Peer Review File
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Reviewer A

Comment	Reply
Line 64: 'should be considered in order to early empirical antibiotic prescription.'. Consider 'in order to expedite antibiotic prescription'	We have modified our text as advised. "COVID-19 pneumonia patients with these associated factors, an early bacterial co-infection should be considered in order to expedite antibiotic prescription." (See Page 3, line 64)
Line 91: I would suggest mentioning earlier that you define early co-infection as within 48 hours of admission & perhaps also include this in the abstract. It may also be valuable to mention in the abstracts a short sentence on how patients were categorised into these groups.	We added a text as advised. "defined by an infection occurring within the first 48 hours after admission" (See Abstract, page 2, line 33) "The definition of early bacterial co-infection may vary among studies, but it generally refers to bacterial infection occurring within the first 48 hours of hospitalization." (See Introduction, page 4, line 85)
Line 162: Was there a reason for why a random sample of this cohort size was used & why a number of 245 was selected?	Sample size estimation was performed based on the previously reported prevalence of early bacterial co-infection and the sample size equation for the descriptive study[1]. Type 1 error, confidence interval width, and expected prevalence were set at 5%, 3%, and 5.5%[2], respectively. The percentage of missing data was set at 10%, thus at least 245 subjects were needed for analysis. (See Methods, Page 6, line 152 and Result, Page 7, line 166) **Reference:** 1. Sharma N, Putman MS, Vij R, Strek ME, Dua A. Myositis-associated Interstitial Lung Disease: Predictors of Failure of Conventional Treatment and Response to Tacrolimus in a US Cohort. J Rheumatol. 2017;44(11):1612. 2. Baskaran V, Lawrence H, Lansbury LE, Webb K, Safavi S, Zainuddin NI, et al. Co-infection in critically ill patients with COVID-19: an observational cohort study from England. J Med Microbiol. 2021;70(4).
Line 181: Were all chest X-rays performed on arrival & within 48 hours?	We added a text as advised.

I presume so but did not see this overtly stated.	"All patients performed chest X-rays within 48 hours of admission." (See Result, page 8, line 187)
Line 278: I would expand on the statement regarding early bacterial co-infection being low - what is this figure being compared to?	The results of our study showed the prevalence of early bacterial co-infection in hospitalized COVID-19 pneumonia was 15.5% which was lower than the prevalence of that in severe influenza which has been reported as high as 30-50%. (See Introduction, page 4, line 90)
I would expand upon the conclusion. It is perhaps worthwhile mentioning why it is that empirical antibiotics are potentially detrimental, drawing upon studies in COVID-19 showing antibiotics may lead to greater morbidity & mortality when given inappropriately.	We added and modified a text as advised. "There have been studies demonstrating that inappropriated use of antibiotics may lead to increased morbidity and mortality in patients with COVID-19. Furthermore, the overuse of antibiotics results in antibiotic-related side effects and development of resistant nosocomial bacterial and fungal pathogens." (See Discussion, Page 12, line 293) **Reference:** 1. Rawson TM, Moore LSP, Zhu N, Ranganathan N, Skolimowska K, Gilchrist M, et al. Bacterial and Fungal Co-infection in Individuals With Coronavirus: A Rapid Review To Support COVID-19 Antimicrobial Prescribing. Clin Infect Dis. 2020;71(9):2459-68. 2. Langford BJ, So M, Raybardhan S, Leung V, Soucy JR, Westwood D, et al. Antibiotic prescribing in patients with COVID-19: rapid review and meta-analysis. Clin Microbiol Infect. 2021;27(4):520-31. 3. Vaughn VM, Gandhi TN, Petty LA, Patel PK, Prescott HC, Malani AN, et al. Empiric Antibacterial Therapy and Community-onset Bacterial Co-infection in Patients Hospitalized With Coronavirus Disease 2019 (COVID-19): A Multi-hospital Cohort Study. Clin Infect Dis. 2021;72(10):e533-e41.
I would also suggest mentioning that use of the mechanisms you used to divide patients into groups (early infection vs. unlikely) are therefore useful in deciding upon the need for empirical antibiotics (e.g. CRP, WCC, and radiological evidence can be used to decide upon the	We added a text as advised. "According to the results of the present study, the initial empirical antibiotics may be considered in COVID-19 pneumonia patients with higher comorbidities, diffuse or mass-liked opacities on chest X-ray, and receiving a high level of

