Author's response to reviews

Title: First multicenter study on multidrug resistant bacteria carriage in Chinese ICUs

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Author's response to reviews: see over
Abstract:
1. Conclusion: MDRO prevalence was high in the ICU, but predominantly originating from patients colonized/infected on admission. With a high rate of MDRO on admission, there is a higher prevalence of admitted patients with MDRO making transmission potentially more likely. Concluding that the prevalence of MDRO is high, may minimize the source of MDRO in Chinese healthcare facilities. If efforts to contain MDRO in China only focused on the ICU (eg. infection control, antimicrobial stewardship, environmental cleaning), would that resolve the problem in the ICU if 1/3 of patients already have an MDRO on admission?

We agree with the reviewer. Indeed, MDRO is a global problem in and out ICUs. However, ICUs have been shown to be a place for high transmission and where antibiotic use is very high. Therefore, it may be a good place to start a program because teams may be more reactive and motivated regarding the MDRO issue. Decreasing transmission in ICUs may help to decrease MDRO outside ICUs where ICU patients are discharged.

We modified the conclusion by underlining the high MDRO admission rate.

Materials and Methods:
1. Surveillance Program, paragraph 2: What was the rationale for defining A. baumannii and P. aeruginosa as MDRO based on resistance to a single agent? Was the 3rd generation cephalosporin specifically resistance to ceftazidime?

There are no universal definitions for MDR A. baumannii and MDR P. aeruginosa. Therefore, we chose resistance to ceftazidime as a marker for multidrug resistance and changed the text accordingly.

2. Surveillance Program: Were all of the facilities included in the study similar (beds, ICU size and infrastructure, patient population, etc.)?

As expected, ICUs were not very similar as it is common in multicentre studies. This is also the interest of multicentre studies to level off differences and make conclusion more generalizable. We have added a brief description of the units and provided data regarding variations by unit in the results section (new Table III).

3. Bacteriology, 2nd sentence: were all colonies that grew on the chromID MRSA considered MRSA? Were there basic tests to confirm the identity of the colony? Did you consider adding a confirmatory test for MRSA?

As stated, no additional test was performed regarding methicillin resistance. The specificity of the test is considered very high (>99%) and we have added a reference regarding this issue.

4. Bacteriology, last sentence: Please state what susceptibility testing method was performed for A. baumannii and P. aeruginosa.

Vitek2 was used as for other gram-negative bacilli. We change the text accordingly.
5. Although surveillance was the primary purpose of the article, discussion of the results related to acquisition in the ICU is dependent on many issues, particularly infection control. It would be important for readers to understand the baseline infection control interventions for MDRO to put the results into context. What are baseline practices (precautions? Single rooms? Isolation?)? What are baseline hand hygiene rates?

Baseline hand hygiene rates were unknown as well as adherence rates to isolation precautions. The main goal of the study was to raise awareness regarding the MDRO issue in these ICUs, and the country as a whole. We did not try to make any intervention regarding hygiene procedures, as we sought to have baseline data regarding MDRO. Single rooms were the most common in the participating ICUs, and isolation precautions was seldom implemented of MDRO carriers.

**Results:**

1. **Paragraph 1:** The stated global prevalence rate of MDRO in the text is 30.5% (results and abstract), but Table 1 reports a percentage for all MDRO as 36%. Please correct.

We have corrected the text.

2. **Paragraph 1:** ESBL-producing Enterobacteriaceae was the most prevalent MDRO. What was the distribution of isolates, with respect to E. coli and K. pneumoniae?)

Unfortunately, only the ESBL-positive status was recorded in the database and not the corresponding species. We have a global distribution of ESBL-positive species among all ICUs during the study period but duplicates have not been taken into account and these data is not linked to the study database to retrieve patients identification to remove duplicates. However, because of the very high proportion of *E.* coli among all ESBL isolates, we provided this data in the manuscript despite the weakness regarding duplicates elimination.

3. **Table 1:** All of the values listed in Table 1 are described as rates (per 100 admissions or per 1000 hospital-days). However, all of the results are reported as percentages. Did you intend to report a proportion of patients with an MDRO (%) or a rate of patients with MDRO (based on admissions or hospital days)?

