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June 24-27, 2008

SESSION VII—CLOSTRIDIUM DIFFICILE: EPIDEMIOLOGY

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CLOSTRIDIUM DIFFICILE: EPIDEMIOLOGY

HEALTHCARE AND COMMUNITY-ASSOCIATED *CLOSTRIDIUM DIFFICILE* INFECTION (CDI) IN NORTH AMERICA

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From 2000 to 2005, both rates and absolute numbers of U.S. CDI-associated hospitalizations increased between two to three-fold, with a 25% increase in 2003-2004 and another 10% in 2004-2005. Meanwhile, mortality is increasing; the rate of death certificates indicating CDI as a cause of death increased nearly four-fold from 1999 to 2004. The mortality attributable to CDI has been 5-16% in recent studies, depending upon consideration of indirect in addition to direct mortality, the time period over which mortality is measured, and whether or not it is measured during an outbreak. This appears increased over historic figures ranging from 1-2%. Excess healthcare costs attributable to CDI total more than $1 billion annually for the United States alone. Increased CDI incidence and mortality appears due to spread of an epidemic strain known variously as North American Pulsed-field (NAP1), restriction endonuclease analysis type "BI", or PCR ribotype 027. NAP1/BI/027 carries an extra toxin in addition to toxins A and B, known as binary toxin, and possesses polymorphisms in the toxin negative regulatory gene, *tcdC*. The latter may be in part or wholly responsible for toxin hyper-production with 16-fold increased toxin A production and 23-fold increased toxin B production documented in vitro. This strain, although historically uncommon, has become an epidemic strain coincident with it becoming more resistant to the fluoroquinolones. It has now been identified in at least 38 states and throughout Canada. Outbreaks caused by NAP1/BI/027 continue to be reported in association with a variety of antimicrobials, but especially fluoroquinolones. This may reflect the prevalence of fluoroquinolone use as well as the selective advantage that NAP1/BI/027 has in environments where fluoroquinolones are used widely. Although substituting one fluoroquinolone for another is unlikely to be successful in controlling outbreaks caused by NAP1/BI/027, there may be a role for restricting all fluoroquinolone use. Previously low-risk populations in which CDI may be becoming more common include peripartum women, in whom disease may be life threatening, and persons living in the community including, but not limited to, otherwise healthy persons. There are now several reports suggesting antimicrobial exposure, a ubiquitous factor for CDI in hospitalized patients, may be absent in a significant proportion of patients with community-associated CDI.

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CLOSTRIDIUM DIFFICILE: EPIDEMIOLOGY

EMERGING *CLOSTRIDIUM DIFFICILE* STRAINS IN NORTH AMERICA

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Prior to 2001, a single *Clostridium difficile* strain, designated PCR ribotype 001/ REA type J/ toxinotype 0 predominated among hospitalized patients with *C. difficile*-associated disease (CDAD) in the United States and England. This strain was highly resistant to clindamycin, use of which was believed to be an important factor in its emergence. Since 2001, however, a new epidemic strain has emerged in association with increases in both incidence and severity of CDAD in the US. This strain, designated PFGE type NAP1/ PCR ribotype 027/ REA type BI/ toxinotype III has been identified throughout the US, Canada, and Europe, and also in Japan. This strain makes an additional toxin, termed binary toxin, produces more of toxins A and B than historic strains with a wildtype pathogenicity locus, and contains truncating mutations in the toxin regulatory gene, *tcdC*. In contrast to historic *C. difficile* strains, including those with indistinguishable REA and PFGE types, current 027/ BI/ NAP1 strains from human disease are uniformly resistant to fluoroquinolone antibiotics. Concurrent with the nationwide increase in human CDAD and the emergence of the NAP1/027/BI strain, outbreaks of *C. difficile* infection have been reported in pork production operations in the US, Canada, and Europe. The strain most commonly associated with disease in neonatal pigs, PCR ribotype 078/ REA type BK/ Toxinotype V, is further divided into types NAP7 and NAP8 by PFGE. Although its role in disease in somewhat unclear, this strain is also common among dairy calves in the US. Like the human epidemic strain, these strains causing disease in neonatal pigs have a variant toxinotype, produce binary toxin, and contain truncating deletions in *tcdC*. These strains were previously considered uncommon causes of disease in humans, but recent reports demonstrate an increase in isolation of 078/ Toxinotype V strains from both healthcare- and community-associated human CDAD in the US and Europe. Finally, isolates from community-associated CDAD in two US studies demonstrate considerable strain diversity, including PFGE types and toxinotypes that were previously rare or unreported, as well as novel combinations of molecular characteristics.

