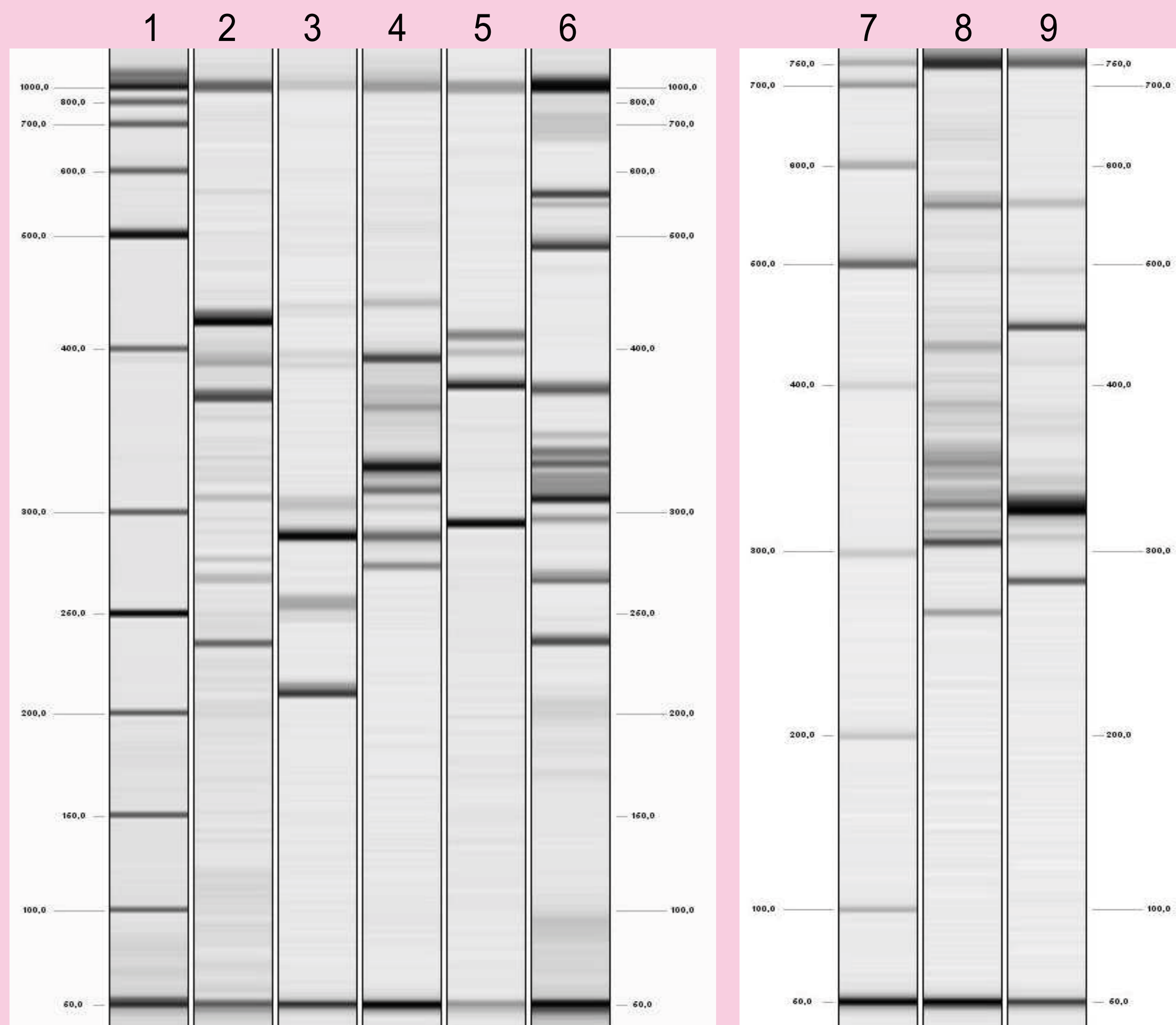


Figure 1. Frequent ribotypes found for Bulgarian *C. difficile* isolates. 1. – Marker 100bp; 2. ribotype 017(isolate 250); 3. 046/isolate 15; 4. 078/isolate 09; 5. unknown ribotype/isolate 223; 6. 014/isolate 256; 7. Marker 50bp; 8. 012/ isolate 225

CONCLUSION

- During the pan European surveillance study Bulgaria initiated a laboratory-based investigation of CDI for Sofia and Plovdiv regions.
- The most prevalent *C. difficile* ribotype was 017 (40%).
- All determined ribotypes were found to be associated with severe CDI and high percentage of lethal outcome.

