Author's response to reviews

Title: Clinical characteristics and risk factors for enterococcal infections in Nagasaki, Japan: a retrospective case-control study

Authors:

Toshiki Kajihara (toshikikajihara@hiroshima-u.ac.jp)
Shigeki Nakamura (moju516@nagasaki-u.ac.jp)
Naoki Iwanaga (naokiwanaga.53.redstar@gmail.com)
Kazuhiro Oshima (d011eb@yahoo.co.jp)
Takahiro Takazono (takahiro-takazono@nagasaki-u.ac.jp)
Taiga Miyazaki (taiga-m@nagasaki-u.ac.jp)
Koichi Izumikawa (koizumik@nagasaki-u.ac.jp)
Katsunori Yanagihara (K-yanagi@nagasaki-u.ac.jp)
Nobuyuki Kohno (nokohno@hiroshima-u.ac.jp)
Shigeru Kohno (s-kohno@nagasaki-u.ac.jp)

Version: 4
Date: 27 July 2015

Author's response to reviews: see over
July 27, 2015

Dear Sir/Madam (Editor in Chief of BMC infectious Disieases),

Thank you for handling the reviews of our manuscript, “Clinical characteristics and risk factors of enterococcal infections in Nagasaki, Japan: a retrospective case-case control study”.

We have dealt with the reviewer’s comments and submitted the revised text. The revised word or sentences are enhanced by red color.

We believe that the findings of the current study are relevant to the scope of your journal and will be of interest to its readership.

This manuscript has not been published or presented elsewhere in part or in entirety, and is not under consideration by another journal. All the authors have approved the manuscript and agree with submission to your esteemed journal. There are no conflicts of interest to declare.

Thank you for your consideration. I look forward to hearing from you.

Sincerely,

Dr. Shigeki Nakamura  
Department of Chemotherapy and Mycoses,  
National Institute of Infectious Diseases  
1-23-1 Toyama, Shinjuku-ku, Tokyo, 162-8640, Japan  
Tel: +81-3-5285-1111  
Fax: +81-5285-1150;  
E-mail: shigekinak@nih.go.jp

(Former) Nagasaki University Hospital  
1-7-1 Sakamoto, Nagasaki 852-8501, Japan  
Tel: +81-95-819-7273  
Fax: +81-95-849-7285  
E-mail: moju516@nagasaki-u.ac.jp, shigemoju@yahoo.co.jp
Response to Reviewer’s comments

Reviewer #1:
The clinical significance of this study is unclear. This research question has been previously addressed in other studies and there is no attempt from the authors to compare their study with others including comparison of study design, methods and statistical considerations such as sample size.

Response: We appreciate your suggestion. Although the number of enterococcal isolation in nosocomial situation is increasing (http://www.nih-janis.jp/report/open_report/2013/3/1/ken_Open_Report_201300.pdf) and the clinical importance of enterococcal infection definitely high, few epidemiological studies about enterococcal infection are published from Japan. Hospitalized patients in Japan has a unique characteristics compared to Europe and United States, for instance, a large amount of elderly patients and the duration of hospitalization is long, and additionally the isolation rate of VRE is quite low. We believe, this is the first study to identify the clinical features and risk factors of enterococcal infection in Japanese population and the comparison of the epidemiological data between different countries is useful for better understanding of enterococcal infection. In addition I added the statistical description like as “sex and age matched case-control study with colonized patients” in abstract and methods since it is considered one of the features of our study design (Page 3, line 41-42, Page 6, line 92-93).

The definition of “infection” is vague. Why did the authors pick up a white blood cell count >9100/mm$^3$ and C-reactive protein > 0.17 mg/dL as cut-off? There is no justification. What about other comorbidities that may affect these parameters (e.g. use of steroids)? The term “organ specific symptoms” is vague. Were the charts reviewed by clinicians? What about selection and misclassification biases?

Response: We appreciate your comments. To determine the “infection cases”, two clinicians, who were infectious diseases specialists, reviewed carefully the medical chart of patients and defined infection or colonization. We were defined as infections a patient with clinical symptoms (temperature > 37.5°C and organ-specific symptoms), laboratory data (white blood cell count > 9100/mm$^3$ and C-reactive protein > 0.17 mg/dL; standard value of our hospital). The “organ specific symptoms” are defined by reference to criteria of CDC/NHSN for specific types of infection (Am J Infect Control 2008;36:309-32.) as followings: Intra-peritoneal infections: Patient has at least 2 of the following signs or symptoms with no other recognized cause: nausea, vomiting, abdominal pain, or jaundice. Urinary tract infections: Patient has at least 1 of the following signs or symptoms with no other recognized cause: urgency, frequency, dysuria, or costovertebral angle tenderness. Bone and soft tissue infections: Patient had at least 2 of the following signs or symptoms with no other recognized cause: localized swelling, tenderness, redness, heat, or drainage at suspected site of bone and soft tissue infection. Bloodstream infections: Patient has at least 1 of the following signs or symptoms: chills, or hypotension. Pulmonary infections: patient has the following signs or symptoms with no other recognized cause: cough, new or increased sputum production, rhonchi, wheezing. Vascular grafts infections: Patient had at least 1 of the following signs or symptoms with no other recognized cause: localized swelling, tenderness, redness, heat, or drainage at suspected site of vascular graft infection. Febrile neutropenia: patient had the following signs or symptoms: neutrophils count < 500/mm$^3$ and chills, or hypotension.
The matched controlled design is poorly described and there are no power sample size calculations. The colinearity between predictors is not considered. Did the authors look for interactions? For example there is collinearity between diabetes, steroids, kidney disease etc.