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CLOSTRIDIUM DIFFICILE: EPIDEMIOLOGY

MOLECULAR EPIDEMIOLOGY OF *CLOSTRIDIUM DIFFICILE* INFECTION IN THE UNITED KINGDOM

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Since 1996, PCR-ribotyping has been applied to several thousand isolates of *C. difficile* at the UK Anaerobe Reference Laboratory and more than 180 distinct ribotypes have been detected.

In the late 1990's, with diagnosis of *C. difficile* infection based upon direct detection of toxins, few UK laboratories cultured for *C. difficile* and hence, relatively little typing or susceptibility testing was performed. Although epidemiological data from these years is patchy, general trends are clearly apparent. In 1996 ribotype 001 dominated in ~50% UK hospitals sampled and, subsequently, ribotype 106 slowly gained prominence. Only 2 cases of ribotype 027 were detected, and these were geographically and chronologically distinct.

However, in 2004, interest was awakened by escalating numbers and severity of cases and possible association with usage of new fluoroquinolones. The previously rare ribotype 027 predominated in a major high-profile outbreak in South-East England and was soon detected in many other regions. This strain was epidemiologically indistinguishable from the hyper-toxin producing strain of major significance in Canada and the USA and now designated BI/NAP1/027.

In 2005 the government instigated mandatory surveillance throughout England to determine ribotype and susceptibilities to eight antibiotics of ~1000 *C. difficile* isolates (~2% cases) per annum. This on-going study provides a more reliable 'snapshot' of prevalent types and antimicrobial susceptibilities and may detect any significant changes in these patterns over following years. The first year's data demonstrated that three ribotypes; 001, 106 and 027 accounted for the majority of *C. difficile* infections in the UK with some regional variations in relative prevalence. Comparative analyses for 2006 and 2007-2008 are in progress. Ribotypes 001, 027, and 106 are significantly more resistant than other UK ribotypes to fluoroquinolones (moxifloxacin MIC_{50} 32 and 2mg/L, respectively) and this may be an important factor in strain selection.

Increased morbidity and mortality associated with strain BI/NAP1/027 may be linked to an 18bp deletion in the *tcdC* gene. However, at the Anaerobe Reference Laboratory, similar or larger gene deletions have been detected in 26 'non-epidemic' ribotypes, while no deletions in this region were detected in 'epidemic' ribotypes 001 or 106. From the limited clinical data available, no clear association of ribotype with severe, relapsing, or fatal disease can be made but the ribotypes responsible for cases with poor outcomes broadly reflect the relative prevalence of those strains.

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CLOSTRIDIUM DIFFICILE: EPIDEMIOLOGY

CHANGING CLINICAL EPIDEMIOLOGY AND PREVENTION INITIATIVES FOR *CLOSTRIDIUM DIFFICILE* INFECTION IN EUROPE

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Clinical epidemiology is the science of making predictions about individual patients by assessing clinical events in similar groups of patients. For *Clostridium difficile* infections (CDIs), it has generally been assumed that elderly, hospitalised patients receiving antibiotics are the at-risk patient group, and they can present with disease with a spectrum ranging from mild-self-limiting diarrhoea through to fulminant life-threatening colitis. Treatment has been withdrawal of the precipitating antibiotic—if possible, followed by treatment with metronidazole or vancomycin. Most epidemiological studies have concentrated on such patients and mandatory reporting has tended to require only patients over 65 to be included in returns. However, there is now a perception that both the patient groups and the spectrum of disease are changing—with involvement of younger patients—with more cases of serious disease.

A prospective study carried out in 14 European countries in 2005 established baseline data for the EU in respect to the types of strains present (66 ribotypes, four major toxinotypes), the mean incidence of CDI (2.45 cases/10000 patient days but wide variation) and the antibiotic resistance patterns. The emergence of the epidemic 027/NAP1/BI strain was seen in several countries and counted for 6.2% of all isolates.

Since the emergence and continuing increase of this epidemic strain in both Europe and North America – which certainly seems to cause more severe disease—and the increased clinical awareness of CDI, many studies have been started to (re-)investigate the at-risk groups and if the spectrum of disease is changing. However, many of the studies done in the early 1980s identified risk factors, patient susceptibility, and epidemiology in different groups, and much of this work seems to have been forgotten! In some countries, particularly the UK and Canada, the news media have been exceptionally active, and CDI is now well recognised by the general public. This has resulted in pressure from politicians to define why so many patients are dying from this previously unheard-of "superbug".

