**Author's response to reviews**

**Title:** Characteristics of invasive Staphylococcus aureus infections in three regions of Korea, 2009-2011: a multi-center cohort study.

**Authors:**

- Kyoung-Ho Song (khsongmd@gmail.com)
- Eu Suk Kim (yonathan@hanafos.com)
- Hye-yun Sin (angel-sin@hanmail.net)
- Kyung-Hwa Park (medkid@dreamwiz.com)
- Sook-In Jung (sijung@cnu.ac.kr)
- Nara Yoon (shinenara@gmail.com)
- Dong-Min Kim (drongkim@chosun.ac.kr)
- Chang Seop Lee (icsmd@chonbuk.ac.kr)
- Hee-Chang Jang (haroc153@naver.com)
- Yoonseon Park (yoonseony@gmail.com)
- Kkot Sil Lee (mdflorid@empal.com)
- Yee Gyung Kwak (philmed202@hanmail.net)
- Jae Hoon Lee (john7026@wku.ac.kr)
- Seong Yeon Park (psy99ch@hanmail.net)
- Minkyo Song (mksong@snu.ac.kr)
- Sue K Park (suepark@snu.ac.kr)
- Yeong-Seon Lee (yslee07@nih.go.kr)
- Hong Bin Kim (hbkimmd@snu.ac.kr)

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Philippa Harris,

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Incidence and characteristics of invasive *Staphylococcus aureus* infections: a population-based study in 3 regions of Korea, 2009-2011.

Dear Dr. Harris,

Thank you for the helpful comments on our manuscript. The following is our response to these comments.

**Reviewer #1.**

Song et al present interesting data on invasive *S. aureus* infection from 3 different regions in South Korea. They examine the incidence, clinical presentation and outcome. Overall, the data is presented in a clear and logical way. However a few issues remain.

**Major compulsory revisions:**

1) 10 of 44 hospitals with clinical microbiology labs were included in the study. This is a potential bias. Have the authors assessed this potential bias? Did this select for example for larger hospitals?

A> In this study, the median number of beds in the total of 44 hospitals (group 3) was 235
The median numbers of beds in the 10 participating hospitals and 34 non-participating hospitals were 257 (IQR: 201-493) and 235 (IQR: 193-294), respectively. Fortunately, there was no significant difference in number of beds between the participating hospitals and the others \( (P=0.303 \text{ by } t\text{-test}) \).

However, we agree with the reviewer’s concern about a selection bias (especially, a volunteer bias) and the representativeness of the participating hospitals, because we depended on voluntary reporting of number of ISA infection by these hospitals.

To clarify this matter for readers we have added the following highlighted sentences to the Results and Discussion sections:

**Results**

*Estimating incidence rates*

In addition to the 1,198 cases of ISA infection that were identified by the 9 ASCs, 724 were reported from the 5 major commercial laboratories. Of the total of 44 hospitals with clinical microbiology laboratories, 10 (23%) volunteered to respond to our approach, and reported 884 cases of ISA infections. Thus during the 2-year period, a total of 2,806 cases of ISA infection were recorded by surveillance, including 1,820 (64.9%) MRSA infections. Median numbers of beds in the participating 10 hospitals and 34 non-participating ones were 257 (IQR: 201-493) and 235 (IQR: 193-294), respectively \( (P=0.303 \text{ by } t\text{-test}) \). After adjusting by the number of total beds in the 44 hospitals, we estimated the number of ISA infections in those hospitals to be 3,170.

**Discussion**

“Although we tried to evaluate all cases of ISA infection in the 3 Korean regions, we
were unable to collect ISA cases from a large number (34) of the 44 hospitals in group 3. While there was no significant difference in number of beds between the 10 participating hospitals and other 34 hospitals, our group 3 data could be biased because we depended on voluntary reporting of numbers of ISA infections by these hospitals (i.e. volunteer bias). Nevertheless, because these were community-based hospitals whose patients have similar clinical characteristics, we were obliged to estimate the rate of ISA infection in the 44 hospitals from the cases in the 10 hospitals that responded to our approach, based on the difference in number of beds………

2) The measured incidence of invasive infection is dependent on the number of diagnostic tests performed (e.g. the number of blood cultures per patient). Is there any data available on the number of blood cultures taken per hospitalized patient, or any similar data?