Data in Table 1 are indeed rates and are given as rates during the period of inclusion for each ICU or overall. We provided the definition in the text. It should be noted for instance that the global incidence density rates are given as /1000 hospital-days during the 6 months of the study (for instance, 71.1‰). Therefore, we do not think changes are requested.

4. **Table II/III/IV:** Length of stay. In the brackets, does this represent the range or an interquartile range?

Here, we chose to present ranges (low-high) into the brackets as it is written in the tables. Therefore, these are not interquartile ranges.

5. **Table III/IV:** As stated in the text, the data for ESBL and MRSA were comparable to any MDRO acquisition. Given this, I think it may be more suitable to either condense these tables or provide them as supplementary tables.
We agree with the reviewer that these two tables do not bring much additional data but leave it as it because some readers may need it. Therefore, we will leave the decision to the editor to provide Tables IV and V as supplementary tables.

6. Paragraph 1: In the discussion of median time to acquisition, was this based only on screening swabs? Were any clinical isolates taken into account (eg. positive tracheal aspirate or blood culture)?

Clinical samples positive for MDROs were also taken into account for the time to acquisition and also for defining the MDRO status and we agree with the reviewer that this issue should be clearer. We give now more precisions regarding this issue in the method section.

Discussion:
1. Paragraph 2 and 3: There is a lot of text describing a comparison between the study ICUs and French/Dutch studies. Are the practices in these countries (and facilities) comparable to China? Perhaps further discussion related to the epidemiology of ARO’s in other Asian countries may provide a more comparable comparator.

We agree with the reviewer, and practices in Europe are likely to be different from those used in China. However, it is also likely that practices used in other country from Asia are different from those used in Mainland China. Finally, because of the size of the country and differences observed in the different Chinese regions, it is likely that practices vary even within China. Very few similar data regarding our study are available in Asia and we tried to cite the one that were relevant and we were aware of.

As underlined by the reviewer, practices in the Netherlands or France do not compare to those of China, especially regarding hygiene practices. However, we are confident that the comparisons are of interest to highlight the differences in MDRO prevalence.

2. Paragraph 3: As mentioned in the results, although hand hygiene and isolation precautions were not recorded, it would be very helpful to know the underlying policies and baseline procedures at these facilities.

We are now explaining in the text that antibiotic policy and isolation precaution were deficient at the time of the study.

- Minor Essential Revisions
1. In several instances, Enterobacteriacea was spelled enterobacteria.
We changed all “enterobacteria” for “Enterobacteriacea”

2. Standardize the MDRO acronym as both MDRO and MDROs is listed.
Now, only MDRO is used.

3. Verb tenses need to be corrected (eg. line 72, ‘infection control teams are in place since 2000.’, line 76: ‘most of the reports are dealing with…”).
We tried correcting this issue.

4. Introduction: line 70, ‘multidrug resistance’; line 89, consider rewording to ‘available data on bacterial resistance may overestimate resistance rates.’
We changed the text as suggested.
Materials and Methods:

1. Surveillance Program, paragraph 2: For MRSA colonization, only nasal carriage was performed for patients. Sensitivity of detecting MRSA can increase with more sites sampled. Perhaps that may have resulted in an underestimate of MRSA colonization?
   Indeed, we agree that other clinical sites may be positive with MRSA. However, it increases the cost of the program and the program may be less cost effective. However, the fact that screening was repeated decreases underestimation. We have added a sentence regarding this issue in the paragraph dealing with weaknesses of the study.

2. Surveillance Program, paragraph 2: I think that this surveillance tool would have been enhanced by including vancomycin-resistant Enterococci, and for the follow-up studies, to include carbapenemase producing organisms.
   We agree that VRE is an important MDRO and should ideally be included in a MDRO surveillance program. However, VRE is not known as a major issue in China (Pathol Biol (Paris). 2015 Feb;63(1):21-3).
   On the opposite, the emergence of carbapenemase-positive Enterobacteriaceae will impose to include such MDRO in future studies.

