Reviewer A

This manuscript looks at the effect of perioperative respiratory colonization on outcome after lung transplantation, the microbiome and incidence of early pneumonia in LTx-patients were analysed in a retrospective analysis. Specimens from bronchial washing, bronchoalveolar lavage, and sputum aspiration collected before and after surgery were used for determination of perioperative colonization.

In a total of 76 LTx-patienten, 34 donors (44.7%) and 28 recipients (36.8%) had a preoperative respiratory colonization. Post-LTx-pneumonia was lower in the survivor group (38.5% vs. 70.8%, P=0.009). Only 57.1% of patients with preoperative respiratory colonization, were alive at 1-year after lung transplantation, versus about 82% without colonization. Perioperative respiratory colonization of donors was not associated with 1-year survival

The authors conclude that perioperative colonization of recipients was a factor for PTP and associated with 1-year mortality in patients after LTx, and they state that this result may be significant in setting the donor acceptance criteria.

The topic is interesting and important, since identification of risk factors for decreased prognosis might improve limited outcome after lung transplantation. Similar data have been published previously. The impact of bacterial colonization before LTx has been shown in especially patients with cystic fibrosis and bronchiectasis previously.

There are several points to be addressed:

Comment 1. The abstract should be structured clearer, e.g. the total number of LTX-patients and the survival rate of the noncolonized patients should be mentioned.

The effect of pre-LTX colonization is pronounced, however this might be due to the relatively low number of patients.

Reply 1: I agreed your comment. I added total number of patients and the survival rate to clear result. (See Page 2, line 10)

Change in the text: Result: Among total of 76 patients underwent lung transplantation, 34 donors (44.7%) and 28 recipients (36.8%) showed positive respiratory cultures with respect to preoperative respiratory colonization. A separate analysis of donors and recipients showed that 42 donors and 48 recipients were in respiratory non-colonized state, and 28 (53.8%) patients and 36 (69.2%) patients survived at 1-year after lung transplantation, respectively.

Comment 2. A surprisingly high number of carbapenem-resistant Acinetobacter baumannii (13 of 76 patients) needs some explanation, please comment on this and give some information about the microbiological setting of your hospital.

Is there a temporal trend in the spectrum of bacteria?

Reply: MDR gram negative pathogen including CRAB is a uprising problem in a Korean and Asian ICU (1,2). Mostly, the structure of a Korean ICU is a mixture of open ward and single room. In that, donor can be colonized with CRAB at the time of graft harvesting. As well, Acinetobacter strains naturally inhabits water and soil, and other possible reservoirs including health care environment, even in respiratory and GI tract. In addition, the proportion of high urgent recipients were very high in this population. This could be another potential reason for high rate of CRAB colonization.

The colonized CRAB was highly likely to be activated and caused host infection. In general, several processes have been demonstrated on how colonized microorganisms actually become true pathogen of infection. Maintenance of the ventilator (1,2), impaired sputum drainage due to lower ciliary movement of the bronchus (3,4), damaged barrier due to the mucosal injury (5,6) and micro-aspiration (7-9) have bed found to be the leading causes of infection. Acinetobacter baumannii has additional characters for better activation of the infection than the above known reasons. A. baumanni has the ability to adhere and form biofilm on both biological and non-biological surfaces. That ability can activation colonization to infection as true pathogen. (10,11) In all lung transplant patients, there is a period in which ventilator treatment is required, so the ability of A. baumannii to attach to non-biological was more important role to cause nosocomial infections (10-12).

MDR-AB is one of serious ICU acquired pathogens in the eastern Asian country and it has become a major challenge to solid organ transplant recipients. Therefore, when MDR colonization was found, antibiotics were actively applied and the results showed

that MDR was not related to the 1 year mortality rate. Importantly, the proportion of MDR among the group with PTP was high. MDR itself was not associated with 1year mortality, but when PTP occurred, it is associated with 1 year mortality. In summary, caution is needed in patients with pre sputum MDR colonization.

Change in the text: This comment did not change the text, because it explains the reason why CRAB is high in our hospital.

Comment 3. Suppl Table 1 needs a legend for abbreviations.

Reply: I add the abbreviations.

