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SESSION VIII: CLOSTRIDIUM DIFFICILE: EPIDEMIOLOGY

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CLOSTRIDIUM DIFFICILE: EPIDEMIOLOGY

CONTROVERSIES IN *CLOSTRIDIUM DIFFICILE* INFECTION EPIDEMIOLOGY

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Current interim surveillance definitions for *Clostridium difficile* infection (CDI) categorize cases into healthcare facility-onset and community-onset, with the later further sub-categorized into healthcare facility and community-associated cases. Although available evidence suggests a relatively short (i.e. <3 days) incubation period, patients appear at increased risk for CDI for several weeks following discharge from an inpatient healthcare facility (i.e. community-onset, healthcare facility-associated disease); this may represent community-acquisition of *C. difficile* among patients at increased risk from recent antimicrobial exposure. Patients with community-associated CDI appear overall healthier than patients with healthcare facility-onset disease; the role of increased community-associated disease in recent increases in overall CDI will be discussed. While CDI continues to affect primarily elderly, hospitalized persons who have had recent antimicrobial exposure, there are several controversial findings in the epidemiology of CDI that have emerged in recent years. These include the proportion of all CDI cases that appear not to be associated with prior antimicrobial exposure, the proportion associated with stomach acid suppressing medications, and the degree to which true disease occurs in young children and infants.

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CLOSTRIDIUM DIFFICILE: EPIDEMIOLOGY

TRENDS OF *CLOSTRIDIUM DIFFICILE* INFECTION (CDI) IN VA HOSPITALS AND PROPOSED SYSTEM INTERVENTIONS

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Clostridium difficile infection (CDI) is a serious healthcare-associated complication which can contribute to significant morbidity, and even mortality. National trends within the U.S. have demonstrated an increase in CDI, and these trends have been mirrored within U.S. Department of Veterans Affairs' (VA) Veterans Health Administration (VHA) healthcare system which is made up of 153 medical centers and over 800 community-based outpatient clinics nationwide, and which provided health care to over 5 million Veterans and had ~ 605,000 discharges last year. Hospital discharge trend data nationwide for a 17-year period have revealed a slowly increasing rate from 1994-2000, followed by an abrupt increase from 2001-2005, and then by a significant decrease from 2006-2009. There has been some regional variation of rates with the Northeast having the highest rates and the South having the lowest, as well as variable age rates, with older Veterans more likely affected than younger ones. VHA has engaged in a number of nationwide initiatives which could be contributing to the recent decrease in CDI, including generalized dissemination of information about CDI, a hand hygiene Directive, development of an ICU outcomes project (Inpatient Evaluation Center, IPEC) which has a focus on healthcare-associated infection reduction and most recently its methicillin-resistant *Staphylococcus aureus* (MRSA) Prevention Initiative. The success of VHA's MRSA Prevention Initiative has resulted in the proposed expansion of this process to include CDI in the next phase. Several of the components of the MRSA Prevention Initiative have direct impact on CDI; these components include application of hand hygiene, use of contact precautions, and espousing cultural change. Along with adapting those three components to expand to include CDI, an additional emphasis on environmental cleaning is being evaluated. With this proposed expansion to include CDI, VHA hopes to be able to continue to reduce the rates of CDI.

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CLOSTRIDIUM DIFFICILE: EPIDEMIOLOGY