Results:

1. Did the results differ depending on the ICU being analyzed? Were all ICUs in the study having similar rates of MDRO (at baseline before admission, during and on discharge)?
   We did not try to assess this issue and separate analysis for each ICU was not planned in the study. One of the reasons was to avoid political issues and personal opposition to the study. Nevertheless, we are now providing data allowing for minimal comparison of the participating ICUs.

2. With a prevalence rate of 51.2% on discharge, was there analysis of transmission? Were they epidemiologically related, or perhaps genetic investigation into relatedness (eg. PFGE)?
   Although of great interest, no further analysis was performed. As the reviewer has noticed, the present study was very pragmatic to be able to gain adherence and to serve as baseline study to bring evidence for action.

3. The presentation of data with ‰ is not commonly used. Perhaps writing it out may be clearer to readers.
   We fill that it is not confusing. However, we changed it in the text and kept it in the tables.

Finally we tried to address the other points raised by the reviewer by rewording the text.
Reviewer 2- MP Muller

Abstract:
The methods section of the abstract is too brief. More information on the methods used, particularly the multivariate analysis, should be included. In the results section, it would be worth including more of the key results (e.g. results of multivariate analysis for both baseline prevalence and acquisition and some data on the individual pathogens assessed separately if space permits).

We tried to address the remarks done by the reviewer. In the results section of the abstract, the results of multivariate analysis regarding MDRO carriage on admission and MDRO acquisition were already presented. We are now providing more data regarding the results.

In the conclusion, it should be mentioned that in addition to a high baseline prevalence of MDRO there was also a high attack rate, exacerbating this problem. These appear to be the two key findings of this report. Many studies have evaluated risk factors for MDRO acquisition but what is unique about this report is that it represents Chinese data and therefore these are the key results (i.e. prevalence and attack rates in Chinese ICU – not the risk factor data).

We tried to improve the abstract following the directions given by the reviewer by adding more data regarding MDRO rates in the abstract.

Materials and Methods:
Please provided data about the ICU’s involved in the study other than their geographic location. Data should be presented on the type and size of these ICU and ideally a ‘table 1’ that described the population of each ICU in terms of mean age, gender, a severity of illness score, common diagnoses, and % ventilated would allow comparison with ICU outside China.

Most of the data requested by the reviewer are not available because ICUs do not collect such data in a routine manner. Nevertheless, we tried to provide more information regarding the participating ICUs.

Results are presented in terms of prevalence rate, prevalence density rate, attack rate, etc. These terms should be defined in the methods.

The definitions are now provided to make it clearer for the readers.

In the Methods section, Surveillance program subsection, it states that patients were swabbed on admission and then weekly. How were clinical specimens dealt with (e.g. what if a urine or blood specimen obtained for clinical purposes was positive for an ESBL? Was this data included?)

Clinical samples positive for MDRO were taken into account for determining the carrier status but also to compute time to acquisition (see reviewer 1).

The text has been changed accordingly.

In the Methods section, Analysis subsection, it is mentioned that multivariate analysis was done. In fact, it appears that there were at least 2 and possibly more multivariate analyses done and each one should be specified in terms of the outcomes studied in each meta-
In the Methods section, Analysis subsection, it is stated that a backwards regression was done. More details should be provided about how this was done. What criteria were used to remove variables from the model? Was this an automated process? How was model performance evaluated?

We are now providing more information regarding the building and selection of the logistic regression models in the methods section.

Results:
No results are presented comparing ICUs. Data on the key outcomes should be presented for each ICU to allow an assessment of variability between regions and ICUs. Did some ICU have very high attack rates and some have lower rates or were rates similar? Were some pathogens more significant by ICU or region?

Our study was not performed to do benchmarking and to try explaining differences among ICUs. Nevertheless, we have added some data regarding the differences among participating ICUs. We are providing data regarding all MDRO? Because ESBL-positive Enterobacteriaceae are the most prevalent everywhere, we do not think it is of interest to provide detailed data regarding each type of MDRO for each ICU.