Public awareness and worries of litigation have probably resulted in over-treatment, and self-limiting diarrhoea is no longer recognised – as treatment is likely to be started if patients in at-risk groups are diagnosed with CDI. Cases in the younger age groups, including paediatrics, peripartum, women and young adults, cases in the community and even possible zoonotic transmission all require further study. On-going surveillance studies, more clear definitions of cases and disease severity, and development of new treatment regimens are rapidly moving fields. This presentation will attempt to summarise the latest situation on the east side of the Atlantic.

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CLOSTRIDIUM DIFFICILE: EPIDEMIOLOGY

PREVALENCE OF EPIDEMIC REA TYPES OF *CLOSTRIDIUM DIFFICILE* FROM A RECENT EUROPEAN CLINICAL TREATMENT TRIAL

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Isolates of *Clostridium difficile* (CD) were obtained from patients enrolled in a large European prospective CD-associated disease (CDAD) treatment trial conducted from 2005-2007 to determine the frequency and distribution of known epidemic groups of CD, as identified by restriction endonuclease analysis (REA) typing.

CD toxin positive frozen stools were submitted to the Microbiology Reference Laboratory at Hines VA Hospital for isolation of CD and REA typing of the recovered isolates as part of a CDAD treatment trial. The trial, sponsored by Genzyme Corp, compared the toxin-binding polymer tolevamer to metronidazole and vancomycin. Samples were collected from 89 sites in 14 countries (AUS, AUT, BEL, CZE, DNK, FRA, DEU, IRL, NOR, PRT, ESP, SWE, CHE, GBR). The pre-treatment samples yielded 332 isolates from 435 stools. Recurrence samples yielded 42 isolates from 46 stools. The REA patterns were compared to 7 major REA groups that have caused CD outbreaks over the past 25 years (Groups B, Y, J, K, G, BI, and CF). Patterns with a 90% similarity index were placed in the same REA group.

Of the 332 pretreatment isolates, 140 (42%) were from 1 of 3 REA groups. REA group J was the most common: 18% (60/332) found in 33 sites in 12/14 Countries. Group Y, 15% (55/332) was found in 32 sites in 11/14 countries. REA Group BI (NAP1/027), 8% (25/332) was found in 8 sites in 3/14 countries (BEL, IRL, GBR). Of the recurrence isolates, Group Y (29%) was significantly more frequent than Groups J (10%) or BI (10%).

REA Group J was the most prevalent group in number of isolates and geographically. Group Y was a close second in both number of isolates and number of countries. REA Group BI (NAP1/027) was seen relatively infrequently in only three countries outside North America. These results contrast with a previous similar trial done in North America in which 35% of isolates were group BI/NAP1, 12% were group J and 9% were group Y.

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CLOSTRIDIUM DIFFICILE: EPIDEMIOLOGY

TACKLING *CLOSTRIDIUM DIFFICILE* IN THE ENGLISH NHS

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The outbreak of *C. difficile* infection (CDI), ribotype 027, at Stoke Mandeville Hospital in 2004-2005 was a watershed in England. A disease considered a clinical nuisance of low priority was thrust into prominence; its prevention and control is now an NHS priority. CDI surveillance had shown a rise in reported cases from *c*.500 in 1990 to 43,672 (England, Wales and N. Ireland) by 2004. Mandatory reporting of CDI in patients over 65 years old began in 2004 and rose to 55,681 in 2006 (England). Type 027 was causing outbreaks elsewhere and the government instructed the Healthcare Commission to investigate Stoke Mandeville. Its report (July 2006) found deficiencies in patient care and hospital management; infection control advice was not accepted, patients were moved frequently, laboratory diagnosis was slow, and patients were not isolated/cohorted appropriately. An earlier HCC/HPA report (Dec. 2005) showed that 66% of hospitals were not implementing the 1994 national guidance and the CMO and CNO issued interim guidance on the investigation, control, and prevention of CDI. In July 2006, a high impact intervention care bundle for CDI was added to the Department of Health (DH) programme for healthcare associated infections. In Dec. 2006, DH announced local targets set by Primary Care commissioners and the acute hospitals for reducing CDI, supported by enhanced surveillance of all patients over 2 years old. The Health Act (2006) Code of Practice gave legal force to responsibility for infection prevention and control. Further CMO and CNO guidance re-emphasised prompt diagnosis; immediate isolation/cohorting of infected patients; antibiotic stewardship; stringent infection control with hand washing; and enhanced cleaning/disinfection. In July 2006 another serious outbreak came to light at Maidstone and Tunbridge Wells. It had lasted over a year but had not been reported; the HCC again investigated. Its report (Oct. 2007), found deficiencies in management, inadequate patient care, poor infection prevention and control and inadequate isolation. There were requirements for changes at the hospitals and national recommendations to raise the recognition of CDI as a principal diagnosis requiring immediate clinical attention and management focus. The recording of deaths involving CDI also needed review. DH announced in Sept. 2007 a national target to reduce CDI by 30% during 2008-2011. Increased funding was allocated for cleaning, and the DH improvement teams that visit hospitals to help with HCAI action plans would address CDI. NHS performance management will focus on reducing CDI through clinical, managerial and government partnership.

