Response to Reviewers' comments

Reviewer #2

C1. This study evaluated the efficacy of adjunctive aerosolized colistin (AS colistin) to IV colistin with LD. Because treatment options are limited in the treatment of CRGNB infection, this study results are important to clinicians. The conclusions of this study were 1) IV colistin with LD was not more effective than IV colistin without LD, 2) AS colistin when it combined with IV colistin with LD reduced 30-day mortality compared with IV colistin alone, 3) AS colistin and IV colistin with LD (AS-LD) did not increase nephrotoxicity. An interesting point of this study is that dose of AS colistin was higher than those of most previous studies (450 mg/day vs 60 ~ 300 mg/day). The daily dose of AS colistin should be emphasized and reviewed in the Discussion section. The paper is well written and easy to follow.

R1. We thank you for all your valuable and positive comments. According to the Reviewer’s comments, we did our best to address the issues. In addition, we emphasized the high dose of AS colistin used in our study in the Discussion section.

Minor comments

C2. AS colistin is usually not effective in non-intubated patients. All patients with HAP received mechanical ventilation in this study. In the Methods section, the sentence such as “AS colistin was administered only in patients who received mechanical ventilation” should be added. In addition, there is a confusing description for methods of colistin inhalation (line 55-60, page 8). Did some non-intubated patients receive AS colistin using a jet nebulizer?

R2. We apologize for the lack of clarity. Yes, you are right. The AS colistin was initiated in patients with mechanical ventilation. However, if the patients were extubated, the AS colistin continued using jet nebulizer. We clarified this in the revised manuscript.

C3. Page 11, Line 50-53: The daily median dose of IV colistin is not correct (wrong unit, mg/day). “mg/kg/day” is probably correct.

R3. Thank you for pointing out our mistake. We closely examined our manuscript again and corrected the type errors in the revised manuscript.

C4. Page 12, Line 23-27: Adjunctive AS colistin to IV colistin (not AS colistin alone) was significantly associated with lower mortality.

R4. We apologize for our carelessness. We corrected the error in the revised manuscript.

C5. In Table 3, 30-day mortality in the non-LD IV group is not correct (36% to 46%). In addition, the definition of clinical response and microbiological response included recurrence, clinical failure, and
microbiological recurrence, failure, respectively. Therefore, clinical failure and microbiological failure should be added in Table 3.

R5. Thank you for pointing out our mistake. We closely examined our data again and corrected the type errors in the revised manuscript. In addition, we added the information on clinical failure and microbiological failure in Table 3.

C6. In Table 5, the unit of IV colistin dose is not correct (from per mg/day to per mg/kg/day).
R6. Thank you for pointing out our mistake. We closely examined our manuscript again and corrected the type errors in the revised manuscript.

C7. In the Discussion section, I suggest more discussion about AS colistin (higher dose than previous studies) rather than about colistin LD.
R7. We added more discussion about AS colistin in the Discussion section as your suggestion.

C8. Page 16, Line 4-10: “Adjunctive AS colistin to IV colistin with LD was related to improved microbiological and clinical outcomes without an increase in nephrotoxicity.” However, the meaningful clinical effect of adjunctive AS colistin was only a decrease in 30-day mortality.
R8. Thank you for your valuable comment. We modified the sentence.

Reviewer #3
In this paper, Junsu Choe and colleagues retrospectively assessed the impact on mortality of inhaled (AS) plus intravenous (IV) colistin vs. IV colistin alone in patients with HAP/VAP. Below are major and minor comments on the technical merits of the paper. It should be acknowledged that I previously reviewed this same paper. The same comments apply.

We thank the reviewer for valuable comments. Addressing them fully has significantly strengthened the manuscript.

Major comments
C1. In my opinion, a major drawback of the paper is that the IV loading dose (LD) was considered for defining groups and not as a covariate possibly influencing the outcome (see following comments)
R1. We understand the reviewer’s concern that intravenous loading dose could influence the better outcome in patients received adjunctive AS colistin in addition to intravenous loading dose and it should be adjusted in our logistic regression model. As described in the footnote of Table 4, the final
model of our logistic regression models adjusted for the intravenous loading dose. But, we should have described clearly the statistical method in original manuscript. We added the details on the logistic regression models in the Method section.