It appears that a large number of variables were assessed in univariate analysis and then included in the multivariate analysis.
Indeed, a large number of variables were assessed in univariate analysis. However, as stated in the methods section, only those variables clinically sounded and significantly associated in univariate analysis were included in the logistic models. In addition, the number of patients included in the study is rather high and the number of events was elevated (overall MDRO acquisition for instance was observed in 104 patients as compared to 335 with no acquisition) and there are not that many variables that were significant in univariate analysis. According to the commonly used rule of thumbs of 1 variable per 10 events, we were able to introduce 10 explanatory variables in the initial model for MDRO acquisition before backward removal.

Is there a potential problem with multiple hypothesis testing when considering the univariate results? Should bonferoni corrections be applied and if not, why not?
As far as we know, Bonferini test may be used when one performs multiple comparisons among multiple groups (although it is discussed). Here, we compared only two groups, and therefore we fill that there is no point using Bonferoni test or another test for multiple comparisons.

Were an appropriate number of variables included in the models relative to the number of outcomes? This should also be mentioned in the limitations section
As explained above, the number of patient is rather high in the multivariate analysis and therefore we were able to introduce up to 10 variables in the models simultaneously. In addition there was no instability in the model presented and the Hosmer-Lemeshov test was used to analyse the fitness of the model. The best model with the least variable is presented and therefore we do not fill there is any reason to comment this issue in the limitations section.

In the results section, paragraph 4 it is stated that “surprisingly, receiving a glycopeptide before MRSA acquisition remained associated with a high risk of MRSA acquisition”. Was
this the outcome of a multivariate analysis – this should be specified? Why is this result surprising?

We deleted “surprisingly”.

The multivariate findings should be presented in their own table.

We decided not to present the results of the multivariate analysis in a table because there are only two analyses, one for MDRO carriage on admission and one for MDRO acquisition, and they do not use the same variables.

However, we are now providing more details in the methods and results sections regarding the multivariate analysis.

Discussion:
In the first paragraph it is noted that “As expected, the risk of acquisition increased with the duration of ICU stay and the use of antibiotics”. Was duration of stay included in the multivariate model? If so, was it significant in the multivariate model?

Yes, duration of stay before MDRO acquisition or for the entire ICU stay for non-carriers was included in the model. We tried to make it clearer in the result section.

Was colonization pressure considered as a potential variable to include in the multivariate analysis addressing attack rate (e.g. was the number of patients colonized with MDRO at admission within a given time period and ICU associated with the risk of acquisition of MDRO within the same time periods and ICU? Could colonization pressure be presented or included in your models?

We agree with the reviewer that colonisation pressure is of interest and has been shown to represent a major indicator for cross-transmission. However, colonisation pressure could not be computed because data collected in the database do not allow such computation.

Therefore, it was not assessed by itself or in the multivariate models as an explanatory variable.

MRSA rarely occurs ‘de novo’ and the high attack rates seen in your study therefore suggest potential deficiencies in infection control practices. It is stated that hand hygiene and other infection control metrics were not assessed but it should be stated at some point that, in addition to antibiotic use, poor infection control practices likely contributed to the high attack rates seen.

We agree with the reviewer and have underlined this issue in the discussion.

In the 3rd paragraph, it is stated that “besides length of stay, the most important risk factor for MDRO acquisition was antibiotic exposure”. What was the basis for this conclusion – were these variables compared together in the multivariate analysis? Or is this simply ‘obvious’?

This comes out from the logistic regression analysis comparing factors associated with MDRO acquisition in the ICUs. Obviously we could not evaluate infection control practices because they were generally poorly followed and we did not record any data regarding the latter issue. We changed the text, deleting “the most important” and underlining the role of poor hygiene that is likely to highly contribute to cross-transmission.
Also, did length of stay vary between the 8 ICU?

We are now providing data regarding each participating ICU and the median length of stay for patients included in the study for each of the 8 ICUs.

Tables and Figures:
Table 1 should include numerator and denominator data in addition to the percentages to allow complete interpretation.

We have added the complete figures for MDRO to ease interpretation.

Minor Comments:
In the introduction, last sentence of the 3rd paragraph (“consequently, available data on bacterial resistance based may bias results...”). This sentence is unclear and should be revised.
This sentence has been modified and we hope that now it is clearer.