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CLOSTRIDIUM DIFFICILE: EPIDEMIOLOGY

CLOSTRIDIUM DIFFICILE IN THE COLORECTAL SURGICAL POPULATION

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Aims: *Clostridium difficile* infection (CDI) cases are increasing, particularly within the surgical in-patient population who are a high-risk group for the development of CDI. Our aim was to review the prevalence of *C. difficile* in the in-patient population presenting to Edinburgh University Hospitals Trust Colorectal Surgical Unit from 2000-2006 inclusive and to assess further the data set for 2006.

Methods: Patients were identified via Medical Microbiology and Lothian Surgical Audit databases. All patients admitted to the colorectal surgical unit who were tested for *C. difficile* by EIA for Toxins A+B were included. Retrospective analysis of prospectively collected outcome data was then performed.
Results: There has been an exponential increase in the number of colorectal surgical in-patients diagnosed with CDI from 2000-2006, commencing with 4 patients in 2000 and increasing to 128 patients in 2006.

Further analysis of data for 2006 revealed that 945 faecal samples were tested for *C. difficile* toxins of which 151 samples were positive. Taking into account multiple samples testing for individual patients, 128 patients were diagnosed with CDI out of 643. Of the 128 positive patients there was a slight male preponderance (male: female = 75:53) and the median age was 74 years (range 18-92 years). Only 62% of patients were found to be toxin A+B positive on their first sample. For the other patients a median period of 10 days (range 2-80 days) was conceded prior to detection of *C. difficile* toxins. From the time of their first negative sample being assessed to the ultimate positive sample, a median of 3 (range 2-6) samples were sent prior to detection.

Conclusion: The number of individual patients treated for CDI by the Colorectal Surgical Service has markedly increased. Culture in conjunction with toxin testing may have led to an earlier diagnosis in this high-risk group of patients. Mandatory data reporting is only required for patients over 65 years in Scotland resulting in 23% of these patients being excluded from national figures.

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CLOSTRIDIUM DIFFICILE: EPIDEMIOLOGY

CLOSTRIDIUM DIFFICILE: WHICH SPECIALTIES ARE AFFECTED?

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Aims: *Clostridium difficile* infection (CDI) is primarily associated with specialties catering to an elderly population. Our aim was to assess the various specialties affected by CDI in the in-patient population presenting to Edinburgh University Hospitals Trust (EUHT) in 2006.

Methods: Patients were identified via Medical Microbiology and Lothian Surgical Audit databases. All patients admitted to EUHT who were tested for *C. difficile* by EIA for Toxins A+B were included. Retrospective analysis of prospectively collected outcome data was then performed

Results: 14598 faecal samples were tested for *C. difficile* toxins in 2006, and 2024 of these were positive. Medicine for the Elderly patients accounted for 36% of all positive samples. Both Gastrointestinal Medicine and Colorectal Surgery were responsible for a further 16% (8% each), followed by Oncology and Transplant patients responsible for 6% each of positive samples. When all medical specialties with the exception of Medicine for the Elderly are considered together, 34% of positive samples belonged to these patients. Additionally when all surgical sub-specialties are combined together, 30% of all positive samples belonged to surgical patients. Finally, 2% of positive samples belonged to Paediatrics.

Conclusions: Whilst high proportion of elderly patients are being diagnosed with CDI, other specialties previously categorised as low risk, such as paediatrics and maternity, are now also being affected. One third of all CDI cases are diagnosed in the surgical population when the surgical specialties are considered together, and equally one third of cases affect the other medical specialties. CDI is therefore affecting all specialties and can no longer solely be considered a disease of the elderly.