CONTRIBUTION OF A GOVERNMENT TARGET TO CONTROLLING *C. DIFFICILE* IN THE NHS IN ENGLAND

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The introduction of mandatory surveillance of *Clostridium difficile* infection (CDI) in 2004 showed the scale of the challenge: cases in patients >65 years old reached 55,681 in 2006. The first type 027 outbreaks had been in 2005 and CDI was a headline issue. The prevention and control of CDI requires a tripartite partnership between clinicians, health service managers, and the government/ Department of Health which needs to set standards, ensure that CDI is a priority, set targets and monitor outcome. Government can also legislate; the Health Act 2006 introduced a statutory Code of Practice for infection prevention and control for the NHS and extended to all independent health and care settings in 2010. In 2008, a national target was set for a 30% reduction in CDI by 2010/11 (baseline 2007/8). It was population-based and set a standard (ceiling) rate /10,000 in each area, within which acute hospitals had a target /1,000 admissions (diagnosed after day 3). In the first year (2008/9), a 35% reduction was achieved from 55,499 to 36,079 cases in all ages. The January-March figure (>65yo) for 2009 was 6776 compared with 15349 in 2006 and 15644 in 2007. The July-September 2009 total was 6423, 52% down from 2007 (61% fall in acute hospital cases, 40% in others); cases >65yo were 29% down from 2008 but only 9% down in the 2-64yo group; cases in acute hospitals (3100) were slightly fewer than in other settings (3323). Death certification showing CDI fell for the first time in 2008; 5931 total mentions (8324 in 2007) of which 2502 (42%) were as underlying cause (4056, 49%, in 2007). The reductions in CDI have been achieved by a raft of measures. Crucially, the targets focused management emphasis on infection prevention and control. This was supported by enhanced surveillance. Clinical practice protocols were implemented through the high impact interventions (care bundle) approach, and there was a major emphasis on cleanliness and hygiene (particularly hand washing for clinical staff and environmental cleaning and disinfection in patient areas). Achievement of the target is not the end of the road; it is to be transformed into an objective (benchmark) for 2011 and beyond based on median rates to maintain pressure for reduction.

Anaerobe 2010
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Anaerobe Society of the Americas

Philadelphia, PA USA • July 7-10, 2010

CLOSTRIDIUM DIFFICILE: EPIDEMIOLOGY

MOLECULAR EPIDEMIOLOGY AND SUSCEPTIBILITY PROFILES OF *CLOSTRIDIUM DIFFICILE* ISOLATES IN NEW ZEALAND, 2009

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Little is known about the *Clostridium difficile* strains that are currently circulating in New Zealand or whether the epidemic hypervirulent strain, PCR ribotype 027 is present.

Eight laboratories, from throughout New Zealand, participated in the survey. Faecal specimens that were *C. difficile* toxin positive were submitted for culture. Specimens were cultured on to CCF agar and isolates were identified by their colonial appearance and typical biochemical profile. Susceptibility testing was carried out using the agar dilution MIC method and, where appropriate, CLSI interpretive criteria were applied. The antimicrobial agents tested were penicillin, piperacillin-tazobactam, vancomycin, ciprofloxacin, moxifloxacin, clindamycin, clarithromycin, meropenem and metronidazole. Isolates were PCR ribotyped according to the method used by the National Public Health Service for Wales. Reference strains of ribotypes 001, 017, 027 and 106 were included.

Between 1st February and 2nd June 2009, 159 toxin-positive faecal specimens were cultured. *C. difficile* was isolated from 103 specimens collected from 97 patients. Most isolates were fully susceptible to the range of antimicrobial agents tested. Isolated resistance to macrolides, clindamycin and fluoroquinolones was seen. Forty-one distinct ribotyping profiles were identified among the 103 isolates. These profiles are currently being reviewed by the Wales reference laboratory to identify those that match known ribotypes.

There was a wide range of *C. difficile* ribotypes circulating in New Zealand. Some types were common to several patients and more than one geographic area. A cluster of patients with the same type was only evident in one area. The hypervirulent strain, PCR ribotype 027, is not present in New Zealand and antimicrobial resistance currently is uncommon.

Anaerobe 2010
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CLOSTRIDIUM DIFFICILE: EPIDEMIOLOGY

CHARACTERIZATION OF *CLOSTRIDIUM DIFFICILE* STRAINS ISOLATED FROM INPATIENTS AND ENVIRONMENT OF PUBLIC AND PRIVATE HOSPITALS IN RIO DE JANEIRO, BRAZIL

