# EMERGENCE OF *CLOSTRIDIUM DIFFICILE* INFECTION IN TUBERCULOSIS PATIENTS DUE TO A HIGHLY RIFAMPICIN RESISTANCE CLONE, POLYMERASE CHAIN REACTION RIBOTYPE 046 IN POLAND

**Piotr Obuch-Woszczatyński [1], Grzegorz Dubiel [2], Celine Harmanus [3], Ed Kuijper [3], Urszula Duda [4], Dorota Wultańska [1], Hanna Pituch [1].**

[1] Department of Medical Microbiology, Medical University of Warsaw, Poland, [2] Hospital of Lung Diseases and Tuberculosis, Bystra Śląska, Poland, [3] Department of Medical Microbiology, Leiden University Medical Center, Leiden, The Netherlands and [4] Sanitary Epidemiological Station, Bielsko-Biała, Poland

## OBJECTIVE

The objective was to determine the characteristics of *C. difficile* isolates associated with an outbreak of patients hospitalised in Hospital of Lung Diseases and Tuberculosis in Poland.

## METHODS

*Clostridium difficile* infection (CDI) was diagnosed by C. difficile TOX A/B test II. Faecal samples from *C. difficile* toxin positive diarrhoeic patients were grown anaerobically on CLO agar. For detection of *tcdA*, *tcdB* and binary toxin genes and deletion in *tcdA* gene PCR were conducted. Isolates of *C. difficile* were typed by the PCR-ribotyping. Banding patterns were compared with those in the PCR ribotypes library at the ARL, Cardiff.
MICs were measured by the E-test, as recommended by the CLSI or EUCAST.

## RESULTS

From September 2009 through December 2010, *Clostridium difficile* infection was suspected in 23 symptomatic patients. Ten patients were diagnosed as a CDI (six patients hospitalized in the tuberculosis unit, 2 in pulmonary, 1 in internal and 1 in thoracic surgery unit). Watery diarrhoea was observed in all patients, and was the major clinical manifestation. Five patients had active pulmonary tuberculosis. These patients were treated with isoniazid, rifampicin and pyrazinamide. Four patients developed CDI recurrence. Five patients died because of CDI.

All *C. difficile* isolates (10), contained *tcdA* and *tcdB* genes. PCR detecting repeating sequences in toxin A gene for one isolate generated a 700 bp product similar to that obtained for the Japanese control GAI 95601 strain. Of ten *C. difficile* strains, PCR-ribotyping could be classified into visually distinct 4 ribotypes: 046 (n=7), 001 (n=1), 002 (n=1), 017 (n=1). All strains were resistant to ciprofloxacin, 8 to moxifloxacin, 7 strains to clindamycin and erythromycin and 7 to rifampicin. All strains were susceptible to metronidazole. MIC range susceptibility to vancomycin were 0,75-1 mg/L. Three strains were intermediate susceptible to vancomycin. All strains belonged to PCR-ribotype 046 were highly resistant to moxifloxacin, clindamycin, erythromycin and rifampicin.

## CONCLUSION

Our findings suggested that (1) patients who are treated with anti-tuberculosis agents, especially rifampicin, who developed acute diarrhoea during or after therapy, should be evaluated for *C. difficile* infection, (2) prolonged treatment with rifampicin can lead to high-level resistance to rifampicin in *C. difficile* strains, (3) the emergence of multidrug-resistant *C. difficile* PCR ribotype 046 may be detrimental to anti-tuberculosis chemotherapy.

**This work was supported by National Science Center, Grant no. UMO-2011/01/B/NZ7/02720 (3G27).**

**Table 1 Clinical summary for 10 cases of CDI in Specialized Hospital of Lung Diseases and Tuberculosis (SHLDT)**

| Date of hospitali-zation | Age (years)/ sex/number of strain | Unit | Underlying diseases | Anti-TB drugs | Other AB | Date of diagnostic CDI | Symptoms of CDI | Fatality associated with CDI | PCR-ribotype/ rifampicin RI (R) or RI (S) |
|---|---|---|---|---|---|---|---|---|---|
| 27.11.09-17.12.09 | 82/F/CD14 | TBU | Non active TB, atherosclerosis, hypertension | - | AMOX | 09.12.09 | Diarrhoea, hypovolemic shock, extreme dehydratation | Yes | 046/RI (R) |
| 13.10.09-04.01.10 | 86/F/CD12 | TBU | AP TB, chronic congestive heart diseases | RI, I | CI | 09.12.09 | Recurrent diarrhoea, heart failure (associated with CDI) | Yes | 046/RI (R) |
| 03.11.09-08.01.10 | 80/F/CD15 | TBU | AP TB, failure, chronic obstructive pulmonary | RI, I, P | - | 11.12.09 | Diarrhoea | - | 046/RI (R) |
| 19.11.09-02.02.10 | 48/M/CD13 | TBU | AP TB, alcoholism, cirrhosis of the liver | RI, I, P | - | 09.12.09 | Recurrent diarrhoea, neurological symptoms (associated with CDI) | Yes | 046/RI (R) |
| 17.02.09-09.04.10 | 67/F/CD18 | TBU | AP TB, lung empyema, cirrhosis of the liver | RI, I, P | - | 30.12.09 | Recurrent severe diarrhoea | Yes | 046/RI (R) |
| 19.11.09-12.02.10 | 51/M/CD4 | TBU | AP TB, pulmonary mycetoma | RI, I, P | CI | 12.01.10 | Diarrhoea | - | 046/RI (R) |
| 04.02.10-03.03.10 | 86/M/CD22 | INT | Respiratory systems diseases | - | AMOX, CI | 25.02.10 | Diarrhoea | - | 046/RI (R) |
| 17.03.10-25.03.10 | 72/MCD30 | PUL | Pneumonia, lung empyema | - | AMOX, CI, CM, MZN | 22.03.10 | Severe diarrhoea | Yes | 017/RI (R) |
| 29.11.10-05.02.11 | 88/M/CD61 | PUL | Pneumonia, chronic obstructive pulmonary renal failure | - | AMOX, TX | 30.12.10 | Diarrhoea | - | 001/RI (S) |
| 14.09.10-23.09.10 | 69/M/CD50 | TS | Lung tumor | - | CEF | 23.09.10 | Diarrhoea | - | 002/RI (S) |

*AB* antibiotics, *CDI* C. difficile infection, *TBU* tuberculosis unit, *INT* internal unit, *PUL* pulmonology, *TS* thoracic surgery, *TB* tuberculosis, *AP TB* active pulmonary tuberculosis, *RI* rifampicin, *I* isoniazid, *P* pyrazinamide, *AMOX* amoxicillin, *CI* ciprofloxacin, *CM* clindamycin, *MZN* metronidazole, *TX* trimethoprim/sulfamethoxazole, *CEF* cefazolin (perioperative prophylaxis), *RI* (R) rifampicin resistant, *RI* (S) rifampicin sensitive