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CLOSTRIDIUM DIFFICILE: EPIDEMIOLOGY

RISK FACTORS FOR *CLOSTRIDIUM DIFFICILE*-ASSOCIATED DIARRHOEA IN A PERTH HOSPITAL

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Given recent changes being seen world-wide in the epidemiology of *Clostridium difficile*-associated diarrhoea (CDAD), we investigated if any changes were evident at Royal Perth Hospital (RPH), a 900-bed Perth teaching hospital. Our aim was to determine risk factors for CDAD. A retrospective matched case-control study was performed. Cases were *C. difficile* culture and faecal toxin positive, with onset of symptoms at least 72 h after admission. Hospital controls (3 per case) were matched by age (within 5 yrs), date of admission (< 30 d) and duration of exposure. A total of 90 cases and 261 controls was included. Data were collected on demographics, co-morbidities, pre-admission location, antibiotic use (within preceding 3 months), gastrointestinal and immunosuppressant medication, gastrointestinal procedures, and outcomes. The mean age of cases and controls was 64 yrs. More controls were male (58% vs 50%), although this difference was not statistically significant. Cases had a higher prevalence of malignancy (31% vs 18%, $p<0.01$), and peptic ulcer disease (9% vs 3%, $p<0.05$). The mean Charlson Comorbidity Index was higher for cases (2.0) than controls (1.7) ($p<0.05$). Cases had significantly higher rates of endoscopy (17% vs 7%, $p<0.01$), nasogastric tubes (18% vs 8%, $p<0.01$) and PEG feeding (12% vs 5%, $p<0.01$). Anti-diarrhoeal medications were more commonly used by cases in the preceding 3 months (13% vs 5%, $p<0.05$) and laxatives were less commonly used by cases (42% vs 59%, $p<0.01$). All-cause mortality was higher amongst cases: 14% of cases died within 30 d of onset of symptoms and 2% of controls died during the admission, and mortality in cases remained higher after controlling for age and cancer. There was no difference between cases and controls in requiring a colectomy. Piperacillin/tazobactam, ICU stay, endoscopy, anti-diarrhoeal medications and peptic ulcer disease were independently associated with CDAD on multivariate analysis. Laxatives and roxithromycin were associated with a reduced risk of CDAD. There was no association between quinolones and CDAD, which may reflect the presence of a different strain(s) of *C. difficile* in Perth. However, there was an association with piperacillin/tazobactam which needs to be considered by antibiotic stewardship committees.

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CLOSTRIDIUM DIFFICILE: EPIDEMIOLOGY

DETECTION OF BINARY TOXIN POSITIVE *CLOSTRIDIUM DIFFICILE* STRAINS AMONG CLINICAL ISOLATES FROM PATIENTS HOSPITALIZED IN NEPHROLOGY UNIT IN PAEDIATRIC HOSPITAL, WARSAW, POLAND

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The recent emergence of the epidemic binary toxin positive *Clostridium difficile* strains PCR-ribotype 027 in Europe could lead to important changes in the epidemiology of *C. difficile*-associated diseases (CDAD) among adult patients. A three years (2003-2006) study on *C. difficile* was conducted in nephrology unit of Paediatric Hospital in Warsaw.
Objective. This study investigated the frequency of binary toxin positive *C. difficile* strains among paediatric patients with antibiotic-associated diarrhoea (AAD) hospitalized in nephrology unit.

Method. The presence of *C. difficile* was investigated in 120 stool samples from 116 patients collected between 2003-2006. The isolation of *C. difficile* from fecal samples was performed using CCCA medium. Alcohol shock was performed prior the specimens inoculation on medium. Specific PCRs were used for detection of binary toxin genes (*cdtA* and *cdtB*), toxin genes A and B. PCR-ribotype of binary positive *C. difficile* strains was determined at the Reference Laboratory in The Netherlands. MICs for clindamycin, erythromycin, ciprofloxacin, moxifloxacin, gatifloxacin, metronidazole, and vancomycin were determined by E-test.

Results. Of 120 stool samples, 12 (10%) were positive for *C. difficile* A^+B^+, 4 (3,3%) for $A^+B^+CDT^+$, 5 (4%) for A^-B^-, and 4 (3,3%) for A^-B^+. All CDT strains were isolated form the same patient with hemolytic uremic syndrome (HUS) of unknown etiology and persistent gastrointestinal tract infection. All binary toxin positive *C. difficile* strains were highly resistant to newer fluoroquinolones (MIC=32 mg/L) and were susceptible to clindamycin, erythromycin, metronidazole and vancomycin. Data obtained by PCR-ribotyping confirmed that a single CDT positive ribotype 045 *C. difficile* strain was involved.

