Author’s response to reviews

Title: Factors influencing microbial colonies in the air of operating rooms

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Author’s response to reviews:
EDITORS’ Comments:
We found a second reviewer for your manuscript. They have made some salient points that need to be addressed.

Ans: Thank you for giving the opportunity to revise the manuscript. We have already interpretation for the reviewer’s comments.
Please see below.

REVIEWER 1 (Xiaoling Ma, M.D.): comments
Authors have revised their manuscript according to the comments carefully. More details and more discussion have been added. The manuscript has been improved. Although some conclusions, for example, the number of personnel influence the microbe concentration, coagulase-negative staphylococcus was the most common pathogen, have been reported, authors tried to make the plentiful content. They demonstrated some new important factors and their level affecting the number of microbial colonies. So I think it is suitable for publication after revision.
Ans: Thank you for accepting our manuscript amendment and agreeing to publish it.

REVIEWER 2 (Prof. José Luís Alfonso Sánchez): comments
From the paper entitled "Factors influencing microbial colonies in the air of operating rooms" I have to emphasize the following aspects.

The paper focuses on "determine factors affecting air pollution in a tertiary referral medical center", and therefore, is an interesting work and still is a question that remain unanswered, however I kindly have to make the following comments about the paper:
Ans : Thank you for giving the guidance and the opportunity to revise the manuscript.

1.- First, the sample number is low, since they use only 250 samples collected during surgical procedures, which, given the availability of 28 operating rooms, means about 10 samples per OR, which makes it even less representative.

Ans 1: This study was performed in a medical center with 28 ORs, and the settings and equipment of the each OR environment were similar for all working ORs. (Please see METHORDS page 5, the last paragraph and page 6, the first and the second paragraphs; and DISCUSSION section page 15, lines 15-20)

The authors wanted to assess the important factors (such as procedure-related operative characteristics and surgical environment [environmental- and personnel-related factors] characteristics) and their degree affecting the number of microbial colonies in each OR of similar environment. (Please see BACKGROUND page 4, lines 25 to page 5, lines 1-5) In addition, the same surgical procedures maybe performed in different ORs. Therefore, the present study mainly evaluated the number of air microbial sampling based on surgery and its type, rather than on the number of samples per OR. Thank you for the careful review. For further research in the future, we will take this design of air sampling into consideration.

For the question of 'about 10 samples per OR ', please continue to see the second point (Ans. 2).
2. So, the time was extremely long from May through August 2015. It means 2 samples per month per OR.

Ans 2: This study carried on the number of air microbial sampling within 3 months. Four types of surgical procedures were selected every workday and excluded on holidays. The numbers of air sampling for the types of surgical procedure were selected according to its proportion in 2014, and the ORs were randomly selected. (Please see METHODS page 5, lines 13-18)

3. The information related to the type of interventions collected are very different, including from high-risk interventions, such as transplant surgery or orthopedics surgery, with an excessively small number of samples, mixed with other interventions with lower risk such as urology surgery and others.

Ans 3: Please refer to the description of Ans 1 and Ans 2.

In addition, the distribution of the number of airborne microbes for the types of procedure and other variables were shown in the univariate analysis (Table 1).

As the reviewer mentioned, such as transplant surgery (high-risk interventions) with an excessively small number of samples; however, the data also showed the highest number of colonies for transplant surgery. In the multiple analysis (Table 3), but also due to the numbers of air sampling of part types of procedure were less frequently (such as transplant surgery). Therefore, this variable in the model was divided into the adult group and the pediatric group for comparison.

4. The mean bacterial count (cfu / m3) for transplant surgery are 2 or 3 times higher than those of urology or general surgery which is incomprehensible, and normally with those figures we need to close OR in order to clean them.

Ans 4: Most reports suggest that an acceptable bacterial limit for a working OR is below 180 cfu/m3[6,10,16,21]. The suggested alert values of fungi were 4 cfu/m3[20]. In our study, the mean colony counts obtained in ORs were 78±47 cfu/m3 (with the lowest counts in general
surgery of 67 cfu/m³ to the highest in transplant surgery of 123 cfu/m³), which was much lower than 180 cfu/m³. (Please see DISCUSSION page 10, lines 9-10; and lines 19-22).

