PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	Risk factor of intestinal carriage of carbapenem-resistant Acinetobacter baumannii and the impact on subsequent infection among patients in an intensive care unit: an observational study
AUTHORS	Qiao, Fu; Huang, Wenzhi; Gao, Shan; Cai, Lin; Zhu, Shichao; Wei, Li; Kang, Yan; Tao, Chuanmin; Zong, Zhiyong

VERSION 1 - REVIEW

REVIEWER	Axel Kola, MD Charité – Universitätsmedizin Berlin, Germany
REVIEW RETURNED	13-Dec-2019

GENERAL COMMENTS	The manuscript presents the results of a case control study which was performed to identify risk factors for intestinal CRAB colonization and a retrospective cohort study to analyze the impact of intestinal CRAB colonization on subsequent CRAB infection in a Chinese 4 300 bed universitary hospital. Background, P4L57: "A. baumannii is one of the most common nosocomial pathogens [1]." According to Ref1., please add: "...in Asia and South America." Background, P5L84: "It is well known that A. baumannii incl. CRAB usually colonize the respiratory tract of hospitalized patients…": Please replace "usually" with "may". Discussion, P12L271ff: "There are few limitations in this study." Please add the following: Due to the poor sensitivity of rectal swabbing, a single negative test result could overlook carriers. Moreover, no molecular strain typing was performed. Though reasonable, it was not proven that CRAB isolated from intestinal colonization and site of nosocomial infection were identical.

REVIEWER	François Barbier, MD PhD Medical ICU La Source hospital CHR Orléans Orléans, France
REVIEW RETURNED	20-Dec-2019

GENERAL COMMENTS	In this single-ICU study conducted in China, Qiao and coworkers sought to describe the prevalence of CRAB intestinal carriage (imported or acquired in the ICU), risk factors for ICU-acquired CRAB colonization, and impact of CRAB carriage on the risk of subsequent CRAB infection in critically ill patients. A total of 961 patients were included in the study cohort. CRAB intestinal carriage was detected at admission or acquired during the ICU stay in 6.9% and 12% of patients, respectively. Pancreatitis, hematological disease, gastric tube feeding, and carbapenem exposure were independent risk factors for carriage acquisition. CRAB carriage was an independent risk factor for subsequent CRAB infection (OR 1.75, 95% CI 1.16–2.62). Notwithstanding a large number of included patients, the results provided here are not really original since several prior studies conducted in the critical care setting yielded similar findings, notably for risk factors of acquired CRAB carriage (e.g., prior carbapenem exposure), and the increased risk of CRAB infection in carriers (this is now well established in ICU patients for all MDR Gram-negative pathogens, including ESBLE, CRE, MDR P. aeruginosa, and MDR Acinetobacter baumannii). Moreover, several major points should be considered by the authors to improve the quality and interpretability of this article. ESSENTIAL COMMENTS 1. The design of this observational study is not adequately described. Indeed, the authors state that this work included a case control study (for risk factors of CRAB carriage) and a retrospective cohort study (for CRAB infection in carriers and non-carriers). No matching was performed on the first point; therefore, the term “case-control study” is not adequate here. When reading the manuscript, we understand that this is simply a retrospective cohort study based on rectal swabs routinely sampled for the surveillance of CRAB carriage in this ICU. This may explain the high proportion of patients with missing samples. Please clarify in the Methods section and in the Abstract. 2. Was the study protocol approved by an Ethical Committee? This does not appear in the manuscript. 3. Along with colonization pressure and antimicrobial exposure, one on the main risk factor for acquisition of MDR pathogens in critically ill patients in the length of the ICU stay. This major variable - not provided in the manuscript - should be compared between patients with and without acquisition and should be included in the multivariate model. Ideally, the competing risk of death or ICU discharge should have been taken into account in multivariate analyses. 4. Important data are lacking to describe this cohort of ICU patients: mortality rates (in-hospital, or at least in-ICU), ICU LOS, RRT, vasopressor use, reason for ICU admission (it is unclear whether “underlying diseases” in Table 1 are related to chronic comorbidities as detailed by the Charlson comorbidity score ad/or acute disease leading to ICU admission). 5. Antimicrobial exposure should be integrated in multivariate analyses as a continuous variable (that is, treatment duration in days) and not as a nominal variable. Indeed, the risk may increase with treatment duration. In other words, 2 days of cephalosporin use may not have the same impact on the gut microbiota and intestinal colonization resistance than 8 or 10 days. 6. Figure 2 (risk of CRAB infection in carriers and non-carriers): In patients with acquired carriage, does Day 0 correspond to ICU admission or to the day of the first positive rectal sample? Also, if