A> We are afraid of that we did not investigate how many blood cultures per patient were performed at each hospital. However, Kim EC et al. recently reported the number of blood cultures per 1,000 patient days at seven Korean university-affiliated hospitals in 2010 (Korean J Clin Microbiol 2012;15(2):67-69.) Because the characteristics of the 7 participating university-affiliated hospitals were similar to those of our 9 ASCs, we could assume that our number of blood cultures was similar. The mean number of blood cultures per 1,000 patient days was 110 ±23 (SD), range 65 to 129 [31], which is similar to or somewhat lower than the number (103-188) of blood cultures per 1,000 patient days suggested by Cumitec 1C (Baron EJ, Weinstein MP, Dunne Jr. WM, Yagupsy P, Welch DF, Wilson DM. Cumitech 1C, blood cultures IV. Washington, DC; ASM Press, 2005.).
difference might be secondary to a difference in the proportion of patients with acute illnesses or to different blood culture ordering systems. Most hospitals (about 70%) in Korea examine two blood cultures per adult patient and one culture per child patient (about 80%) rather than three sets.

To clarify this matter for readers, we have incorporated the above statements into the Discussion section and added the relevant references.

**Table 1.** Statistics of yearly requested blood culture at 7 university-affiliated hospitals in 2010

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<tbody>
<tr>
<td>Blood culture performed (A)</td>
<td>76,062</td>
<td>70,763</td>
<td>45,925</td>
<td>35,404</td>
<td>29,940</td>
<td>22,649</td>
<td>16,372</td>
<td>297,115</td>
</tr>
<tr>
<td>Admitted patient numbers (B)</td>
<td>75,477</td>
<td>94,040</td>
<td>39,161</td>
<td>32,843</td>
<td>24,151</td>
<td>32,618</td>
<td>28,605</td>
<td>332,895</td>
</tr>
<tr>
<td>Average admission days (C)</td>
<td>7.8</td>
<td>6.9</td>
<td>8.8</td>
<td>8.37</td>
<td>11.3</td>
<td>9.7</td>
<td>8.8</td>
<td>7.9</td>
</tr>
<tr>
<td>Total admitted patient days (B×C)</td>
<td>588,718</td>
<td>669,571</td>
<td>334,619</td>
<td>299,985</td>
<td>269,823</td>
<td>219,695</td>
<td>251,726</td>
<td>2,634,137</td>
</tr>
<tr>
<td>Adjusted blood culture by 1,000 patient days* (1,000×A/B×C)</td>
<td>129.2</td>
<td>105.7</td>
<td>137.2</td>
<td>118.0</td>
<td>111.0</td>
<td>103.1</td>
<td>105.0</td>
<td>109.9</td>
</tr>
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</table>

*Mean, SD, and 95% confidence intervals are 110, 23, and 95-125, respectively.


3) In table 4 data on appropriate antibiotic therapy is shown. However, exact definitions are missing and it is unclear whether the choice of substance, dose and duration were assessed. Furthermore, this data is not discussed in the text. Interestingly, inappropriate definitive antibiotic therapy did not have an effect in the multivariate model. How do the authors explain this finding?

A> In this study, the antimicrobial therapy was considered 'appropriate' if the treatment
regimen included antibiotics that were active in vitro, and the dosage and route of administration were in conformity with current medical standards. However, we did not take into account in the analysis the duration of the appropriate definitive antibiotic treatment, and data on definitive antibiotics were absent for 54 (6.3%) patients.

