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Dear Professor Lian-Sheng MA**,**

Please find our revised manuscript entitled:

ESPS Manuscript NO: 14617
Title: The burden of Clostridium difficile infection between 2010 and 2013: trends and outcomes from an academic center in East Europe

Authors: Zsuzsanna Kurti, Barbara D. Lovasz, Michael D. Mandel, Zoltan Csima, Petra A. Golovics, Bence D. Csako, Anna Mohas, Lorant Gönczi, Krisztina B. Gecse, Lajos S Kiss, , Miklos Szathmari, Peter L. Lakatos

Once again thank you for inviting us (Editorial Board ID: 0001303) to contribute to the *World Journal of Gastroenterology* with the above paper and offering to waive the publication fee in case of final acceptance.

We are grateful for the reviewer for the detailed and helpful comments that helped in further clarifying the manuscript and for finding the paper interesting and adding new data on the incidence , management and outcomes in CDI in the region.

We are sending revised version as requested be the editorial office.

Detailed answers to reviewers:

reviewer 2982286

This is an epidemiological study regarding *C. difficile* infection in Eastern Europe where its incidence is unclear. The study data were mainly descriptive with some analysis, such as that of the risk factor associated with CDI. However, the risk factors noted in the study were already examined in multiple studies of CDI, and the paper noted no major new findings. However, the incidence data could be important if the global burden of CDI were considered. Major/minor concerns are noted below. The manuscript requires major modifications to strengthen it.

1. Overall major comment

The manuscript requires editing by a native English speaker or editing service since there are many typographical and grammatical errors in the abstract.

The focus of the study is a current descriptive epidemiology of CDI and the risk factors associated with developing CDI in Eastern Europe. Since the burden of CDI in Eastern Europe is not well known, the findings in the study will heighten awareness of this issue. Although the data were prospectively obtained, because this is a single-center study, it may not represent the current state of CDI epidemiology in Eastern Europe.

I felt that the discussion section of the manuscript could be more tightly written, especially the paragraph regarding risk factors associated with CDI, given the lack of novel findings in this study.

The general knowledge on the incidence of CDI in this geographic region is suboptimal, this is why we added the scant data in comparison with data from Western Europe and North America, to enable the readers to assess the significance of our results. We found an astonishing high incidence, almost an epidemic!

2. Specific comments.

Methods:

Page 4, first paragraph

The authors defined CDI as 'acute diarrheal disease' (more than three liquid stools per day based on reference 19. Do you have any data regarding the Bristol stool chart? Since 'liquid stool' is very subjective, objective parameters for liquid stool should be noted.

Acute onset diarrheal disease was based on clinical symptoms and on physicians' opinion, which was defined as more than three liquid stools per day. It is equals to Bristol type 6 or 7, but it is not used scale in Hungary and that's why we haven't got results from present database.

Page 4, first paragraph

The authors stated that, "In our department we apply standardized medical protocols." What does 'standardized medical protocol' mean?

In 1st Department of Medicine, we used standardized protocols, approved by the Semmelweis University. It means that chose of antibiotics and length of antimicrobial treatment is harmonized in all wards in the hospital and all Department in the University. The use of this protocol enables us to compare treatment outcomes directly.

Page 5 method section

How did the authors track *mortality information*? The authors should describe this in the methods section.

Inpatient records were collected and comprehensively reviewed, including medical reports and final reports, in case of transfer different ward, e.g. ICU to assess different outcome. Short term mortality and 30 day mortality rates were defined from medical records and death markers by health insurance number.

Results:

Page 5, paragraph 1 (incidence of CDI and severe CDI section)

The author stated that the "Community acquired infection rate was 45.3%." *How do you define community-acquired CDI in this study?* This is extremely important as the denominator in information for hospital onset CDI and community onset CDI is different and these incidence densities should be separately reported to help understand CDI epidemiology better. The definition of community-acquired CDI should be included in the methods section. The definition of the onset of CDI is available in the current US and European CDI guidelines.