need for early antibiotics within 48 hours, and otherwise they should not be given).	respiratory support while waiting for confirmation from microbiological tests." (See Discussion, Page 12, line 304)
It may also be pertinent to mention that routine culturing for patients with COVID-19 should be performed, given within your sample size the low number of patients with a culture taken (25.3%).	Collecting respiratory specimens for microbiological studies should be considered depending on the clinical suspicion of bacterial co-infection. We added a text to mention our opinion based on data from literatures and our results. "However, these radiological features can overlap and cannot be totally distinguished from COVID-19 pneumonia, combining with the clinical data and other laboratory tests would be necessary to consider microbiological tests to confirm the presence of early bacterial co-infection" (See Discussion, Page 11, line 265)
The sample size used by random selection does lose some statistical power given here we are talking about rates of co-infection from a group of 62 patients who had cultures performed. Could microbiological data have been collected for all patients within your timeframe, and those studied could be only those with cultures taken? As this would give a greater significance to your figures.	• We did not perform microbiological tests in all patients because of the followings: 1. The present study was a retrospective study. We did not have a routine protocol for collecting respiratory specimen in all patients with COVID-19 pneumonia. 2. There was a limitation in respiratory specimen collection due to the risk of spreading the virus to healthcare providers and other patients. • These appear to be a limitation of the present study but it is a real-life situation in the pandemic era (see reference). • In a subgroup analysis of patients who had microbiological confirmation, the prevalence of early bacterial co-infection was 9.7% (6 of 62), which was not markedly different from the prevalence reported in our results and conclusions. (See Discussion, Page 10, line 242; and Page 11, line 279) **Reference:** Russell CD, Fairfield CJ, Drake TM, Turtle L, Seaton RA, Wootton DG, et al. Co-infections, secondary infections, and antimicrobial use in patients hospitalised with COVID-19 during the first pandemic wave from the ISARIC WHO CCP-UK study: a multicentre, prospective cohort study. Lancet Microbe. 2021;2(8):e354-e65.

Reviewer B

Comment	Reply
• It would appear to me, if I understand the timing of these "early co-infections" as defined by the authors, that these are not co-infections, but rather superinfections (some may have occurred early and others later). • It seems to me that many of these patients may have had SARS-CoV-2 infection for several days before the diagnosis was made, at which time the investigations for "co-infections" were undertaken. I would therefore suggest that these were "superinfections" • All the additional investigations performed (as per the secondary objectives) would appear to be invalid based on a consideration that the definition of these "early co-infections" is incorrect.	I agree with your comment. The definitions of co-infection and nosocomial superinfection are clearly defined in literature and guidelines. However, the differentiation between co-infection and superinfection might be difficult in clinical practice because most patients usually have symptom onset for several days before visiting the hospital or admission. We thus followed the definition as it was widely used in several studies on COVID-19 and influenza which focused on community pathogens.
Most of the common microorganisms found in the patients were more typical of nosocomial pathogens (superinfections) than community-acquired infections.	*S.aureus* (17.8%), *H.influenzae* (12.7%), and *S.pneumoniae* are common pathogens reported in various studies. Gram-negative organisms were more reported as causative pathogens in nosocomial infection or superinfection. However, gram-negative organisms have also been reported as the causative pathogens in early bacterial co-infection with variable prevalence, including *P.aeruginosa* (9.3%), *K.pneumoniae* (3.4%), and *E.coli* (7.6%). In the present study, two patients had *S.maltophilia* mixed with *K.pneumoniae* and MSSA on their sputum culture. Therefore, the authors classified these patients into the early bacterial co-infection group. (See Discussion, Page 12, line 286) **References:** 1. Russell CD, Fairfield CJ, Drake TM, Turtle L, Seaton RA, Wootton DG, et al. Co-infections, secondary infections, and antimicrobial use in patients hospitalized with COVID-19 during the first pandemic wave from the ISARIC WHO CCP-UK study: a multicentre, prospective cohort study. Lancet Microbe. 2021;2(8):e354-e65.