In response to the reviewer’s comment we have reviewed our statistical procedures. To calculate the risk factors for 30 day mortality in the multivariate binary logistic regression model, we had checked the value of the Hosmer-Lemeshow goodness-of-fit $\chi^2$ test to exclude interaction between variables. In the multivariate model including both inappropriate empirical antibiotic and inappropriate definitive antibiotic, the $P$-value of the Hosmer-Lemeshow goodness-of-fit test was 0.308, indicating good fit. However, among the 122 patients receiving inappropriate definitive antibiotic, 108 (89%) also received inappropriate empirical antibiotic. When we rechecked the relationship between inappropriate empirical and definitive antibiotics and 30-day mortality, there was significant interaction between the two variables ($P$-value of the Hosmer-Lemeshow goodness-of-fit, $P=0.000$). Hence, we reanalyzed inappropriate empirical antibiotic and definitive antibiotic in separate logistic regression models to avoid interaction in the multivariate analysis. When we did this, inappropriate empirical antibiotic was no longer an independent risk factor for mortality, and inappropriate definitive antibiotic acquired significance as a risk factor for mortality (aOR, 3.199 [2.352-4.350]). Although the adjusted odds ratios of other variables were altered, none of the other changes affected the matters considered in the Results and Discussion sections.

To clarify these matters, we have added the following highlighted sentences to the Methods, Results, Discussion sections and footnote of Table 3 and changed the adjusted odds ratios in Table 3:
Methods

“…Clinical isolates were confirmed as *S. aureus*, and were tested for antimicrobial susceptibility by standard techniques. The antimicrobial therapy was considered 'appropriate' if the treatment regimen included antibiotics active in vitro, and the dosage and route of administration were in conformity with current medical standards. Metastatic infection was defined as the presence of microbiological or radiographic evidence of *S. aureus* infection caused by hematogenous seeding.[14] ……

Results

“…In multivariate analysis, extreme age (≥65 or <6 years), steroid use within 1 month, severe underlying disease (Charlson’s WIC ≥3), presentation with severe sepsis or septic shock, presence of metastatic infection of the lung, and inappropriate definitive antibiotics were significantly associated with 30-day mortality due to ISA infection…”

Discussion

“…As in previous studies of *S. aureus* bacteremia, older age, use of immunosuppressant, severe underlying disease, high severity of illness, and inappropriate definitive antibiotics were independent risk factors for mortality.[8, 20-22] In contrast to other studies, respiratory tract infection itself did not increase the risk of death, whereas metastatic infection of the lung was an independent risk factor.[21, 22]”

Footnote of Table 3.

“Data indicate no. (%) of patients. Total no. of cases was 1,024, because detailed clinical information was not available for 174 patients. Charlson’s weighted index of co-morbidity
and each underlying diseases were analyzed in separate logistic regression models to avoid data overlap in the multivariate analysis. Inappropriate empirical antibiotics and definitive antibiotics were also analyzed in separate logistic regression models to avoid statistically significant interaction between the two variables.

Minor essential revisions:

4) Methods: What is meant by “new town”?  
A> New towns are planned urban communities that combine residential, commercial, and recreational areas, and include satellite towns. Goyang is a new town built to decentralize the Seoul metropolitan area, established in the early 1990’s. We use the expression “new town”, because there may be an epidemiologic difference between new towns and naturally-formed “old” towns. Actually, in our data, the incidence rate of ISA infection in Goyang was only one half to one-third of that in the other regions. As we mentioned in the Discussion section, this difference may have been due to the proximity of Goyang to the Seoul metropolitan area and/or some aspect of the new town itself.

5) In some places in the manuscript it is unclear what is meant by the numbers in brackets (e.g. epidemiologic profiles paragraph line 9). This needs to be clarified.  
A> These numbers (in brackets) are mean incidence rates (lowest incidence rate in the 3 regions ~ highest incidence rate in the 3 regions). We now think that this format could be confusing to readers, as it resembles confidence intervals etc. For clarity, we have removed the numbers in brackets and simply present the mean numbers in the 3 regions in the Results and Discussion sections, as follows:
Results

“The crude annual rates of ISA and invasive MRSA infections in the 3 regions of Korea in 2010 were estimated to be 43.3 and 27.7 per 100,000 populations, respectively. Estimated incidences of ISA infection, *S. aureus* bacteremia, invasive MRSA infection and MRSA bacteremia by surveillance site are listed in Table 4.”