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There are a few reports concerning the role of *C. difficile* as antibiotic-associated diarrhea (AAD) agent and investigating the spread of clonal types inside hospital units in Brazil. The aim of this work was to isolate *C. difficile* strains from stool and hospital environmental samples in two hospital units in the city of Rio de Janeiro and characterize them. All samples were submitted to standard culture techniques and the stool samples were tested for the presence of toxins A and B using a commercial immunoenzimatic assay (ELISA). Positive samples were identified by phenotypic and genotypic methods. PCR was used to confirm the presence of *tcd*A, *tcd*B, binary toxin genes and to investigate possible deletions at *tcd*C. PCR-ribotyping and PFGE were used as molecular typing tools and the MIC of the isolates to antimicrobials was accessed with E-test. Samples from hospital 1 (public university hospital) were obtained between March of 2008 and September of 2009 and samples from hospital 2 (private hospital) from March to July of 2008. We received 81 stool samples and 67 from the environment. Concerning hospital 1, it was not possible to recover *C. difficile* from environment samples, but 27.1% (19/70) of the stool samples were ELISA-positive and eight of them were also culture-positive. Regarding hospital 2, thirteen stool samples were ELISA-positive and it was possible to isolate the bacteria from one environment sample (lavatory sink) (2.5%) and from three stool samples (23%). All the isolates were positive for the presence of *tcd*A and *tcd*B genes, and negative for *cdt*. They also did not present any significative deletions on *tcd*C. Molecular typing revealed two clonal types at hospital 2, being one type isolated from two different stool samples (belonging to the Brazilian ribotype 135) and the other isolated from one stool and other environment sample. In hospital 1 four clonal types were detected and 50% (4/8) of them belonged to the Brazilian ribotype 133. All the isolates were sensitive to metronidazole, vancomycin and moxifloxacin and resistant to clidamycin, ciprofloxacin and levofloxacin. Resistance genes are under investigation. This work shows the presence of *C. difficile* in inpatients presenting AAD and in the environment of hospitals in Rio de Janeiro, demonstrating the spread of this microorganism and warning against possible development of outbreaks.

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Anaerobe 2010
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Anaerobe Society of the Americas

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CLOSTRIDIUM DIFFICILE: EPIDEMIOLOGY

ANALYSIS OF PREVALENCE, RISK FACTORS AND MOLECULAR EPIDEMIOLOGY OF *CLOSTRIDIUM DIFFICILE* INFECTION IN KUWAIT OVER 3 YEAR PERIOD

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Background: The incidence of *Clostridium difficile* infection (CDI) has been increasing in recent years. The aim of the study was to investigate the prevalence, epidemiology and risk factors of CDI and to determine the ribotypes responsible for CDI and ribotype 027 in Kuwait.
Materials and methods: Stool samples of patients with diarrhoea were collected and sent to the Anaerobe Reference Laboratory for *C. difficile* toxin A and B detection and culture over 3 year period 2003-2005. Demographic data and data on risk factors for CDI were carefully recorded. PCR ribotyping was performed for the isolates.
Results: A total of 697 patients were investigated of which 73 (10.5%) were positive for *C. difficile* toxin A and B. Of these, 56 (76.7%) were hospital-acquired and 17 (23.3%) outpatient cases. The prevalence of hospital-acquired CDI was c.8%. About 43% of CDI patients were aged >=60 years and 79% of these were aged >=71 years. Only 23.2% of patients were in younger age group (41-60 years). About half of the patients developed CDI within 4-10 days of admission to the hospital. Nasogastric tube feeding (P<0.025), immunosuppressive drugs (P<0.031) and exposure to specific antimicrobial therapy (P<0.001) in CDI patients versus controls were statistically significant risk factors. *C. difficile* was isolated in only 38 (67.9%) patients. These isolates belonged to 16 different ribotypes. Ribotype 002 (18.4%) was the commonest, followed by ribotype 001 (15.7%), 126 (10.5%) and 140 (10.5%). No PCR-ribotype 027, 017 or 078 was encountered in this series.
Conclusion: Prevalence of hospital-acquired CDI was c.8%. Exposure to the third-generation cephalosporins, nasogastric tube feeding and immunosuppressive therapy were the most significant risk factors for CDI. The most common ribotypes were 002 and 001 and no hypervirulent 027 strain was encountered.