Community acquired CDI defined as symptoms developed before hospital admission or less than 48 hours after

ref: Lee L, Cohen SH Community-Acquired Clostridium difficile Infection: An Emerging Problem , Current Emergency and Hospital Medicine Reports , 2013, Vol 1, 3, 149-153

Page 5,
Do you have the data for testing densities? Since the incidence is correlated with the frequency of testing in the previous European study (Bauer MP et al. Lancet 2011), the testing density (number of tested /10000 patient-days) is needed when evaluating CDI incidence.
Total 601 stool sample tested for Clostridium difficile infection in Microbiology Department of Semmelweis University, microbiological serology test, including 168 positive and 433 negative result and including recidive cases. Testing density was 5.11/10000 patient-days. This is now added to the results.

Page 5
The authors should provide information regarding "time to CDI" for patients with hospital onset CDI.
Mean time to presence of CDI symptoms was 2.75 days (SD: 5.3) from hospital admission.

Page 5
Regarding severe CDI, the author stated:

"The incidence of severe CDI was 12.6% (2.63/1000 of all cause hospitalizations). In severe CDI patients were older (severe: 84.2% vs all: 69.6% of patients were >65 years, p<0.001) and duration of hospitalization was longer (18.4 (SD 11.7) vs 17.3 (SD 10.3) inpatient days, p<0.001). "

It is unclear which population(s) were compared with those with severe CDI (the control population? Or non-severe CDI patients?). The author should clarify this.
The primary aim was to compare severe CDI with non-severe CDI cases, with regards to treatment strategy, relapse rates and outcomes.

If the authors compared severe CDI patients with non-severe CDI patients, they should explain what the difference in length of hospital stay was after diagnosis of CDI in each group.
Total length of hospital stay in severe CDI was longer in severe cases compared to non-severe CDI cases(18.4 (SD 11.7) vs 17.3 (SD 10.3) inpatient days, p<0.001). Diagnosis of severe CDI was based on laboratory test performed the closest to CDI serological diagnosis.

Discussion
Page 8
Why was the incidence density of CDI in this institution extraordinarily high among European countries? The data in the manuscript reflected much higher values than even the data from Poland. Is this biologically plausible? The authors should discuss why the incidence of CDI was so high.
Incidence of CDI was high in the present study. Possible reasons of this high incidence are high comordity rates of inpatients and primary internal medical care territory of our institution presents many nursery homes with elderly population. These objectives may responsible for higher incidence rates.

Page 8
As noted in the general comments, discussion about the risk factors associated with CDI should be shortened, given the lack of new findings in this study.
Thank you for the comment, as noted previously, the study found a surprisingly high incidence with a high rate of severe infections as well as no data on routine management, treatment and

outcomes are systematically available from the region. Thus we felt that a detailed comparison with other regions is needed. The paper will also serve as the base data for quality of care assurance in the region

Page 9
The author stated that higher mortality might be due to a higher Charlson comorbidity index and elderly population. However, even in the US, Europe, and other industrialized regions, the elderly population with multiple comorbidities comprise a major proportion of CDI patients. Thus the authors' comment may not fully explain the higher mortality rate in CDI patients in Eastern Europe. More detailed insights should be given regarding higher mortality rate.
The interpretation was based on the results from the statistical analysis as no other significant factors were found. Age is of course the other major factor.

Page 10, limitations
In this study, the author used the definition of severe CDI from the SHEA guidelines. There were several definitions differentiating CDI by severity, but none of the definitions were clinically validated. The author should state this in the discussion.
Thank you for the comment, we corrected as recommended.

Minor comment
1. Clostridium difficile should be written in *italics.*
Thank you for the comment, it is corrected as recommended.

2. **The authors spelled out *Clostridium difficile* infection even after introducing the abbreviation, "CDI". Please correct *Clostridium difficile* infection to CDI in the appropriate areas.**

Thank you for the comment, it is corrected as recommended.