Discussion

“In the present study we tried to estimate incidence rates of ISA infection in Korea for the first time. Based on the 2,806 observed cases of ISA infection, we estimated that over 21,000 ISA infections and 13,000 invasive MRSA infections occurred in Korea in 2010…”

6) Some definitions used need to be reported: How were infective foci defined (esp. respiratory tract infection)? As an example, respiratory tract infection may be overestimated when *S. aureus* colonizing the airways is counted as infection.

A> We agree with the reviewer’s concern about distinguishing real infection from simple colonization. However, in this study, we included only invasive *S. aureus* infections defined by the isolation of *S. aureus* from a normally sterile site. If *S. aureus* was only isolated from sputum, the case was not enrolled in the study. Hence, we believe that our data are free of this overestimation problem. A primary diagnosis was made on the basis of the clinical, radiological, and microbiological information, according to Jang et al. (*Clin Infect Dis* 2009;49(3):395-401). We have added that reference to the Methods section.

7) How was “metastatic infection” defined?

A> In this study, metastatic infection was defined as the presence of microbiological or
radiographic evidence of \textit{S. aureus} infection caused by hematogenous seeding (Jenkins \textit{et al.} \textit{Clin Infect Dis} 2008;46(7):1000–8). We have added this definition and reference to the Method and Reference sections.

8) Table 2: “treatment failure” is not defined. The data presented in the table is not discussed in the text. It could be left out.

A> We assessed the initial treatment response at 72 hours after starting antimicrobial therapy. We defined 'treatment failure' as patients who died or where there was deterioration or absence of abatement of fever, leukocytosis or all other signs of infection (Lautenbach E \textit{et al.} \textit{Clin Infect Dis} 2001;32(8):1162-1171). To make this point clearer to readers, we have added this definition and reference to the Methods and References sections.
Reviewer #2.

In this report, Song and colleagues aim to report a population-based study of invasive Staphylococcus aureus infections. This is a significant undertaking. Data is collected from a multiple sites within a large population in Korea during a two-year period. While a number of population-based studies of invasive/bacteremic S. aureus infections have been reported from centers in North America, Europe, Australia/New Zealand, there is a lack of data in this regard from the Asian region.

Population based surveillance studies are ideal to define the epidemiology of an infectious disease. This is because in these designs usually all incident cases of disease occurring in residents of a well-defined (geographically and demographically) population are included and non-residents are excluded. As a result, sampling bias is minimized. Incidence rates can be calculated because the number of cases and population at risk are known.

My main comments regarding this study relate to the population-based design. Many of these have been recognized and discussed by the authors and include:

1. Residency status was not known but estimated for group 2 and 3 patients (ie non-ASC). This is a significant major bias.

   Because the ISA cases in groups 2 and 3 came from community-based clinics and hospitals with acute presentation and severe ISA infections, we believe that the effect of imported cases was minimal. However, we agree with the reviewer’s point that this is a significant bias. To make this limitation clearer, we have added the following sentences to the Methods section:

   Methods
“We also requested the 44 hospitals (group 3) to report their number of cases of ISA infection. In contrast to the 9 ASCs (group 1), the clinical microbiologic laboratories in group 2 and group 3 were requested to volunteer to participate in this surveillance. In addition, the number of cases was only passively reported without residency data, and separate audits to identify ISA cases were not performed in groups 2 and 3.”

2. Only 10/44 hospitals acute care labs participated in the study. These were not randomly selected from the overall cohort and hence represent a major potential (“volunteer”) bias. The number of estimated missed cases (3170) exceeds that of included cases (2806).