Conclusion. We conclude that AAD in a paediatric population is associated with toxinogenic *C. difficile* in approximately 16%. Binary toxin positive strains can also be found as causative agents of AAD in children, but their prevalence is low.

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CLOSTRIDIUM DIFFICILE: EPIDEMIOLOGY

TYPING AND SUSCEPTIBILITY OF BACTERIAL ISOLATES FROM THE OPT-80 (PAR-101) PHASE 2A STUDY FOR *CLOSTRIDIUM DIFFICILE*-ASSOCIATED DIARRHEA

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Background: OPT-80 (PAR-101) is the first development candidate in a new class of antibiotic, the 18-membered macrocycles. It is currently in phase 3 trials for the treatment of *C. difficile*-associated diarrhea (CDAD). An open-label, phase 2A study comparing 3 different doses of OPT-80 (50 mg, 100 mg, and 200 mg bid x 10 days) for the treatment of patients with mild to moderate CDAD was conducted previously, and the clinical results were presented in 2005. An epidemic hypervirulent *C. difficile* strain has been found to be associated with higher severity of disease and generally poorer outcomes. This strain has been typed as BI by restriction enzyme analysis (REA), as North American PFGE type 1 (NAP1), and as PCR ribotype 027. We analyzed the clinical samples from our phase 2A study to determine whether the BI strain was represented in this trial and its response to the treatments.

Methods: Fecal samples were plated on CCFA agar for isolation of *C. difficile*. These isolates were tested for susceptibility to OPT-80, vancomycin, and metronidazole using CLSI agar dilution methods. Isolates were also subjected to REA typing which designates the epidemic strain as BI group.

Results: Samples from 38 subjects (of 45) were sufficient for bacterial isolation. Of these, 16 (42%) were the epidemic *C. difficile* BI group. Antibiotic susceptibilities were consistent with the previously reported MIC_{90} values for all three antibiotics. In the top dosing group, for which 16 of 16 subjects were cured at the end of therapy, 5 of the12 (42%) positive cultures yielded *C. difficile* strains that were REA type BI.

Conclusion: These results underscore the rising incidence of the BI epidemic strain and demonstrate that the BI strain was well represented in the prior phase 2A study of OPT-80. Despite the presence of 42% BI overall and in the top dosing group, only 2 subjects in each of the two lower dosing groups and none in the top dosing group failed to achieve clinical cure.

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CLOSTRIDIUM DIFFICILE: EPIDEMIOLOGY

HAVE ANTIBIOTICS INFLUENCED THE MOLECULAR EPIDEMIOLOGY OF *CLOSTRIDIUM DIFFICILE?* A SCOTTISH STUDY OF 194 ISOLATES OVER A 27-YEAR PERIOD

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As has been described in numerous studies, the incidence of *Clostridium difficile*-associated disease has been rising over the past 30 years with the relative incidence of epidemiological types differing between regions. This study was conducted in order to determine the changes in the local epidemiology of the organism in the southeast region of Scotland over the last 27 years and to identify a possible role for antibiotic resistance in the evolution of the organism.

A total of 194 clinical isolates of *C. difficile* from 1978-2004 were selected from our extensive collection of freeze-dried cultures. Those selected were from adults with clinical diseases with only a single representative from any suspected outbreak to avoid any duplication. The isolates were typed by the PCR ribotyping technique originally described by O'Neill et al. as modified by the Anaerobe Reference Unit in Cardiff, Wales. Antibiotic susceptibility was determined by the CLSI reference agar dilution method.

Results indicated that the currently most prevalent ribotype (RT) 001 strain had gradually increased from fewer than 10% of the early isolates to more than 50% of the total in the later period. As compared to the non-predominating isolates, the resistance of RT001 to several key antibiotics has increased over time disproportionately, with higher MICs than the other strains: erythromycin, early RT001 fully sensitive as compared to 50% resistance among the later isolates; ceftriaxone, early RT001 25% resistant and later 50% resistant and moxifloxacin, early RT001 isolates fully sensitive, later ones 50% resistant. Although clindamycin resistance was very high among all ribotypes, 100% of 001 ribotypes were resistant. Interestingly, a high proportion (18.4%) of early strains of all ribotypes were resistant to tetracycline, yet none of the later strains were resistant probably reflecting the decline in tetracycline usage. Resistance to metronidazole or vancomycin was not detected. No RT027 strains were present among the tested isolates, reflecting the relative absence of outbreaks due to this strain in Scotland.

The observed increase in antibiotic resistance to some antibiotics may have enabled RT001 to out compete the other ribotypes in the presence of antibiotic selection pressure. Further studies on spore survival, virulence and genomic stability are required to understand the predominance of some epidemiological types over others.