3. **As noted in the general comments, there were many typographical errors. The authors should use request an English editing service for help.**

Thank you for the comment, it is corrected as recommended.

reviewer 02731212
This is a case-control study conducted from 2010-2013 among inpatients with community- or healthcare-acquired CDI who were admitted to a large, academic medical center in Budapest. The authors seek to evaluate the morbidity and mortality of patients with CDI as well as risk factors associated with CDI. They identified 247 cases of CDI and matched these 1:3 with control patients by age, sex, care period, and unit. They found that antibiotics and PPIs were associated with CDI, which confirms the results of previous studies. The epidemiology of CDI is important because CDI remains a major nosocomial infection in the western world and the epidemiology of CDI appears to be shifting more from healthcare- to community-acquired disease. However, there are important methodological questions that should be addressed in this study.

Major:
I would describe the study design as retrospective because it was conducted as a database review, however the authors describe it as a prospective in several places including the abstract. Unless case verification was done in real time (e.g., by going to the bedside to ascertain diarrhea) the study should be described as a retrospective study.
The data collection was prospective in the last 5 years in inpatients. Patients and treatment was registered prospectively as well as the characteristic of CDI.

The paper does not adequately distinguish between community-acquired CDI and healthcare-acquired CDI, yet this is the major question in CDI epidemiology. (Also, some risk factors such as unit type are only relevant for healthcare-acquired CDI since unit type could not possibly affect diagnoses that were already present at the time of admission.) I suggest the authors stratify outcomes into community- and healthcare-

acquired disease. Alternatively, they may wish to exclude community-acquired disease since this is an inpatient study.
Thank you for your comment and suggestions. In the present study we distinguished community and healthcare associated diarrhea according to onset of symptoms within 2 days from hospital admission. Treatments strategy, but not outcomes was different according to onset type in our study. In addition, in our department we apply standardized medical protocols and surveillance guidelines for HAI including CDI, and thus evaluation of symptomatic patients and treatment strategy is harmonized

The Methods section should begin by clearly defining the outcome—including healthcare-acquired vs community-acquired CDI—and then the matching criteria. For example, was age matched within the categories shown in Figure 2? Or by quartile?
In the present study selection of control patients, one of the matching criteria was age within decades for easier comparison of results we used age groups in results section <40 years, 40-60 years and >60 years what is clearly correlated with higher mortality rate in elderly patients.

Then the Methods should clearly define all the exposure variables. For example, what was the time window for antibiotics exposure? Was this ascertained from admission notes only?
What if the patient could not give a history?
Antibiotic exposure was registered from medical history and electronical database at the time of admission notes and previous documentation during the year before time of CDI diagnosis.

The Methods section should also state the criteria used to evaluate variables for the multivariable model.
As usual, univariate p values of.1 were included in the multivariate models.

Minor:
Say "general medical inpatients" instead of 1st Department of Medicine.
Our hospital is working as a referral center for both in and outpatients and also a primary and secondary and tertiary referral internal medical care for a geographical region.
Unless it conflicts with the journal's style, the p-values and ORs should be given with just 2 significant figures.
Thank you for your comment, where it is possible it is corrected as recommended, but in certain place longer figures are maintained for exact significance level determination.

Table 1: Is this data skewed? Probably better to give the median and IQR rather than the mean and SD for continuous variables.
Table 2: Include relevant definitions within the table.
For example, was PPI exposure any dose/duration of PPI? Within one year?
Thank you for your comment, the data follow normal distribution, thus median and mean are comparable, we did not modify the presentation.

Also, were these the only variables in the model?
For defining possible risk factors patient ward, comorbidities, age, gender, medication use, living in nursery home and laboratory parameters were analysed.

The tables should describe the matching criteria and explicitly state which variables were in the model.
Matching criteria and variables analysed were described in details in methods section, relevant variables were extracted and showed in tables section.

Figure 1: List a p-value comparing the 2 survival curves.
Thank you, it is indicated as recommended.

Figure 2: Show the mortality of the control patients as a comparison, and the p-values for CDI cases vs controls within each age bracket.

Also: include a figure showing the flow of patients into the study.
Corrected as appropriate

Reviewer 2458689
The authors present prospective data regarding incidence, risk factors, treatment and outcomes of Clostridium difficile infection. The paper covers an interesting topic and includes a considerable number of patients. However, I have some questions for these authors:
Major points:

1. **I have a question regarding the laboratory parameters given in table 1.:**
   When were the samples taken?
   Are these mean values of all tests during hospitalization or during acute infection?
   Laboratory samples were taken at time of CDI diagnosis or based on the first available laboratory test results after CDI symptoms onset (max within 2 days).