	this curve represents CRAB infection-free survival, how were handled patients who died from another cause than CRAB infection? 7. It is briefly stated in the Limits sections (Abstract and main text) that not all CRAB isolated on selective media were confirmed as CRAB through usual antimicrobial susceptibility testing (Vitek II): what do the authors mean? How many “false positive” CRAB were identified? How were classified the corresponding patients: carriers or non-carriers??? This essential point must be developed, in the Methods section and in the Results section. Patients not confirmed as CRAB carriers through AST should be classified - and analyzed - as non-carriers. ADDITIONAL COMMENTS 1. The Introduction is quite long and could be substantially shortened. 2. Methods, page 6, “Patient and public involvement: Patients were involved in this study”: what do the authors mean here? 3. Clinical isolates and carriage isolates of CRAB were not compared through molecular methods (or at least PFGE) in carriers with subsequent infection: this could have been smart to confirm the link between colonization and infection. A comment on this point could be added in the Limits section.

REVIEWER	Dervla Kelly University of Limerick, Ireland
REVIEW RETURNED	27-Feb-2020

GENERAL COMMENTS	Overall a well written paper examining risk factors for CRAB infections contributing to the understanding of the likely origin of CRAB infections. Line 51: implies causality. Suggest replace with was associated with… Line 271: limitations: analyses of strain no carried out… were patients infected by their own strain? Table 2: item 1: CRAB intestinal carriage… please specify whether on admission or at any point during stay

REVIEWER	Majdi Al-Hasan University of South Carolina School of Medicine
REVIEW RETURNED	28-Feb-2020

GENERAL COMMENTS	The statistical methods used in this study are appropriate and clearly described. I have only one comment regarding the Methods section: - How did the authors account for the competing risks of death in ICU and subsequent infections in Cox model? Were patients censored if they died in the ICU prior to infection? If so, please specify that the study examined infections in survivors. If otherwise, please explain.

VERSION 1 – AUTHOR RESPONSE

Reviewer: 1
Background, P4L57: "A. baumannii is one of the most common nosocomial pathogens [1]." According to Ref1., please add: "...in Asia and South America."
Revised.

Background, P5L84: "It is well known that A. baumannii incl. CRAB usually colonize the respiratory tract of hospitalized patients…": Please replace "usually" with "may".
Revised.

Discussion, P12L271ff: "There are few limitations in this study."
Please add the following:
Due to the poor sensitivity of rectal swabbing, a single negative test result could overlook carriers. Moreover, no molecular strain typing was performed. Though reasonable, it was not proven that CRAB isolated from intestinal colonization and site of nosocomial infection were identical.
Revised.

Reviewer: 2
1.The design of this observational study is not adequately described. Indeed, the authors state that this work included a case control study (for risk factors of CRAB carriage) and a retrospective cohort study (for CRAB infection in carriers and non-carriers). No matching was performed on the first point; therefore, the term “case-control study” is not adequate here. When reading the manuscript, we understand that this is simply a retrospective cohort study based on rectal swabs routinely sampled for the surveillance of CRAB carriage in this ICU. This may explain the high proportion of patients with missing samples. Please clarify in the Methods section and in the Abstract.

This study is indeed a retrospective cohort study. The exposure factor is whether there is CRAB intestinal carriage, and the outcome is whether CRAB infection occurs. However, in addition to this content, we also explored the risk factors for intestinal carriage of CRAB in ICU patients. For risk factor analysis, case-control study is used. Case group is defined as acquisition of CRAB intestinal carriage in ICU, and control groups is defined as those without CRAB intestinal carriage during their ICU stay. In order to make full use of the sample information and to achieve statistical efficiency, we use a non-matched case-control study instead of a matched design. And influencing factors are adjusted by multivariate analysis.
So, we finally set the study design as an observational study, including two design types, a case-control study and a cohort study. We have added the study design in the Abstract.

2. Was the study protocol approved by an Ethical Committee? This does not appear in the manuscript.

Yes, this project was approved by the Ethics Committee of West China Hospital of Sichuan University. We have added this in the manuscript.
And we confirm that consents were not obtained from the patients. First, active screening is part of the routine care for ICU patients in our hospital. In other words, no matter whether we analyzed the data, the patients would receive the screening. Second, this is a retrospective study, in which we looked back the patients' data and did not perform any interventions. Third, before we performed this study, we have obtained ethical approval from the Ethical Committee and inform consents were waived due to the retrospective nature of this study.

3. Along with colonization pressure and antimicrobial exposure, one on the main risk factor for acquisition of MDR pathogens in critically ill patients in the length of the ICU stay. This major variable - not provided in the manuscript - should be compared between patients with and without acquisition

and should be included in the multivariate model. Ideally, the competing risk of death or ICU discharge should have been taken into account in multivariate analyses.