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CLOSTRIDIUM DIFFICILE: EPIDEMIOLOGY

STRAIN DIVERSITY AMOUNG *CLOSTRIDIUM DIFFICILE* ISOLATES FROM HEALTHCARE- AND COMMUNITY-ASSOCIATED DISEASE

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Background: *Clostridium difficile* associated disease (CDAD), historically considered a healthcare associated disease, has been reported with increasing frequency among patients with no healthcare facility (HCF) exposure. Increases in incidence and severity of CDAD in HCF have been attributed to the emergence of an epidemic strain (NAP1). However, the importance of NAP1 in community-associated CDAD (CA-CDAD) remains unclear. We isolated *C difficile* from patients with healthcare facility onset–Healthcare Facility Associated (HO-HCFA CDAD) and CA-CDAD, and compared the strain types and diversity between these two groups.

Methods: All *C. difficile* toxin-positive stools at a single HCF collected between March 2005 and May 2007 were stored at -20°C. Stools were sent to the Centers for Disease Control and Prevention (CDC) anaerobe lab for culture and molecular characterization. Pulsed field gel electrophoresis (PFGE) was performed using SmaI restriction enzyme and analyzed with Bionumerics 4.01 software (Applied Math, Austin, TX). Strain types were established at ≥80% similarity using Dice coefficients and UPGMA. Other characterization included toxinotyping and PCR for the presence of binary toxin and deletions in the *tcdC* gene. Patients with symptom onset >48 hours after admission to a HCF were classified as having HO-HCFA CDAD; patients with symptom onset in the community or less than 48 hours after admission to a HCF were classified as having CA-CDAD, provided that this was >12 weeks after the last discharge from a HCF. Medical record review was performed on all patients, and patients with CA-CDAD were interviewed to rule out prior HCF exposure.

Results: *C. difficile* was isolated from 335/486 (69%) of the toxin positive frozen stools processed. Forty five percent (151) of isolates recovered were from HO-HCFA CDAD patients, 9.0% (30) were from CA-CDAD patients, and the remainder were unknown and therefore excluded. Of the HO-CDAD isolates, 54% (81) were NAP1, 11% (16) were a single unnamed group, 5% (7) were NAP7, 3% (5) were NAP3, and 26 other named and unnamed types accounted for less than 3% each. Of CA-CDAD isolates, 50% (15) were NAP1 and the remainder were made up of 14 other named and unnamed types. Simpson's index of diversity for strain types associated with HO-HCFA CDAD and CA-CDAD were 0.70 and 0.76, respectively.

Conclusion: The current epidemic strain, NAP1, was the predominant strain isolated from both HO-HCFA CDAD and CA-CDAD and thus appears to be an important cause of disease in the community as well as in healthcare facilities. *C. difficile i*solates recovered from both HO-HCFA and CA-CDAD in this study demonstrated diverse PFGE types, with slightly higher diversity among isolates from community-associated disease compared to healthcare facility-onset disease.

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CLOSTRIDIUM DIFFICILE: EPIDEMIOLOGY

CHANGING PATTERN OF *CLOSTRIDIUM DIFFICILE* EPIDEMIOLOGY IN HUNGARY

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Objectives: The 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 feces was determined, the main PCR ribotypes of the examined strains were identified, and the emergence of more virulent ribotypes was sought; the obtained results were compared with the results of the previous study periods.

Methods: 150 *C. difficile* strains were isolated in various Hungarian laboratories from diarrhoeal feces of both inpatients and outpatients. The presence of toxin genes (*tcd*B, *cdt*B and the 3'end of the *tcd*A) were detected by PCR in the Anaerobe Reference Laboratory (Szeged, Hungary). The ribotypes of these strains were determined by PCR ribotyping method.

Results and conclusion: On the basis of comparison of the present results with the data from 2001 and 2004, the prevalence of binary toxin producing strains among toxin positive isolates was increased. In 2004, three binary toxin producing strains were isolated from severe cases of diarrhea, these cases and Dr. F. Barbut's (Paris, France) data also suggested that the binary toxin producing isolates may cause more severe clinical form of CDAD than toxin A/B positive and binary toxin negative strains, however B. Geric *et al*. (Lubljana, Slovenia) showed that binary toxin alone was not sufficient to cause disease in hamster model. The increase in the prevalence of binary toxin positive isolates may be explained with the fact that the presence of binary toxin or changes in the toxin production may increase the fitness of this bacterium. Results of our present survey and previous studies have also revealed that the presence and distribution of *C. difficile* ribotypes vary from country to country, and we have shown that there can additionally be regional differences within a given country and changes in the distribution of various ribotypes were also detected.