A> We agree the reviewer’s comment and have dealt with this matter in our response to comment 1) of reviewer #1.

3. I am not clear whether there may be any other labs (community based, small hospital, nursing home, minor commercial labs, etc) that process invasive specimens; if so failure to include them imparts a bias. Most previous population-based studies on invasive/bacteremic S. aureus infections have estimated coverage of 90-100% of all labs in a region.

A> In our study we tried to cover all available clinical microbiologic laboratories. As we described in the Methods section, we classified the laboratories in the surveillance area into 3 groups, according to their clinical environment. Community-based hospitals which have clinical microbiologic laboratory were identified by the Korean Association of Quality Assurance for Clinical Laboratories (KAQACL) and enrolled as group 3. Most small local clinics, nursing homes and long-term care facilities do not have their own laboratories. They
usually contract major commercial laboratories to carry out microbiologic examinations (group 2).

Although quality assurance was not mandatory for the clinical laboratories, a total of 1,347 laboratories participate in the KAQACL in Korea and we requested them to report the number of ISA infections in all microbiologic laboratories in the studied region (The Korean Association of Quality Assurance for Clinical Laboratory, Available at: http://www.lab-qa.org). In view of the special difficulties facing clinical microbiologic laboratories, such as the labor-intensive nature of the work, the difficulty of maintaining high quality, and the low profit margins, it is unlikely that there are many small microbiologic laboratories that do not participate in KAQACL.

In addition, because of the acute presentation and severity of ISA infections, we think that most patients suspected of having ISA infections are transferred to larger hospitals which have clinical microbiologic laboratory. Hence, we believe that the effect of missed laboratories is minimal.

4. Surveillance regions were not captive. As many as one half of patients in one of the regions (Goyang) seek care elsewhere.

A> The data on major diseases (rates of internal use of medical treatment facilities by the National Health Insurance Corporation) which are cited in the Discussion section were based on chronic illnesses, such as diabetes, hypertension and cancer. Considering the acute presentation and severity of ISA infections, the proportion of exported cases may not be as high as 43.9%. However, we agree with the reviewer’s point that the new town (Goyang) was not suitable for a population-based study. We have therefore changed the title of the manuscript to a multicenter cohort study, as implied by the reviewer.
5. The detailed clinical data is obtained from the ASC academic cohort. These patients have been demonstrated in multiple studies to be markedly different from patients in populations at large. Inclusion of all patients, with minimization of selection bias is a major key aspect of population-based studies for which this study does not fulfill.
A> We agree the review’s comment. As mentioned in the response to comment 4, we refer to our study as a multicenter cohort study with an attempt to estimate incidence rates.

6. It is not clear if the same criterion to define a resident (is this a postal code, census tract, community name etc) is matched to the denominator data.
A> In Korea, a national resident registration system has been in use since 1968. The residence data for the patients with ISA infections (numerator) and the census data (denominator) were based on the same criterion, namely the location given in the resident registration system.

7. Audit was performed at ASC but not in the same degree at the other labs. It is known that audit frequently identifies different incident cases as compared to passive report.
A> As the reviewer points out, we did not perform a separate audit to identify ISA cases in groups 2 and 3. To make this limitation explicit, we have added the following highlighted sentence to the Methods section:

Methods

“…In contrast to the 9 ASCs (group 1), the clinical microbiologic laboratories in group 2 and group 3 were requested to volunteer to participate in this surveillance. In addition, the...
number of cases was only passively reported without residency data, and separate audits to identify ISA cases were not performed in groups 2 and 3.”

8. The results report an estimated rate for all of Korea. Sampling of surveillance regions within Korea was not random. Intra-regional variability occurs within countries. While such an estimate is reasonable to propose, in my opinion this should not be part of the study results but suggested in the discussion.

A> We agree and have removed the estimated rate for all of Korea from the Results section and have retained it in the Discussion section, as suggested.

