Recent outbreaks of *Clostridium difficile*-associated diarrhoea (CDAD) with increased severity, high relapse rate and significant mortality have been related to the emergence of a new, hypervirulent *C. difficile* strain referred to as North American pulsed-field type 1 (NAP1) and PCR ribotype 027 (McDonald et al., 2005). This epidemic strain produces toxins A (TcdA) and B (TcdB) and binary toxin (CDT), is resistant to erythromycin and newer fluoroquinolones (Kuijper et al., 2007). The increased virulence of this epidemic strain might be associated with overproduction of toxins A and B (Warny et al., 2005).

Until May 2008, *C. difficile* type 027 has been reported in 16 European countries (including Poland). It has been responsible for outbreaks in Belgium, Germany, Finland, France, Ireland, Luxembourg, the Netherlands, Switzerland and the UK. It has also been detected in Austria, Denmark, Sweden, Norway, Hungary, and Spain. (Kuijper et al., 2006; 2007; 2008; Brazier et al., 2007; Joseph et al., 2005; Tachon et al., 2006; Indra et al., 2006; Lyytikäinen et al., 2007; Zaiss et al., 2007). One isolate of PCR-ribotype 027 was found in Japan (Kato et al., 2007). We report the first isolation of *Clostridium difficile* type 027 in Poland.

On 3 February 2005, a 66-years old woman was admitted at the Central Clinical Hospital (1025 beds) in Warsaw with persistent diarrhea, foul-smelling stool, abdominal pain and high fever. She was known with chronic, inflammatory, autoimmune disorder (Sjögren’s syndrome), rheumatoid arthritis. She was known with chronic, inflammatory, autoimmune disorder (Sjögren’s syndrome), rheumatoid arthritis. Recently, diabetes mellitus was diagnosed. Soon after admission, antimicrobial therapy was started with ciprofloxacin. Probiotics, sulfasalazine and prednisolon were also included, but the patient’s condition did not improve. Stool cultures at admission were negative for enteropathogens as *Salmonella* sp., *Shigella* sp. Stool culture of specimens taken at 14 February yielded *C. difficile*. Metronidazole was prescribed for 7 days and the diarrhoea resolved in few days. We consider it very likely that the disease was acquired in the community and was therefore not recognized at admission. Patient was not hospitalized in the 3 previous months.

**First Isolation of Clostridium difficile PCR-ribotype 027/toxinotype III in Poland**

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**Abstract**

Of 175 *Clostridium difficile* strains isolated from patient hospitalized in one academic hospital in Warsaw between 2005–2006, one isolate belonged to PCR-ribotype 027/toxinotype III. This isolate had tcdA, tcdB, binary toxin genes (cdtA and cdtB), a 18-bp deletion and a 1 bp deletion at 117 position in the tcdC gene. Antimicrobial susceptibility tests revealed high level resistance to erythromycin, moxifloxacin and gatifloxacin. This is a first report of the 027 strain of *C. difficile* in Poland.

**Key words:** *Clostridium difficile*, binary toxin, PCR-ribotype 027/toxinotype III, resistance to newer fluoroquinolones

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A total of 175 _C. difficile_ strains isolated between 2005–2006 from diarrheal patients hospitalised in Central Clinical Hospital in Warsaw were sent to the Department of Medical Microbiology, Medical University of Warsaw for determination of toxigenicity and antimicrobial susceptibility. _C. difficile_ strains were isolated and identified by the standard procedures (Pituch et al., 2005). Toxigenicity of _C. difficile_ strains were confirmed with the using PCR to the detection of _tcdA_ (TcdA), _tcdB_ (TcdB) and _cdtA_ and _cdtB_ (CDT) genes in addition to immunoenzymatic assay _C. difficile_ TOX A/B II ™ (TechLab, Inc., Blacksburg, VA, USA) for both or either of toxins TcdA /TcdB and _C. difficile_ OxoTest for detection only TcdA. For comparative reasons, we included a toxigenic control _C. difficile_ strain VPI 10463 (A+B+) and the non-toxigenic reference _C. difficile_ strain NIHBRIGGS 8050 (A-B+CDT−). One reference strain, _C. difficile_ CCUG 20309 (A-B’CDT−), was used as a control for PCR testing of _cdtA_ and _cdtB_ genes (Pituch et al., 2005).

Susceptibility to metronidazole (MZ), vancomycin (VA), erythromycin (EM), clindamycin (CM), ciprofloxacin (CI), moxifloxacin (MX), and gatifloxacin (GA) was tested on Brucella blood agar medium, using E-test method. Resistance was defined according to Clinical and Laboratory Standards Institute (CLSI) recommendations. For metronidazole testing, _Bacteroides thetaiotaomicron_ ATCC 29791 and _Bacteroides fragilis_ NCTC11295 were included as reference strains, as were _Escherichia coli_ ATCC 25922 and _Staphylococcus aureus_ 268 Pituch H., M. Rupnik, P. Obuch-Woszczatyñski, A. Grubesic, F. Meisel-Miko‡ajczyk and M. £uczak.

Of 175 _C. difficile_ strains, 166 (95%) were toxigenic. Among these strains 35% produced only toxin B (A-B’CDT−) and only one strain possessed toxin A, toxin B, and binary toxin genes (A-B’CDT+). Resistance to erythromycin, clindamycin, ciprofloxacin, moxifloxacin, gatifloxacin, was found in 45%, 42%, 97%, 33%, 33%, respectively. All strains were fully susceptible to metronidazole and vancomycin. PCR-ribotyping showed that all strains A-B’CDT− belonged to PCR-ribotype 017, and were highly resistant to CM, EM, CI, MX and GA.

Only one strain, isolated from a 66-years old woman, was classified as PCR ribotype 027/toxino-type III, which was confirmed at Leiden University Medical Center. This isolate was positive for binary toxin genes, had an 18-bp deletion and a 1bp deletion at position 117 of _tcdC_. As determined by E-tests, the isolate (PCR-ribotype 027) was highly resistant to newer fluoroquinolones as ciprofloxacin, gatifloxacin and moxifloxacin (MIC≥32 mg/l) and also to erythromycin (MIC≥256 mg/l). The strain was _ermB_ negative and sensitive to clindamycin (MIC = 6 mg/l), metronidazole (MIC = 0.38 mg/l) and vancomycin (MIC = 0.75 mg/l).

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**Literature**