C2. Only patients receiving an intravenous loading dose (LD) of colistin were included in the IV-AS group, while it seems that patients treated both with and without LD were included in the IV group considered for the multivariable analyses for mortality. Since according to the most recent PK/PD models a LD is necessary in critically-ill patients to guarantee rapid attainment of steady state concentrations (and thus possibly favorably influencing survival), the study might be biased towards observing a better survival in the IV-AS group. Of note, this possible selection bias involves the primary outcome of the paper and it might exist despite the fact that mortality in the IV-AS group was apparently lower than in the LD-IV group (since this comparison was not adjusted).

R2. As responded to the first comment, intravenous loading dose was adjusted in the final logistic regression model for determining an association of AS colistin in addition to intravenous loading dose with 30-day mortality.

C3. Propensity to receive AS might have also influenced the results and should thus be considered in the analyses

R3. According to the reviewer’s comment, propensity score (PS) matching was conducted to control differences in baseline characteristics between patients with LD IV and AS-LD, because the decision to use adjunctive AS colistin was left to the individual physician’s discretion. A PS was generated for each patient from a multivariable logistic regression model based on pretreatment covariables as independent variables with LD IV versus AS-LD as a binary dependent variable. Covariables included in the PS model were gender, age, SOFA score, type of pneumonia, respiratory component of the SOFA score and mechanical ventilation at the time of IV colistin initiation. Each case of AS-LD was matched with up to 3 cases of LD IV using a caliper of 0.25. Standardized mean difference (SMD) was demonstrated in the unmatched and matched patients, highlighting that PS matching successfully decreased the SMD for 6 variables to an absolute value of ≤ 0.2. The rate of microbiological eradication was significantly higher in the AS-LD group (60%) than in the LD IV (39%) group (P = 0.039). In addition, 30-day mortality was lower in the AS-LD group (23%) than in the LD IV (47%) groups (P = 0.016).

C4. As regards nephrotoxicity, logistic regression is probably not the correct model to be adopted because of the potential presence of patients who died early in absence of nephrotoxicity.

R4. We thank the reviewer for valuable comment. As the Reviewer pointed out, death is a competing risk which precludes the possibility of experiencing the nephrotoxicity. To consider death as a competing risk for nephrotoxicity, we performed Fine-Gray competing risk models. After adjusting for other risk factors and accounting for the competing risk of death, only predictor of increased cumulative incidence of nephrotoxicity was use of vancomycin (HR 1.652, 95% CI 1.023 - 2.668, P = 0.040). We added this information in the Method and Results section.
C5. An important limitation of retrospective studies is the lack of standardization as regards the timing of microbiological sampling, which might severely impair the reliability of the assessment of microbiological eradication.

R5. We totally agree with your concern. It should have been acknowledged as a major limitation of our study. We added this important limitation in the Discussion section.

C6. After an initial selection of variables according to their potential association with mortality in univariable comparisons, the authors performed different multivariable models with arbitrary selection of the variables to be included (table 3). This could be ok, but the rationale should be provided.

R6. We apologize for the lack of clarity. To adjust for potential confounding factors, variables with a P value less than 0.2 on univariate analyses, as well as a priori variables that were clinically relevant were entered into the forward stepwise multiple logistic regression model. We added this information in the Method section.

Minor comments

C7. Please report colistin dosage both in mg of CBA and in MU of CMS

R7. As recommended, we added the equivalent dose of CBA to CMS and conversion factor in Table 1.

C8. Results. “Of the 191 patients eligible for the analysis”. How many patients were not eligible? Please report the work-flow of the patients inclusion process.

R8. We provide a flow diagram to report number of patients at each stage of study.

C9. Adding Kaplan-Meier curves might provide interesting additional information

R9. We thank the reviewer for valuable comments. As the Reviewer pointed out, we added Kaplan-Meier curves for survival and probability of culture positive by each method of colistin treatment as presented in Figure 2 of the revised manuscript.

C10. Tables. Variables “immunocompromised” and “nephrotoxins” should be defined better

R10. As the Reviewer pointed out, we defined the immunocompromised status in the Method section. However, details of nephrotoxic agents were described in the Table and footnote.

C11. No efficacy data can be inferred from a non-interventional study

R11. We absolutely agree with your concern. We modified our sentences to reflect this concern.