2. **Methods: Please give more detailed information regarding your statistics: *Six tests are mentioned, but it is not clear which test was used for which analysis.***

   Thank you for the comment, we modified the statistical paragraph:
   D-test, ANOVA-Scheffe test were used to compare continuous variables, Khi2, Fischer-exact tests were used to compare categorical variables. Categorical variables if appropriate were further tested in a multivariate analysis by using logistic regression analysis. Variables with a p of <0.1 were included in the multivariate testing. Kaplan-Meier curve was plotted to analyse mortality outcomes with LogRank test. A p-value of <0.05 was considered significant.

3. **Results: Risk factors for CDI and Table 2 In the text, you only give the results of the univariate analysis. What are the factors you adjusted for in the multivariate analysis in table 2? Confidence interval of "Previous Clostridium difficile infection" is not reported in table 2**

   Factors with a p value of <0.1 in univariate analysis were enrolled in multivariate analysis, we added 95%CI and now use 2 decimal speces

4. **Results, Outcome of CDI infection: In this section you describe duration of hospital stay, mortality and recurrence rates. Please address in a separate paragraph how many patients (with severe CDI) were admitted to emergency surgery, and what kind of surgery (ileostomy creation, colectomy, subtotal colectomy), and what was the outcome of these patients, because early surgical intervention is critical in patients with severe CDI not responding to medical and ICU-treatment. Please also discuss these results.**

   In our patients, there was no need for surgical intervention due to CDI, because severity of CDI did not completed the criteria of acute surgery indication.

5. **Results, Outcome of CDI infection: As CDI was more severe in elderly patients (e.g. highest mortality rates) it is not clear to me why length of hospitalization was not different between age groups. These data should be shown or explained.**
   In our study treatment strategy was differed according to severity of CDI and this was based on the harmonized CDI and infection control protocol of the university. More aggressive treatment strategy was characteristic in patients with severe CDI

with higher rate of combined antibiotic treatment or vancomycin alone. The interpretation of the findings

Minor points:

1. **Please check the manuscript for several mistakes in punctuation marks (e.g. Results, Treatment strategy, line 5: "vancomycin alone.)" and typographical mistakes. Maybe the manuscript should be corrected by a *native English speaker*.**
   The paper was again read by native speaker, linguistics were corrected.

2. **Methods: Please describe your criteria for recovery after CDI. As "*Recovery after CDI*" is one of your three endpoints/outcomes, this should be addressed in your section "Outcome after CDI infection."**
   Results of present study showed 21.9% mortality rate and rest of the patients were recovered, in 8.1% of patients after ICU therapy, while recurrence of infection was observed in 11.3%.

3. **Results, Treatment strategy, line 4: SD for length of antibiotic treatment is not given**.
   Thank you for comment, it is corrected.

4. **Results, Treatment strategy, line 6: I don't understand what the authors mean with the sentence: "*The length of the treatment was 13.6 days (SD: 5.9 days), and 12.6 days (SD: 7.1 days) in severe cases*." Was this the length of treatment after change in the antibiotic therapy?**
   YES, the reviewer is correct

5. **"CDI infection" in your manuscript is duplicate: The "I" already stands for "infection"**
   Thank you for your comment, it is corrected as recommended.

6. **In the Results you say that mortality rate was 21.9%, but in the Discussion it is 20.2? What is correct?**
   Thank you for the comment, correct mortality rate in present study is 21.9%, it is corrected in discussion section.

We would like to thank you again for the helpful comments and for considering our paper. We do hope that the changes that have been made, have improved the quality of the manuscript with regards to clarifying the methodology and data presentation.

All authors have fulfilled the criteria of authorship and seen and approved the final version of the manuscript and they have authorized the first author to grant on behalf of all authors to transfer exclusive copyright to *World Journal of Gastroenterology* in case of acceptance.

We hope that the article could provide useful new information to the readers of *World Journal of Gastroenterology*.

Sincerely yours,

Laszlo Peter Lakatos, MD, PhD
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