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CLOSTRIDIUM DIFFICILE: EPIDEMIOLOGY

CHARACTERIZATION OF *CLOSTRIDIUM DIFFICILE* STRAINS ISOLATED FROM HIV POSITIVE PATIENTS IN A HOSPITAL IN RIO DE JANEIRO, BRAZIL

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Clostridium difficile is recognized as a major cause of nosocomial antibiotic-associated diarrhea (AAD) and pseudomembranous colitis. Recent reports from many countries have been associating a more severe form of AAD with a hipervirulent strain characterized by PCR-ribotyping and pulsed-field gel electrophoresis (PFGE), known as PCR ribotype 027/NAP1. Pathogenic strains of *C. difficile* produce, as main virulence factors, an enterotoxin (TcdA) and a cytotoxin (TcdB). PCR ribotype 027/NAP1 strain overproduces TcdA and TcdB, due to a deletion in its negative regulator gene *tcdC*, and also produces the binary toxin (CDT). In Brazil, there are a few reports concerning the role of *C. difficile* as AAD agent and the spread of clonal types inside hospital units. The aim of this work was to search for *C. difficile* strains from stool and hospital environmental samples and characterize them. The samples were obtained from Evandro Chagas Institute of Clinical and Research, located at Oswaldo Cruz Foundation (Fiocruz), Rio de Janeiro/Brazil, over a period of one year (2006-2007), when it was detected an outbreak of AAD. Forty environmental and 21 stool samples were analyzed and submitted to standard culture techniques. Stool samples were also tested for the presence of toxins by using immunoenzimatic assays (ELISA). We could not recover any *C. difficile* strains from the environmental samples, but 28.5% (6/21) of the stool samples were positive. From these, four were positive both in culture and ELISA, and two were only positive in ELISA. The four *C. difficile* isolates (1575, 1598, 1599 and 1602) were obtained from AAD cases and three of these patients were HIV positive. PCR was performed to confirm the presence of toxins A and B genes, and also to investigate the presence of the binary toxin gene, being the four strains positive for TcdA and B and negative for CDT. PFGE analysis showed that the strains 1575 and 1598 had a similar genetic fingerprint, while 1599 and 1602 had the same genetic pattern. These results were confirmed by PCR-ribotyping, which indicates that 1575 and 1598 strains belongs to ribotype 014, and 1599 and 1602 belongs to ribotype 106. For the first time the ribotype 106 was detected outside the United Kingdom. This work suggests the spread of *C. difficile* and the presence of two clonal strains involved in an outbreak of AAD in Rio de Janeiro.

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CLOSTRIDIUM DIFFICILE: EPIDEMIOLOGY

INCREASING DETECTION OF *CLOSTRIDIUM DIFFICILE* POLYMERASE CHAIN REACTION RIBOTYPE 027 IN FINLAND

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Background: In October 2007, the first case of *Clostridium difficile*-associated disease (CDAD) caused by the hypervirulent PCR ribotype 027 was reported in Finland. To assess the extent of the spread of this particular ribotype, all clinical microbiology laboratories were requested to send *C. difficile* isolates from severe cases and/or suspected outbreaks to the National Public Health Institute (KTL).

Material and Methods: During November-December, a total of 153 isolates from 7 out of 20 health care districts were sent to the Anaerobe Reference Laboratory, KTL, to be typed by PCR ribotyping (median, 6 strains/sender; range, 1-86). PCR ribotyping was performed according to the protocol of the Anaerobe Reference Unit in Cardiff, using their PCR ribotypes 001 and 027 as the reference. After gel electrophoresis, the band patterns were analyzed using the BioNumerics software (Applied Maths NV, Belgium).

Results: Of the 153 isolates, 76 (50%) represented PCR ribotype 027 and 27 (18%) were identified as PCR ribotype 001; 50 (33%) were of other ribotypes. The isolates of PCR ribotype 027 came from 4 out of the 7 health care districts, and originated from 21 different health care facilities, locating in southern and south-western Finland and most of them providing primary or long term care. All except 5 of the 76 subjects positive for PCR ribotype 027 were at the age of 60 years or older.

Conclusion: Despite the still limited material, it is obvious that the hypervirulent *C. difficile* PCR ribotype 027 has spread at least in southern and south-western parts of the country. Further examinations are ongoing.