**Response:** We appreciate your suggestion. This study was a sex and age matched case-control study between “Infection” and “Colonization”. It was difficult to analyze the collinearity because there are many predictors. In multivariate analysis, stepwise variable selection with forward selection demonstrated identical results. (Page 7, line 128-129)

The significance of Table 2 is unclear. The antimicrobial susceptibilities for Enterococcus sp have been described in numerous studies.

**Response:** We deleted Table 2.

Overall poor design, the study lacks novelty, incomplete review of the literature.

**Response:** We appreciate your suggestions. We understand your comments however we would like to clarify the independent risk factors of enterococcal infection in Japanese population since the prevalence of enterococcal infections is increasing in nosocomial situation in Japan. The background of hospitalized patient’s is different compared to European countries and United States, for instance, the number of elderly patients is quite high, the duration of hospitalization is long and the severity of infection is variable, considering that the difference of insurance system might exert influence to the patient’s population. Furthermore the other important feature of enterococcal infection in Japan is low prevalence of VRE. Taken together with those reasons, we added the following sentences in discussion to describe the significance of this study.

The independent risk factors for VRE infection were reported previously. Kim et al. compared the VRE “infected” and “colonized” group and reported combined infection with bacteria other than VRE, presence of hemodialysis catheter, duration of vancomycin use were the independent risk factors for VRE infection. Another study performed Olivgeris et al. reported cortisone use, third- or fourth-generation cephalosporins, enteral nutrition and VRE colonization were the risk factors for developing enterococcal infection in critical ill patients. Zaas et al. reported the use of vancomycin, gastrointestinal procedure, diabetes mellitus and acute renal failure could be the risk factors for enterococcal bloodstream infection in the malignant patients colonized VRE. As shown in these reports, the risk factors of enterococcal infections, especially VRE, are variable depends on patient’s background. In addition the risk factors for vancomycin-susceptible enterococcal (VSE) infections, which have a high proportion in Japan, were still unknown. In our study, structural abnormalities of the urinary tract, abdominal surgery, immunosuppressive agent use, and the use of in situ devices are the risk factors of VSE infections. Structural abnormalities of the urinary tract are unique in comparison with the risk factors of VRE infections, indicating that we should require attention to VSE infection in the patients who has primary or secondary urinary tract abnormalities, stent placement and nephrostomy etc. (Page 12-13, line 206-223)

References

Reviewer #2:
MAJOR COMMENTS
The article focuses on an argument which is relevant for the BMC Infectious Diseases journal and also specifically for nosocomial infections. The English language and writing level is sufficient.
The authors carried out a case-control study to describe clinical characteristics and risk factors for enterococcal infections in a teaching hospital in Japan.
The main finding was that abdominal surgery, structural abnormalities of the urinary tract and use of in situ devices are the most significant risk factors for enterococcal infection.
The study adds useful regional epidemiological information (Japan), and the findings are important to those with related research interests.

Background
1) Page 6, line 80: include one more specific reference (Orsi GB, Ciorba V. Vancomycin-resistant enterococci healthcare infections. Ann Ig 2013; 25 (6): 485-492);
Response: We added the reference according to reviewer’s suggestion (Page 5, line 78) and described the sentences about the added reference in discussion.

Results
2) Page 10, lines 134-138: the phrase is not very clear, … “there were 583 Enterococcus spp case isolates…These corresponded to 181 infected patients …; moreover, 390 cases were colonized”. 181 infected patients + 390 colonized should be 571, please explain;
Response: We added the explanation as bellows in materials and methods according to the reviewer’s suggestion.
A total of 571 cases positive for Enterococcus spp. and 583 isolates were analyzed included twelve duplicated cases (the patients enterococci re-isolated over 3 months after first isolation or multiple enterococci isolation from a patient). (Page 6, line 97-100).

Discussion
3) Overall the Discussion looks a bit too long for the reader and needs to be shortened.
Response: We shortened the discussion according to the reviewer’s suggestion.

4) References cited in the Discussion do not appear in order. After reference number (7) in page 8 in the discussion page 13 line 204 appears reference (20), please check. Also reference (18) appears after reference (20).
Response: We checked and renumbered the references according to the reviewer’s suggestion.

References
5) There are seven references (11, 19, 21, 22, 23, 24, 36) I could not find in the manuscript text. If they have been eliminated during corrections please cancel them. Also renumber appropriately all references as described in the “Instruction for Authors”;
Response: We deleted seven references, and renumbered according to the reviewer’s suggestion.