Change in the text: Abbreviations: CSAB, carbapenem-susceptible Acinetobacter baumannii; CRAB, carbapenem-resistant Acinetobacter baumannii; CSPA, carbapenem-susceptible pseudomonas aeruginosa; CRPA, carbapenem-resistant pseudomonas aeruginosa; MSSA, methicillin-susceptible Staphylococcus aureus; MSRA, methicillin- resistant Staphylococcus aureus; K.pn. ESBL, Klebsiella Pneumoniae extended spectrum beta-lactamase; NTM, nontuberculous mycobacteria; E. faecalis, Enterococcus faecalis; E.coli, Escherichia coli; MRCNS, methicillin-resistant coagulase-negative Staphylococci; E. aerogenes, Enterobacter aerogenes

Reviewer B

This is a retrospective unicenter study in a small cohort of LT patients in a hospital with high incidence of CRAB.

Lack of correlation between colonization pre-transplant and PTP is the major strength. Merging many characteristics is the major weakness, that needs to be detailed in the discussion.

Authors have to do an effort to detail and assess subgroups.

Comment 1: Please provide the lung transplant protocol as ESM.

Reply: Due to the hospital's own regulations, the protocol cannot be disclosed as non-public material. So I will explain it very simply here.

1. Use of prophylactic antibiotics according patient condition.
2. lifelong Pneumocystis jiroveci prophylaxis with trimethoprim-sulfamethoxazole

3. routine anti-CMV prophylaxis for 6 months

4. routine anti-fungal prophylaxis with itraconazole for 6 months

5. maintenance with tacrolimus, mycophenolate mofetil, and prednisolone

6. Routine check CT chest, BAL

Change in the text: This comment did not change the text because this information is confidential in accordance with the internal regulations of the hospital.

Comment 2: Authors have to clarify whether cultures were qualitative or quantitative and the threshold used.

Reply: The diagnosis of pneumonia was defined with clinical criteria or bacteriologic criteria. If respiratory specimen are qualitative, for instance sputum or tracheal aspirates, pneumonia was defined with clinical criteria described in method part. If quantitative respiratory culture is available, positive threshold is above 104 colony forming units/ml. The diagnosis of pneumonia was defined with clinical criteria or bacteriologic criteria according to ATS guideline. In addition, all microbial data were from qualitative results. (See Page 4, line 21)

Change in the text: It was definition that the positive of culture is bacterial was growth. That is qualitative.

Comment 3: Distribution of organisms in sputum, bronchial washing and BAL must be reported. Any difference between sputum vs BAL isolates?

Reply: Bronchoalveolar lavage (BAL) is a procedure used to collect tissue and immune cells from the lower respiratory system. It is mainly used to collect immune cells from the lung bronchial surface. There is no difference between the BAL and bronchial washing in qualitative culture and only in the case of quantitative culture. Therefore, there was no difference in the distribution of organisms in sputum, bronchial washing and BAL.

Change in the text: This comment did not change the text because it is an explanation of the reviewer's question.

Comment 4: What were the differences between bilateral & unilateral LT in organisms, PTP & outcomes?

Reply: Mostly, we did bilateral lung transplant except 3 cases. Because of high

proportion of high urgent recipients, bilateral lung transplant preferred in our hospital. Among 3 cases, we do not find any differences in organisms, PTP incidence and outcome.

Change in the text: No new content was added to the manuscript as it did not affect the results.

Comment 5: What was the distribution of organism depending of the underlying disease: DILD, COPD; Pulmonary hypertension?

Reply: The distribution of organism according to the underlying disease are as follows. There was no statistical significance when the analysis was performed according to the underlying disease.

	Pre colonization SC	**Post colonization SC**
DILD	Candida, K.pn. ESBL+	CSPA, CRPA, K.pn. ESBL+, MRSA, MSSA, candida, NTM, aspergillus, E.coli, , CRAB, other
COPD	Candida, CRAB, K.pn. ESBL-/, other	Candida, CRPA, K.pn. ESBL+, MRSA, CRAB, , CRAB
ARDS	CSPA, CRPA, K.pn. ESBL+, MRSA, MSSA	CRAB, Candida, CRPA, K.pn. ESBL+, MRSA,
BO	CRPA, MSSA, K.pn. ESBL+	K.pn. ESBL+, MRSA, CRAB, other
BE		CRAB
Other	Candida, E.faecalis	Candida, CRAB, MRSA, CRPA, K.pn. ESBL+

Change in the text: No new content was added to the manuscript as it did not affect the results.

Comment 6: How many patients have CF and what were the differences if excluded?

Reply: As your point, CF is a very important issue regarding pretransplant bacterial coloinzatio. However, the prevalence of CF is almost zero in Korea due to genetic and

ethnic difference.