Anaerobe 2010
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Philadelphia, PA USA • July 7-10, 2010

CLOSTRIDIUM DIFFICILE: EPIDEMIOLOGY

EPIDEMIOLOGICAL MODEL FOR *CLOSTRIDIUM DIFFICILE* TRANSMISSION IN HEALTH CARE SETTINGS

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Purpose: *C. difficile* is the leading cause of infectious diarrhea in hospitals. Recent outbreaks of *C. difficile* infection (CDI) have been difficult to control, and data indicate the sources of transmission may have changed. Our objectives were to evaluate the relative contributions of asymptomatically and symptomatically colonized patients to new infections and to determine the most important epidemiological factors influencing *C. difficile* transmission.
Methods: Data from six medical wards at a large tertiary care hospital and published literature were used to develop an epidemiological compartmental model of *C. difficile* transmission. The next generation matrix method was used to quantify the contribution of the different sources of transmission and to estimate the basic reproduction number (R_0). Patients could be in one of five transition states in the model: resistant to colonization (R), susceptible to colonization (S), asymptomatically colonized without protection against CDI (C^-), asymptomatically colonized with protection against CDI (C^+), and patients with CDI (D). Plausible ranges of parameter values were obtained from the available data.
Results: The contributions of C^-, C^+ and D patients to new infections were, respectively, .18 to 1.5, .20 to 2.3, and .45 to 2.10 new cases. These results indicate the relative contribution of asymptomatic carriers is similar to patients with CDI. The distribution of the simulated R_0 ranged from .18 to 1.5, with median .5. These values suggest that for a wide range of parameter values, transmission within the ward alone cannot sustain *C. difficile* colonization, and therefore, the admission of colonized patients plays an important role in sustaining transmission in the ward. The epidemiological parameter that ranked as the most influential was the transmission coefficient for asymptomatic carriers. Other epidemiological parameters that strongly influenced R_0 were those related to patient susceptibility (antibiotic prescription per day rate, proportion of admitted susceptible patients, and restoration rate of the colonization resistance).
Conclusion: Our study underscores the need to further evaluate the role of asymptomatically colonized patients in *C. difficile* transmission and to better characterize patient susceptibility to *C. difficile* colonization.

Anaerobe 2010

The 10th Biennial Congress of the
Anaerobe Society of the Americas

Philadelphia, PA USA • July 7-10, 2010

CLOSTRIDIUM DIFFICILE: EPIDEMIOLOGY

A LARGE DIVERSITY OF *C. DIFFICILE* ISOLATES, INCLUDING NOVEL NAP TYPES, CIRCULATE IN COSTA RICAN HOSPITALS

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The number and severity of cases of *Clostridium difficile* associated disease (CDAD) has markedly increased in some Costa Rican hospitals during the last year. In this study, we analyzed 92 isolates of *C. difficile* from 9 hospitals as regards their macrorestriction patterns (PFGE), toxin genotypes (PCR), and susceptibilities to metronidazole, vancomycin, clindamycin, moxifloxacin, ciprofloxacin and rifampicin (E-test) to elucidate their diversity.

Thirty-one *Sma*I PFGE patterns were observed. Almost half the isolates exhibited the patterns of the already described NAP1 (n=35), NAP2 (n=2), NAP4 (n=3), NAP6 (n=3), and NAP9 (n=2) strains. However, a group of 25 isolates were named North American Pulsovar Costa Rica 1 (NAPCR1).

NAPCR1 were *tcdA*+, *tcdB*+ and contained the same 18 bp deletion in the *tcdC* gene as was found in the NAP1 strains. However, we do not expected these isolates to overproduce toxins because they do not have the 1 bp deletion that causes the loss of *tcdC* function. NAPCR1 isolates, which are more closely related to NAP9 strains than to any other of the NAP types detected, have been found in 6 hospitals so far.

The *tcdA* and *tcdB* genes were detected in 84 isolates. By contrast, the gene *cdtB* was only found in 36 toxigenic isolates from 3 different *Sma*I patterns. Most of these *cdtB*+ bacteria were NAP1 strains. The *Sma*I pattern of a *cdtB*+ isolate was similar to those of some atoxigenic isolates and one atoxigenic strain had a macrorestriction pattern that resembled those of the NAP6 and NAP4 strains.

All isolates were susceptible to metronidazole and vancomycin. In accordance to their genetic relatedness, the NAPCR1 and NAP9 strains were without exception resistant to clindamycin, moxifloxacin, ciprofloxacin and rifampicin. The NAP1 strains, in turn, were categorized as resistant to fluoroquinolones, susceptible to rifampicin, and showed variable MICs of clindamycin.