	2. Elabbadi A, Turpin M, Gerotziafas GT, Teulier M, Voiriot G, Fartoukh M. Bacterial co-infection in critically ill COVID-19 patients with severe pneumonia. Infection. 2021;49(3):559-62.
Microbiological data (including PCR) was only available in 62 of 245 (25.3%) of the patients and, therefore, while this is a study of "co-infections" almost 75% of patients had no microbiological data.	• We did not perform microbiological tests in all patients because of the followings: 1. The present study was a retrospective study. We did not have a routine protocol for collecting respiratory specimen in all patients with COVID-19 pneumonia. 2. There was a limitation in respiratory specimen collection due to the risk of spreading the virus to healthcare providers and other patients. • These appear to be the main limitation of the present study but it is a real-life situation in the pandemic era (see reference). • In a subgroup analysis of patients who had microbiological confirmation, the prevalence of early bacterial co-infection was 9.7% (6 of 62), which was not markedly different from the prevalence reported in our results and conclusions. (See Discussion, Page 10, line 242; and Page 11, line 279) **Reference:** Russell CD, Fairfield CJ, Drake TM, Turtle L, Seaton RA, Wootton DG, et al. Co-infections, secondary infections, and antimicrobial use in patients hospitalised with COVID-19 during the first pandemic wave from the ISARIC WHO CCP-UK study: a multicentre, prospective cohort study. Lancet Microbe. 2021;2(8):e354-e65.
The definition of "probable co-infection" was at least 2 SIRS criteria in patients that were treated with antibiotics for at least 5 days. I am unfamiliar with this definition, which is not referenced. Were these cases included with the "early co-infection" patients?	• We considered to include “probable bacterial co-infection” as an early bacterial co-infection group because, in real-life situation, we did not perform microbiological tests in all patients as described in the above section. • There is no definite definition of “probable bacterial co-infection”. Therefore, we modified the definition from the term “clinically diagnosed bacterial co-infection” in He’s and Coenen’s study, as well as based on our clinical practice. We have added the reference in the text. (See Methods, Page 6, line 135) **Reference:** 1. He S, Liu W, Jiang M, Huang P, Xiang Z, Deng D, et al. Clinical characteristics of COVID-19 patients

	with clinically diagnosed bacterial co-infection: A multi-center study. PLoS One. 2021;16(4):e0249668. 2. Coenen S, de la Court JR, Buis DTP, Meijboom LJ, Schade RP, Visser CE, et al. Low frequency of community-acquired bacterial co-infection in patients hospitalized for COVID-19 based on clinical, radiological and microbiological criteria: a retrospective cohort study. Antimicrob Resist Infect Control. 2021;10(1):155.
I do not agree with the authors' conclusions that "early co-infections" are rare. They quote that the literature shows a rate of these infections between 0-46% of cases and I also do not think the finding in this study of a rate of 15.5% is rare at all, given that almost 75% of cases did not have microbiological data. The denominator for assessing the rate of "early co-infections" should therefore be the 62 patients with microbiological data and not all 245 patients, this may give a very much higher rate of "early co-infections".	• We conclude that early bacterial co-infection in COVID-19 pneumonia in the present study was low (15.5%) (as compared to the prevalence of bacterial co-infection in influenza). • In a subgroup analysis of patients who had microbiological confirmation, the prevalence of early bacterial co-infection was 9.7% (6 of 62), which was not markedly different from the prevalence reported in our results and conclusions. **Reference:** Klein EY, Monteforte B, Gupta A, Jiang W, May L, Hsieh YH, et al. The frequency of influenza and bacterial co-infection: a systematic review and meta-analysis. Influenza Other Respir Viruses. 2016;10(5):394-403.
Furthermore, while being mindful of the need for careful assessment of the need for antibiotics (in line with antimicrobial stewardship), the conclusion that antibiotics are not needed, based on this study would appear not to be correct. In this respect, do the authors have the details of the use of antibiotics.	• According to the results of the present study, we conclude that “there is insufficient evidence to support the empirical use of antibiotics in patients with COVID-19 pneumonia.” • We added a text indicated the details of the use of antibiotics based on our results. “According to the results of the present study, COVID-19 pneumonia patients with higher comorbidities, diffuse or mass-liked opacities on chest X-ray, and receiving a high level of respiratory support, the initial empirical antibiotics may be considered while waiting for confirmation from microbiological tests.” (See Discussion, Page 12, line 304)
It is quite well described in the literature that the PCT can be raised in patients with co-infections, but also in patients with COVID-19 pneumonia that is progressing, so the use of PCT is not always that helpful in including/excluding co-infection.	I totally agree. We found no significant difference in PCT levels in both groups.