Minor essential revisions
1) Page 6, lines 86-87: “This study aimed to describe epidemiology, clinical characteristics and
risk factors for enterococcal infections” please correct;
Response: We revised the word according to reviewer’s suggestion (Page 5-6, line 86-88).

2) Page 7, line 94: “….an 862-bed tertiary care and teaching hospital…” please correct;
Response: We revised the word according to reviewer’s suggestion. (Page 6, line 94).

3) Page 10, lines 145-148: data already reported in the Table 1 should not be repeated in the text. You might briefly underline something you consider important. Please cancel all repeated data;
Response: We revised the sentences according to reviewer’s suggestion. (Page 9, line 151-153).

4) Page 13, line 210: “….from 2010 to 2011 …” or “…during 2010-2011….” please correct;
Response: We revised the word according to reviewer’s suggestion. (Page 13-14, line 229-232).

5) Page 13, line 211: “….indicating this complication incidence is high at 42% …” please correct;
Response: We revised the sentence according to reviewer’s suggestion. (Page 13-14, line 229-232).

Reviewer #3:
Kajihara et al performed a retrospective case-case control study to determine the clinical characteristics and risk factors associated with enterococcal infections in Nagasaki, Japan.

Major:
1. Tables 1 & 2: Please provide chi square analysis between E. faecalis and E. faecium variables to determine if the differences are statistically significant or not and add P values to the text.
   Response: We added the statistics of Bonferroni post-hoc test and mentioned in materials and methods, revised the sentences according to the reviewer’s suggestion.
   —In intra-peritoneal infections after liver transplantation, the number of E. faecium infections was significantly more than that of E. faecalis infections (p < 0.001). On the other hand, In urinary tract infections, the number of E. faecalis infections was significantly more than that of E. faecium infections (p = 0.016). (Page 8-9, Line140-144).
   —Compared with E. faecalis, bone marrow and stem cell transplantation were significantly common in E. faecium infections (p = 0.027). (Page 9, Line157-158)

2. Line 127: Please explain how colonization vs infection were determined.
   Response: We were defined as infections a patient with clinical symptoms (temperature > 37.5°C and organ-specific symptoms), laboratory data (white blood cell count > 9100/mm³ and C-reactive protein > 0.17 mg/dL; standard criteria of our hospital), and bacteriological tests (monomicrobial culture or the same organisms isolated from two organs). The “organ specific symptoms” are defined by reference to criteria of CDC/NHSN for specific types of infection (Am J Infect Control 2008;36:309-32). And we were defined as colonization a patient with Enterococci positive culture, without no clinical symptoms and laboratory data. (Page 6-7, line 104-112).

3. Was there a link between colonization and the risk of acquiring infection?
   Response: We added the following sentences in Introduction according to reviewer’s suggestion.
   —Previous reports have shown pharyngeal or intestinal colonization of enterococci were the risk factors for enterococcal bacteremia which is associated to increase the mortality particularly in the immunocompromised patients (Page 5, Line 80-83).
4. Please comment on the lack of VRE isolated from this study. What is the VRE prevalence rate at the institution?

Response: In this study no VRE was isolated at our hospital during 2010-2011. In Japan the rate of VRE is very low (reference number 12,23).

Minor:
1. Line 42: Should be: …patients with Enterococcus spp infection…

Response: We revised the word according to reviewer’s suggestion. (Page 3, line 39).

2. Line 87: Remove “as” from the sentence.

Response: We revised the sentence according to the reviewer’s suggestion. (Page 5, line 86-88).

3. Line 122 under Statistical Analysis: There should be at least a count of 10 in any category to determine statistical significance and not the use of 5 as described.

Response: Thank you for your suggestion however we consider the statistical analysis made by using the SPSS with expected count < 5 is common (e.g.: Launes C, et al. Clin Microbiol Infect. 2013). If there are another statistical problems for our results, please don’t hesitate to contact us and give us your suggestion.

4. Line 137: Please rephrase the sentence. …; moreover, 390 patients were colonized with Enterococci.

Response: We revised the word according to reviewer’s suggestion. (Page 8, line 140-141).

5. Line 139: Insert “and” between abscess discharge and blood.

Response: We revised the word according to reviewer’s suggestion. (Page 9, line 146).

6. Lines 146-148: Rearrange comorbidities from the most common to the least common in the sentence.

Response: We revised the sentences according to reviewer’s suggestion. (Page 9, line 151-153).

7. Please combine tables 3-5.

Response: We combined Table 3-5 and renamed table 2 according to reviewer’s suggestion.

8. Line 208-212: The sentence needs to be reworded especially the ending.

Response: We added the sentence according to reviewer’s suggestion.

—There was significant relationship between liver transplantations and infections with E. faecium compared to E. faecalis. (Page 17, line 291-292).

9. VanA and VanB need to be corrected in the manuscript. There is no space between Van and the determinant.

Response: We revised the word according to reviewer’s suggestion. (Page 14, line 246-247).