Change in the text: No new content was added to the manuscript as it did not affect the results.

Comment 7: Please, provide details on organisms causing PTP.

Reply: We provide in supplementary table.

Change in the text: Please refer to the supplement table1.

Comment 8: I recommend to use in the multivariate analysis: age as a qualitative variable with breakpoint 55yrs, rather than using age as a continuous variable.

Reply: We accepted your suggestion and revised the article as following: Change in the text:

Table 2. Cox proportional hazard model for 1-year mortality

	Univariate		Multivariate	
	HR (CI)	P	HR (CI)	P
Recipient				
Age*	3.523(1.4-9.2)	0.010	3.954(1.5-10.3)	0.005
BMI	1.063(1.0-1.2)	0.146		
Preoperative sputum colonization	1.917(0.9-4.3)	0.111	2.550(1.2-5.2)	0.010
BTT	2.010(0.6-6.7)	0.258		

	Univariate analysis		Multivariate analysis	
PTP	3.067(1.3-7.4)	0.013	3.239(1.4-7.3)	0.005

Abbreviations: BMI, body mass index; BTT, bridge to transplantation; PTP, post-transplant pneumonia; CI, confidence interval.

*Age was used as a qualitative variable with a breakpoint 55yrs.

Table 4. Binary logistic regression analysis for risk factors of early postoperative pneumonia

	Univariate analysis		Multivariate analysis	
	OR (95% CI)	P	OR (95% CI)	P
Recipient				
*Age	0.974(0.4-2.3)	0.951		
Preoperative SC MDR	2.979(1.0-9.0)	0.052	3.284(1.2-8.8)	0.018
Donor				
Preoperative SC MDR	1.550(0.5-4.9)	0.455		

BTT	1.286(0.4-3.9)	0.657
**Appropriateness of prophylactic antibiotics	0.943(0.3-2.6)	0.909

Abbreviations: BMI, body mass index; SC, sputum culture; MDR, multidrug resistance;

PF ratio, PaO2/FiO2 ratio; TLC DR ratio, total lung capacity donor/recipient ratio; BTT,

bridge to transplantation; OR, odds ratio

*Age was used as a qualitative variable with a breakpoint 55yrs.

Comment 9: Authors do not detail incidence of 72h Grade III PGD, ECC, or PAFI<200 at ICU admission. PTP needs to be correlated with this variable.

Reply: We use ECMO during lung transplant instead of CPB. Our strategy is a continuum of ECMO support in immediate postoperative period until G III-IV is not evident withinn 72 hour. In that, we present the data for ECMO weaning state at 72 hour and we put this data (ECMO weaning state on 72h) on logistic regression model. However, we did not find any difference from the original analysis.

Change in the text: This comment did not change the text because it is an explanation of the reviewer's question and as it did not affect the results.

Comment 10: Also, Mechanical ventilation duration. It is unclear whether PTP is a surrogate of prolonged MV (need to be reported).

Reply: Generally, definition of prolonged MV is the support of MV above 3 weeks. In this data, or mean duration of MV is not different. So, we did not that into regression analysis. As you suggested, we put the duration of MV into multiple logistic regression

and found no association with PTP.

Change in the text: This comment did not change the text because it is an explanation of the reviewer's question

Comment 11: What immunomodulatory regimen was used? What was the effect of using rituximab or developing acute graft dysfunction within the 30 days post-transplant?

Reply: We give a IL-2 receptor blocker as induction therapy and followed triple regimen, steroid, tacrolimus and mycophenolate mofetil. Rituximab is only used in biopsy prove antibody-mediated rejection and we did not experienced AMR within 30 days in this cohort. In addition, we only experienced acute cellular rejection cases within 30 days and it's too small to put into regression analysis.

Change in the text: This comment did not change the text because it is an explanation of the reviewer's question.

Comment 12: Please, detail what antibiotic prophylaxis was used in general and for CRAB.

Reply: We adopt a prophylactic antibiotic protocol based on donor respiratory culture. However, we use a combination of cefepime and tecoplanin if the donor culture are not available. If CRAB is identified in respiratory specimen of donor or recipient side, colistin nebulization is preferred as initial choice. Then, the use of intravenous colistin is dependent on possibility of PTP or bronchoscopic finding or renal function of recipient.

Change in the text: If resistant bacteria were found as a result of antibiotic susceptibility, the antibiotic was determined according to the reported DST. Among them, when CRAB was identified, clositin was used for the initial treatment, and other antibiotics is also used depending on sensitivity.