Reviewer C

Comment	Reply
I recommend making a further revision of the manuscript to fix some small typing/language errors. For example, line 99 “Thailand” is redundant.	I have corrected our statement as advised. “In Thailand, the data on the prevalence of early bacterial co-infection in patients with COVID-19 pneumonia is limited.” (See Methods, Page 4, line 103)
The title is clear and direct. Personally, I believe it could be improved and be more focused on results. For example: “Early Bacterial Co-infection in Hospitalized Patients with COVID-19 Pneumonia is associated with a higher mortality.”	I prefer the previous title because it reflects the primary outcome of the study.
Authors should add other KW, in order to increase the traceability of this paper (and consequently the possibility of the Journal to be cited by Readers and Stakeholders).	Keywords were added as advised. (See Page 2, line 48)
Although the introduction fits the context of the study, it is concise. Sometime, many concepts clearly explicated in an exhaustive introduction could help readers to become passionate about reading the paper and using it as a reference.	I have modified our text as advised by adding some definitions of early bacterial co-infection and some details of nosocomial infection. (See Introduction, Page 4, lines 84-92)
It is important to underline that imaging plays an important role in monitoring these patients, helping detect these complications and, therefore, suggesting further laboratory investigations	We added a text as advised. “Chest X-ray is commonly used and plays an important role in monitoring COVID-19 pneumonia patients to assess the severity and extent of lung involvement, identify complications, and treatment guidance.” (See Discussion, Page 10, line 257) “The present study confirmed the usefulness of chest X-ray in determining the severity and monitoring the disease.” (See Discussion, Page 11, line 262)
It is necessary to state that secondary infections (or superinfections), defined as infections that emerge during the course of the illness or hospital stay (i.e., >48–72 h after admission), are more frequently diagnosed in COVID-19 patients, reaching up to 45% of cases.	We added a text as advised. “One of the most anticipated risk factors is a bacterial infection, including early bacterial co-infection and nosocomial bacterial infection, which the latter appears to be more frequent with a prevalence of 45-71%.” (See Introduction, Page 4, lines 82-85)

	"The most common causative pathogens included *S.aureus*, *H.influenzae*, and *S.pneumoniae* while gram-negative organisms were more reported in nosocomial infection" (See Discussion, Pages 11-12, line 284)
Another main limitation of the study is that no other concomitant viral and/or fungal infections were reported, which are well-known co-morbidities in these patients.	Thank you for your suggestion. The primary objective of the present study is to demonstrate the prevalence of early bacterial co-infection. Although Paparoupa's study demonstrated a high incidence of viral and fungal superinfections occurring more than 7 days after intubation which indicated that these pathogens were nosocomial, so we did not mention these results in the manuscript.