Given that some patterns were common to different hospitals, we conclude that a large diversity of *C. difficile* strains circulate in Costa Rican hospitals. This finding raises questions regarding the hygiene and the effectiveness of the infection control measures taken at the hospitals analyzed.

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CLOSTRIDIUM DIFFICILE: EPIDEMIOLOGY

CLOSTRIDIUM DIFFICILE IN THE HOSPITAL ENVIRONMENT

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Objectives: *Clostridium difficile* Infection (CDI) has become a growing concern world-wide with an increased reported incidence among patients admitted to surgery. Our aim was to assess the presence of *Clostridium difficile* spores within the surgical wards of a large tertiary referral hospital, to assess the common areas contaminated and the effects of introducing new cleaning procedures, from December 2007 to January 2009.
Methods: 4 acute surgical wards were included and 180 CCEY (cefoxitin, ceftriaxone and egg yolk) contact plates were used every 4-6 weeks to sample the environment. The same areas were sampled on each occasion in the same manner over a 14 month period. At the 7 month time point chlorine based cleaning agent was introduced in addition to the original non-chlorine based detergent. The plates were incubated for 5 days and all *Clostridium difficile* colonies were tested for toxins A+B.
Results: Over the study period 5% of all contact plates were positive for toxins A+B producing *Clostridium difficile* colonies. One ward had 80% fewer positive plates than the other wards. This is likely to represent the differing patient cohort within the ward (overnight stay/daycase patients). Contaminated areas included bed frames, patient tables, bedside lockers, door handles, sinks, bathroom floors and electronic sphygmomanometers. Bed frames produced the greatest number of positive contact plates when compared with the other surfaces tested. Only 11% of the positive contact plates were from rooms which were occupied by a patient with CDI at the time of sampling. There was an initial 86% reduction in the number of positive contact plates following the introduction of chlorine based cleaning agents during months 7-8. Following this initial reduction, a plateau of between 48-62% reductions was maintained for the remaining months when compared to the first 6 month period.
Conclusions: The environment is an important reservoir for *Clostridium difficile* spore contamination and hence potential patient development of CDI. Attention to the cleaning of areas which may be overlooked such as electronic sphygmomanometers should be paid. The use of chlorine based cleaning agents and meticulous cleaning procedures are vital components of infection control procedures and the control of CDI dissemination.

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CLOSTRIDIUM DIFFICILE: EPIDEMIOLOGY

IDENTIFICATION OF *CLOSTRIDIUM DIFFICILE* IN COLORECTAL SURGERY

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Clostridium difficile infection (CDI) has become a growing concern world-wide with an increased reported incidence among patients admitted to surgery. Our aim was to review prospectively the role of toxigenic culture in the diagnosis of CDI in colorectal surgical in-patients from December 2007 to January 2009.

All faecal samples, submitted to Lothian University Hospitals Division, were processed in a single enteric laboratory following national guidelines – all diarrhoeal samples from hospital in-patients aged 1 year and above were tested for *C. difficile* toxins A and B by enzyme immunoassay. All faecal samples submitted to the laboratory from colorectal surgical in-patients were reclaimed for toxigenic culture (culture on selective media and EIA for toxin detection).

Samples (632) from 483 adult patients (median age 71 years, ranging from 18-100 years) were reclaimed for toxigenic culture. Of these 105 samples (16.6%) were found by the laboratory to be positive by EIA. Following toxigenic culture a further 72 samples (11.4%) were identified as positive. 38 patients, who were symptomatic at the time of testing, were not identified with CDI during their admission.

Five patients whose samples were not tested as they were not diarrhoeal (≤ 4 on the Bristol stool chart) were toxigenic-culture-positive. These patients were symptomatic of CDI and subsequent diagnosis was delayed in three of these patients and not diagnosed in two patients. Seven samples found to be toxin positive by the lab were culture negative.

CDI diagnosis or recognition at present may be delayed, as with current national guidelines CDI detection is based solely upon *C. difficile* toxin detection. Current resources cannot support toxigenic culture for all suspected samples and this has implications in regards to delayed treatment, further patient management and infection control procedures. With current testing only for diarrhoeal samples, a proportion of patients with CDI may go undiagnosed or have diagnosis delayed.