9. It is not clear as to how the issue of duplicates were handled. For example, if a patient had a MSSA infection with positive cultures of blood (days 1, 3, 5) and pleural fluid (day 4), negative on both on day 7 positive blood again on day 18 following completion of antibiotics, then recurrence 3 months later with septic arthritis due to MSSA and 3 months later MRSA bacteremia, was this considered 1, 2, 3, or 4 incident infections within that year?

A> We counted the number of patients with ISA infections, not the number of isolates. When repeated cultures from normally sterile sites were positive in a single patient within 12 week of the onset of illness, the number of ISA infections was counted as one. Recurrence within 12 week of the onset of illness was defined on the basis of the clinical, radiological, and microbiological information, including the finding that the recurring strain had the same antibiotic susceptibility pattern as the original isolate. ISA infections occurring after more than 12 weeks were considered new cases.

Hence, we would count the patient described by the reviewer as a single ISA case (with
MSSA infection) with recurrence (positive conversion of the MSSA infection and 3 months later septic arthritis, if the joint fluid culture was positive) and a separate ISA case (with MRSA infection).

To make this point clearer, we have added the following highlighted sentence to the Methods section:

**Methods**

**Definitions**

“A case of ISA infection was defined by the isolation of *S. aureus* from a normally sterile site. Normally sterile sites included blood, cerebrospinal fluid, pleural fluid, pericardial fluid, peritoneal fluid, joint/synovial fluid, bone, internal body sites (lymph node, brain, heart, liver, spleen, vitreous fluid, kidney, pancreas, or ovary), and other normally sterile sites. **When repeated cultures from normally sterile sites were positive in a single patient within 12 weeks of the onset of illness, the number of ISA infections was counted as one.**”

This study represents a significant effort for which the authors are commended. However, the majority of the results of the paper are based on the non-population based ASC cohort, with only a small proportion of the study focused on the overall population-based cohort (one sentence of the results in the abstract; first two paragraphs of the main results and Table 1). Given this, and the multiple limitations in design as detailed above, in my opinion, this is questionably a population-based study. Rather, I would consider this a multi-centered cohort study with an attempt to estimate population-based incidence rates.
To this end it is my recommendation that the title of the paper be changed to remove reference to population based design. Furthermore, the text should also be reorganized to indicate that the incidence data were based on a significant number of estimates and assumptions/presumptions. The estimating criteria should be explicitly stated in the methods. Alternatively, the paper could be presented as a multi-centred cohort study with incidence estimate data reserved for presentation in the discussion.

As suggested, we have changed the title to: “Characteristics of invasive *Staphylococcus aureus* infections in three regions of Korea, 2009-2011: a multi-center cohort study”. We have also reduced the scope of our characterization of the ISA infections and have just estimated incidence rates, not identified epidemiologic profiles.

We have made our best effort to reorganize the text and tables to accommodate the reviewers’ suggestions. In the Background section we have clearly defined our study as a multi-center prospective cohort study aimed at characterizing the clinical spectrum of ISA cases and identifying the risk factors for mortality, along with an attempt to estimate population-based incidence rates. We have also explicitly stated the limitation of our incidence rate estimates in the Methods section. Based on the relative priorities and reliability of the data, we have changed the order of the text of the Results, Discussion and Tables to: 1. Clinical characteristics, 2. Clinical outcomes, 3. Risk factors for mortality and 4. Estimating incidence rates. We hope that the changes are considered satisfactory.

We believe we have addressed all the questions and comments in a suitable fashion, but would be happy to provide further information or revision if necessary.
Thank you for your consideration of the revised manuscript.

Sincerely yours,

Hong Bin Kim, M.D.

Associate Professor
Division of Infectious Diseases,
Department of Internal Medicine,
Seoul National University Bundang Hospital
300 Gumi-dong, Bundang-gu, Seongnam-si
Gyeonggi-do, 463-707, Republic of Korea
Tel: +82-31-787-7021, Fax: +82-31-787-4052
E-mail: ksbkhb@chollian.net or hbkimmd@snu.ac.kr