Reviewer D

Comment	Reply
In the title of the abstract, it is written a retrospective review study that needs to be corrected and change to retrospective study	We have modified our text as advised. "The present study is a retrospective study" (See Title, Page 1, line 2; Abstract, Page 2, line 31; Method, Page 4, line 107)
The authors have written in the lines 92-3 : "The most commonly reported causative pathogens included methicillin-susceptible Staphylococcus aureus (MSSA), Hemophilus influenzae, and Streptococcus pneumoniae, respectively" While some other studies consider Gram-negative pathogens to be the most important isolated pathogens from COVID-19 patients, therefore, these studies should also be included.	We added a text as advised. "Gram-negative organisms have also been reported as the causative pathogens in early bacterial co-infection with variable prevalence, including *P.aeruginosa* (9.3%), *K.pneumoniae* (3.4%), and *E.coli* (7.6%)." (See Discussion, Page 12, line 286) **References:** 1. Russell CD, Fairfield CJ, Drake TM, Turtle L, Seaton RA, Wootton DG, et al. Co-infections, secondary infections, and antimicrobial use in patients hospitalized with COVID-19 during the first pandemic wave from the ISARIC WHO CCP-UK study: a multicentre, prospective cohort study. Lancet Microbe. 2021;2(8):e354-e65. 2. Elabbadi A, Turpin M, Gerotziafas GT, Teulier M, Voiriot G, Fartoukh M. Bacterial co-infection in critically ill COVID-19 patients with severe pneumonia. Infection. 2021;49(3):559-62.

The authors should explain why patients with a history of hospitalization in the last 14 days were excluded from the study?	We excluded this group because patients with a history of hospitalization in the last 14 days are at risk of nosocomial infection.
The legend of the tables and figures such as fig1 and table 2 and 5 are not complete and informative and it is necessary to change them	We have modified our text as advised. • **Figure 1** Study flow chart. 1,429 hospitalized COVID-19 pneumonia patients were available for selection, and 245 patients who met the inclusion criteria were randomly selected for the analysis, with the sample size determined based on a calculation. • **Table 2** Clinical features of patients in the Early bacterial co-infection and Unlikely early bacterial co-infection groups • **Table 3** Hospital course and complications of patients in the Early bacterial co-infection and Unlikely early bacterial co-infection groups • **Table 5** Univariate and multivariate analyses of the factors associated with early bacterial co-infection
Why is p value 0.01 written in table number 4 facing the outcome? Please correct it	I correct the table as advised.
In table number 5, it is expected that all the variables that have a p value less than 0.05 in the Univariate analysis will be included in the Multivariate analysis, while this did not happen with the variables such as Male , Coronary artery disease and etc, please explain it	The variable CRP and Brixia score were not included in the multivariate analysis because There are no patients in the early bacterial co-infection group who had CRP < 60 mg/dL and Brixia chest X-ray score < 8. The variable initial ICU admission and vasopressor use were not included in the multivariate analysis because the unlikely early bacterial co-infection group had a small sample size, which could affect the reliability of the results. The other variables were not included in the multivariate analysis because they appeared to be clinically irrelevant, making it difficult to draw meaningful conclusions.
In the results, no information is mentioned about the COVID treatments used in these patients, while some treatments such as corticosteroids or interleukin-6 antagonists can increase the incidence of bacterial infections due to the immunosuppression effect .	Thank you for your suggestion. To determine if corticosteroids/IL-6 antagonists/JAK inhibitors affected the outcome and led to complications such as nosocomial infection, it is important to know the proportion of patients who received these treatments. All patients in the present study received corticosteroids due to the indication of pneumonia and desaturation. Unfortunately, we did not have data on which patients received other

	treatment, such as IL-6 antagonists and JAK inhibitors.
The authors did not explain and discussed why the number of deaths in group with unlikely bacterial infections was greater than patients with bacterial infections	The mortality of patients with unlikely early bacterial co-infection was lower than in patients with early bacterial co-infection, 12.6% and 31.6%, respectively ($p = 0.012$). (See Table 3 and Results Page 8, line 201)