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CLOSTRIDIUM DIFFICILE: EPIDEMIOLOGY

CLOSTRIDIUM DIFFICILE IN THE PAEDIATRIC POPULATION

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Aims: This study was performed to identify and compare the molecular epidemiology of *Clostridium difficile* from stool samples in paediatric patients with symptoms of diarrhoea and establish the patient characteristics of the population from which these isolates were obtained.
Methods and Results: *C. difficile* toxin-positive stool samples obtained in the Lothian area of Scotland between August 2007 and August 2008 from patients with diarrhoea under 17 years of age were included in the study. From 47 patients 54 stool samples yielded positive cultures and were further analysed.

Five colonies from each stool sample were initially typed by S-layer typing to determine if more than one type existed in some samples. No differences were found in the S-layer types within each sample. Type 5238 was the most common identified accounting for 22% of isolates, followed by type 5043. Other types identified included 4945, 4942, 5044 and 5449.

PCR ribotyping was carried out on one colony per stool sample according to O'Neill *et al* (1996) with modifications by the Anaerobe Reference Unit (Cardiff UK).

Ribotype 015 was the most common (34%) followed by 106. Ribotypes 001, 002, 005, 020, 023, were also identified.

Of the 47 patients, 18 were female. The ages of the patients ranged form <1 to 16 years with a mean of 3.4 years and a median of 1 year. Half of patients were potentially immunosuppressed at the time the stool sample was taken and 65% were suffering from an underlying medical condition including cancer, Crohn's disease and cystic fibrosis. Another organism capable of inducing diarrhoea was identified in 15% of samples within one month of the *C. difficile* positive stool. These were identified as rotavirus, norovirus and enterovirus.
Conclusion: The presence of ribotypes 001 and 106 are consistent with data available for the adult population in Scotland, where 106 is the dominant ribotype followed by 001. In 2005 015 was identified as the fourth most common ribotype isolated in UK hospitals however, it is not a common type isolated in South-east Scotland.

This study did not find any cases of 027 which follows data for the adult population that apart from a single isolated case in 2008, Edinburgh is free from the epidemic strain.

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CLOSTRIDIUM DIFFICILE: EPIDEMIOLOGY

EPIDEMIOLOGY AND ANTIBIOTIC SUSCEPTIBILITY OF COMMUNITY-ACQUIRED AND NOSOCOMIAL *CLOSTRIDIUM DIFFICILE* STRAINS IN HUNGARY

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Objectives: Since 2003, rising incidence of *C. diffcile* infections is getting more and more important; this has been attributed to the increased consumption of broad-spectrum antibiotics among hospitalized patients and in the community, the increased number of immunocompromised patients, the ageing population and recently, the presence and the rapid spread of a hypervirulent PCR ribotype 027/NAP1/toxinotype. The increased antibiotic consumption may have impact on the emergence of multiple resistant *C. difficile* strains, therefore our aims of this survey were to continue our previous investigations, in which the presence of the major toxin genes (*tcd*A and *tcd*B) and binary toxin genes of *C. difficile* isolated from human diarrhoeal faeces was determined. At the same, antimicrobial susceptibility and further genotypic feature of the isolated strains, deletions in the *tcd*C were characterized.
Methods: 200 *C. difficile* strains isolated between 2008 and 2009 in various Hungarian laboratories from diarrhoeal faeces of both inpatients and outpatients were analyzed. The presence of toxin genes (*tcd*B, *cdt*B and the 3'end of the *tcd*A), and *tcd*C gene were detected by PCR in the Anaerobe Reference Laboratory (Szeged, Hungary). Antibiotic susceptibility of the isolated strains was determined by E test (Solna, Sweden) for metronidazole, moxifloxacin, clindamycin, erythromycin and rifampicin.
Results: During the recent and earlier study periods, the majority of the isolated strains (80-90%) collected in various laboratories proved to be positive for both toxin A and B using PCR. It seems that the prevalence of binary toxin positive strains is increasing from year to year. Among toxin positive isolates, *tcd*C gene PCR showed various deletions in several isolates. All of the tested isolates were sensitive to metronidazole, while the prevalence of isolates resistant to moxifloxacin, erythromycin, clindamycin and rifampicin is increasing, if we compare recent findings to our earlier results.

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