Comment 13: WHAT WERE THE DIFFERENCES BETWEEN PATIENTS WITH crab AND OTHER ORGANISMS? They can not be merged together!

Reply: As you comment, combining the CRAB group with other organisms groups can be at risk of interpretation. However, after we tried so many methods to analyze this paper, we came to the conclusion that it is most reasonable to divide the analysis based

on PTP. And I think it will help lead to better outcomes for lung transplant patients. We agree that further studies are needed, taking into account your comments as more patients gather. Thank you very much for your comment and I think you pointed out an important point.

Change in the text: This comment did not change the text because it is an explanation of the reviewer's question, and since further analysis is not possible.

Comment 14: It is unclear the statement that prophylactic antibiotics prescribed for CRAB colonization were associated with better outcome. Acinetobacter is a low virulent organism and authors should detail in results what justify this interpretation.

Reply: We agreed with your logical comment. Those contents are over-stated on our result and can cause misunderstanding for readers. We humbly deleted the related contents as following:

Change in the text: Deleted page 9, line 20

Comment 15: Main organism elsewhere is Pseudomonas aeruginosa. It is unclear what was the contribution in this cohort. Authors should detail this information and inform on the subset with Pseudomonas colonization.

Reply: Pseudomonas species were not related to onset of early PTB and the outcome of lung transplant. In that, we did not include the analysis regarding Pseudomonas species.

We analyzed whether the Pseudomonas detected group had an effect on the conclusion, but it was excluded for consistency due to the lack of relevance to the conclusion of the overall paper. Instead, here we described and added general features.

Among MDR bacteria, pseudomonas aeruginosa, burkholderia species, acinectobacter baumannii were the most concentrated. Especially, Pseudomonas aeruginosa is main organism Western population.

Pseudomonas aeruginosa is gram negative aerobic bacillus as tissue invasive pathogen which is caused pneumonia, chronic colonization and severe sepsis. P. aeruginosa is very high rate of colonization in structural disruptive lung disease such as cystic fibrosis and bronchiectasis and it was significant risk of infection after lung transplantation (13). It is also the most common pathogen of pneumonia and can be caused BOS when occurred post-transplant colonization. However, it is not contraindication of

transplantation because there were no significant different bacteria in mortality from other bacteria (14).

Burkholderia is gram negative bacteria including cepacia, gladioli, mallei etc. B. cepacia complex was easily causes chronic infection and colonization in structural damage lung and is considered to be rejected transplantation because it is associated with the highest risk for mortality in previous studies.

Like this, MDR bacteria is a factor that can have a great influence before and after lung transplantation. Therefore, checking for infection or colonization of MDR bacteria in advance and establishing an appropriate treatment strategy will have a huge impact on the outcome.

Change in the text: This comment did not change the text because it is an explanation of the reviewer's question and as it did not affect the results.

Comment 16: Need to add a section on limitations. It is unclear that these findings can be generalized and it has to be explained.

Reply: This data should be cautiously interpreted due to a retrospective design and different clinical settings. As this is a retrospective study conducted only at one center, it is difficult to generalize it. As you comment, we agreed we need to clarify the problem in generalizing. So I modified it like this.

Change in the text: There were several limitations to this study. Since this is a retrospective study conducted only at single center, it is difficult to generalize it. Second, it may have been vulnerable to selection bias because patients with lung transplantation patients were included, but other solid organ transplantation patients were excluded. As mentioned earlier, CRAB colonization rate was higher than that of the Western ICU. For this reason, it is difficult to generalize our results to other lung transplantation centers, and this data should be cautiously interpreted. Despite these limitations, this study will be evidence that illustrates the nature of infections, which will be important for transplants.

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3. Bertrand B, Collet S, Eloy P, et al. Secondary ciliary dyskinesia in upper respiratory tract. Acta Otorhinolaryngol Belg 2000;54:309-16.

4. Cao Y, Chen M, Dong D, et al. Environmental pollutants damage airway epithelial cell cilia: Implications for the prevention of obstructive lung diseases. Thorac Cancer 2020;11:505-10.

5. Du X, Meng Q, Sharif A, et al. Surfactant Proteins SP-A and SP-D Ameliorate Pneumonia Severity and Intestinal Injury in a Murine Model of Staphylococcus Aureus Pneumonia. Shock 2016;46:164-72.

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14. Shoham S, Shah PD. Impact of multidrug-resistant organisms on patients considered for lung transplantation. Infect Dis Clin North Am 2013;27:343-58.