**Author’s response to reviews**

**Title:** Clinical characteristics of and risk factors for enterococcal infections in Nagasaki, Japan: a retrospective study

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Response to Reviewer’s comments

#Reviewer 1

The clinical significance of this study is still unclear. As mentioned earlier this research question has been previously addressed in other studies.

“Hospitalized patients in Japan has 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. “ These are not unique characteristics since there are many elderly patients in Europe and USA with prolonged hospitalization. If indeed these are unique characteristics the authors should cite an epidemiological study that has directly compared these characteristics in Japan vs Europe vs USA before making these conclusions. Rather than providing specific references they just make vague conclusions. To my knowledge there is no such epidemiological study. In any case this study is more targeted towards a Japanese Journal if this is why this study is so important.

Response: We consider it important to inform the world of an epidemiological study on enterococcal infections in Japan, one of the most rapidly aging countries worldwide. Individuals aged ≥ 65 years account for 26.3% of the population as of 2015, the highest value in the world. The values of Italy and Germany, the second and third highest, are 22.4% and 21.2%, respectively [1]. Our aging rate is also highest in the world. In a 24-year period, the elderly population of Japan increased from 7% to 14%. By comparison, Germany experienced the same rate increase in a 42-year period. Thus, aging in other advanced countries is also likely to progress rapidly as in Japan. Therefore, we consider studies coming out of Japan, the most rapidly aging country, to comprise invaluable information. As you indicated, we think it would be a more useful study if we were able to investigate Japan vs. Europe vs. USA. Unfortunately, it is currently difficult for us to conduct such a study due to the absence of connections with research institutions in these countries.

The methods should be revised to clarify the definition of infection with the appropriate references as outlined by the reviewers. In addition the authors did not address the question of the reviewer about selection and misclassification biases. There are statistical methods to address this e.g. a score to quantify agreement between the reviewers of the charts etc. In addition many factors that can affect white cell counts,
medications, liver disease or other comorbidities are not considered.

**Response:** Patients were defined as having an infection on the basis of their clinical symptoms (temperature > 37.5°C and organ-specific symptoms) and laboratory data (white blood cell count > 9100/mm$^3$ and C-reactive protein > 0.17 mg/dL; standard value of our hospital). “Organ-specific symptoms” were those corresponding to the CDC/NHSN criteria for specific types of infection (Am J Infect Control 2008;36:309-32). These criteria were as follows:

1. **Intra-peritoneal infections:** The patient has at least 2 of the following signs or symptoms with no other recognized cause: nausea, vomiting, abdominal pain, or jaundice.

2. **Urinary tract infections:** The patient has at least 1 of the following signs or symptoms with no other recognized cause: urgency, frequency, dysuria, or costovertebral angle tenderness.

3. **Bone and soft tissue infections:** The patient has 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.

4. **Bloodstream infections:** The patient has at least 1 of the following signs or symptoms: chills or hypotension.

5. **Pulmonary infections:** The patient has the following signs or symptoms with no other recognized cause: cough, new or increased sputum production, rhonchi, or wheezing.

6. **Vascular grafts infections:** The patient has 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.

7. **Febrile neutropenia:** The patient has the following signs or symptoms: neutrophils count <500/mm$^3$ and chills, or hypotension.

Because this is a retrospective study using only items that can be evaluated in a medical record, some patients did not undergo a successful evaluation of their hepatic and/or respiratory dysfunction. For this reason, it is difficult to examine liver disease or other comorbidities. Regarding medication, we examined steroids and other immunosuppressive agents involved in infection immunity.

As mentioned previously 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. The authors do not address in the revised manuscript why their study and sample size is superior to other numerous similar
The matched controlled design is poorly described and there are no power sample size calculations. In the revised manuscript there are still no power sample size calculations.

**Response:** As you indicated, we did not mention the sample size. This is because it was difficult to determine the sample size because data on the prevalence of enterococcal infections were unavailable. Therefore, we changed the target from a sex- and age-matched control study to one in which the total number of inpatients in whom enterococci were detected in 2010 and 2011 were compared. We used the same logistic regression analysis with forward stepwise selection as an analytical method.

The colinearity between predictors is not considered. Did the authors look for interactions? For example there is colinearity between diabetes, steroids, kidney disease etc. The authors do not address the question of the reviewer by saying “It was difficult to analyze the collinearity because there are many predictors.”. IN addition multivariate logistic analysis may have biases if they did not take into account the interactions and the colinearity between parameters.

**Response:** As you indicated, there may be collinearity between predictors. However, we believe that we considered the collinearity between predictors because we used stepwise variable selection with forward selection for the statistical analysis.

The authors claim that “Structural abnormalities of the urinary tract are unique in comparison with the risk factors of VRE infections”. There is no clear pathophysiologic mechanism why the structural abnormalities of the urinary tract is a unique risk factor for VSE and not for VRE.

**Response:** In this study, urinary tract infections (UTIs) by E. faecalis were more frequent than those by E. faecium. Regarding the pathogenicity of E. faecalis, various in vitro and in vivo examinations have been conducted. Many factors such as Esp, Ace, Ebp, SrtA, MsrAB, and PerA have been demonstrated to play an important role in UTIs and biofilm formation [2]. On the other hand, the factors of E. faecium involved in UTIs and biofilm formation have not been made as clear as those of E. faecalis. According to
the report on community-acquired enterococcal urinary tract infections by Bitsori et al, the Enterococcus that caused urinary tract infection was E. faecalis in all cases [3]. From these facts, E. faecalis seems to have higher affinity for the urinary tract. Vancomycin-resistant enterococci (VRE) are E. faecium in many cases [4], making us speculate that urinary abnormalities are more closely related to vancomycin-susceptible infections than to VRE infections.

We added following sentences in discussion: Many factors related to the onset of urinary tract infection by E. faecalis have been reported. For example, enterococcal polysaccharide antigen is reportedly involved in the onset of ascending urinary tract infections since it binds to epithelial cells followed by biofilm formation and/or resistance to phagocytosis by polymorphonuclear leukocytes. On the other hand, fewer reports have examined the pathogenicity of E. faecium affecting the onset of urinary tract infection than those on E. faecalis, which may be related to the results of our present clinical study. Structural abnormalities of the urinary tract might be unique compared with the risk factors of VRE infections, mainly caused by E. faecium, indicating that we should closely monitor patients with primary or secondary urinary tract abnormalities, stent placement, and nephrostomy for VSE infections, especially E. faecalis.(Page 15-16, line 256-267).

References