The guideline suggests that conventional OR ventilation systems produce a minimum of about 15 ACH (air changes of filtered air per hour), three (20%) of which must be fresh air [5]. (Please see DISCUSSION page 11, lines 2-5) However, the ORs of this study were taken with a higher standard air conditioning setting (40 ACH and 100% fresh air). (Please see METHODS page 5, the last paragraph).

5.-The study did not distinguish between bacteria and fungi knowing the importance that the latter one have on the surgical infection.

Ans 5: As the reviewer mentioned, bacteria and fungi have known the importance on the SSIs. In the present study, the collected air samples were plated onto trypticase soy agar. (Please see METHODS section, page 6, line second last) Trypticase soy agar is broad spectrum media for the collection and enumeration of microorganisms. This agar is general-purpose, nonselective media providing enough nutrients to allow for a wide variety of microorganisms to grow. They are used for a wide range of applications, including culture storage, enumeration (counting), isolation of pure cultures, or simply general culture.

Microbial culture in this study includes bacteria and fungi, but no fungi were cultured from the air samples. (Please see RESULTS page 9, lines 9) Because the fungus was not isolated from agar in this study, there is no description of fungi in the method section, and the data analyses were presented as bacteria.

We have added about the description of fungi in the METHODS section (Please see page 7, lines 3-4).

6.-Table 3. When speaking of factors affecting bacterial counts in operating rooms using linear regression analysis it is assumed that is a multiple regression, not simple, according to what they say in the text. From this table, it can be assumed that, since the regression coefficients is -1.72 in the case of surgery time (hour), as the time of surgery increases, microbial contamination decreases, something that is against all the scientific evidence that the duration of the
intervention is directly related to the contamination and the increase of surgical infections. But this table contains more contradictory factors with such evidence, such as that wound classification (contaminated-dirty / clean and clean-contaminated) has no statistical significance (0.467) between them and others.

Ans 6: (1) The study divided the perioperative (surgical staging) into three parts, including before incision, during incision to wound closure, and after wound closure. The definition of surgery time in this study referred to during the incision wound to the closure of the period. We have added notes in Tables 1 and 3.

For SSI of patient, as the reviewer mentioned, the scientific evidence that the duration of the intervention (surgery times) is directly related to the contamination and the increase of surgical infections.

For microbial colony counts of surgical environment:
A previous report pointed out that the bacterial count results were the highest during preparation periods (during which the medical staff conducted preoperative procedures and postoperative cleaning) and were the lowest under static conditions. [11] Other report also stated that although turbulent ventilation was not related to wound contamination (p=0.22), it was associated with an increased number of air microbial counts (p<0.001) [26].

Our results were similar to previous studies; counts sampled before the incision stage (99.9±55.7) and after the wound closure stage (110±46.6) were higher than those during incision to wound closure stage [surgery times] (66.9±38.3) with colony values of 36.5 cfu/m3; four-fifths of the microbial colonies detected over the threshold were also at the before incision stage. (Please see DISCUSSION section, page 14, the first and the second paragraphs; Table2 and Table 3)

In addition, the ventilation systems are running continuously during this period (surgery times). The ventilation systems of the ORs produced a minimum of 40 air changes of filtered air per hour (ACH) and 100% fresh air.

It may be these reasons that in the regression model after controlling for other variables, the affect of surgery time (during incision to wound closure) on bacterial counts in ORs was a
negative regression coefficient. However, it had no statistically significant differences, and the 95% CI (-6.25 ~ 2.80) included positive regression coefficients.

We have added the described about negative regression coefficient of the surgery time in the DISCUSSION section. (Please see page 14, the third paragraph)

(2) Wound classification is also a similar reason, affecting the patient's infection, but has little effect on the amount of bacteria in the air.

Looking forward to your acceptance of our explanation and revisions,

Thank you for your guidance again.