To address whether intestinal CRAB carriage could lead to an increased likelihood of subsequent CRAB infection, the multivariate model we use is Cox regression, and time factor has been considered in this model as “survival time”. In patients with CRAB intestinal carriage, Day 0 corresponds to the day of the first positive rectal sample. While in patients without CRAB intestinal carriage, Day 0 corresponds to the day of the first rectal sample.
Death in ICU could indeed prevent us from observing the occurrence of the infection, and the two are competing events. As a result, traditional Cox proportional hazards model may falsely evaluate the effects of covariates. Therefore, data are reanalyzed using sub-distribution hazard model considered death in ICU as a competing risk event. According to the result, we update the contents of Figure 2, Table 2 and supplementary tables.

4. Important data are lacking to describe this cohort of ICU patients: mortality rates (in-hospital, or at least in-ICU), ICU LOS, RRT, vasopressor use, reason for ICU admission (it is unclear whether “underlying diseases” in Table 1 are related to chronic comorbidities as detailed by the Charlson comorbidity score ad/or acute disease leading to ICU admission).

Our main purpose is to explore whether CRAB carriage is a risk factor for subsequent infection. The mortality rate, ICU LOS, and risk factor for them are not concerned, so they are not listed in our research. The relevant content is listed as “attached Table” for your reference.
Variables such as RRT, vasopressor use and reason for ICU admission were not included in this study. Underlying diseases are obtained from the diagnosis of ICU provided by researchers, and they are not the first diagnosis of ICU (not the disease leading to ICU admission). Charlson comorbidity score is provided by ICU doctors. Charlson score may partially overlap with underlying diseases.

5. Antimicrobial exposure should be integrated in multivariate analyses as a continuous variable (that is, treatment duration in days) and not as a nominal variable. Indeed, the risk may increase with treatment duration. In other words, 2 days of cephalosporin use may not have the same impact on the gut microbiota and intestinal colonization resistance than 8 or 10 days.

We agree with your opinion that antimicrobial exposure should be analyzed as a continuous variable. However, antimicrobials are strictly managed by our hospital to prevent antimicrobial use data from being obtained by commercial companies. It’s difficult for us to obtain antimicrobial usage duration and amount data.

6. Figure 2 (risk of CRAB infection in carriers and non-carriers): In patients with acquired carriage, does Day 0 correspond to ICU admission or to the day of the first positive rectal sample? Also, if this curve represents CRAB infection-free survival, how were handled patients who died from another cause than CRAB infection?

In patients with CRAB intestinal carriage, Day 0 corresponds to the day of the first positive rectal sample. While in patients without CRAB intestinal carriage, Day 0 corresponds to the day of the first rectal sample. Maybe the figure is not clearly stated. The survival curve represents the cumulative probability of CRAB infection. We redraw the survival curve and consider death in ICU as a competing risk event using Fine and Gray model as Figure 2. in the attached files.

7. It is briefly stated in the Limits sections (Abstract and main text) that not all CRAB isolated on selective media were confirmed as CRAB through usual antimicrobial susceptibility testing (Vitek II): what do the authors mean? How many “false positive” CRAB were identified? How were classified the corresponding patients: carriers or non-carriers??? This essential point must be developed, in the

Methods section and in the Results section. Patients not confirmed as CRAB carriers through AST should be classified - and analyzed - as non-carriers.

Patients' rectal swabs were inoculated onto modified CHROMagar Acinetobacter colorimetric plates containing 2 mg/L meropenem using the partition-and-streaking method. Plates were then cultured at 37°C for 18–24 h. And then if red bacterial colonies grow on the plate, we consider it CRAB positive. The picture could be seen as attached Picture in the attached files. This method is different from Vitek II test method.
At the beginning of this study, we confirmed that the 58 CRAB strains grown on the chromogenic medium were indeed all CRAB using Vitek II method retested. This result shows that there is no "false positive" CRAB. As a result, although not all screened CRABs are confirmed using Vitek II test, we consider the method is accurate.

ADDITIONAL COMMENTS
1. The Introduction is quite long and could be substantially shortened.
Revised.

2. Methods, page 6, "Patient and public involvement: Patients were involved in this study": what do the authors mean here?
BMJ Open now requires a Patient and Public Involvement (PPI) statement for all submissions. So, we revised as required.

3. Clinical isolates and carriage isolates of CRAB were not compared through molecular methods (or at least PFGE) in carriers with subsequent infection: this could have been smart to confirm the link between colonization and infection. A comment on this point could be added in the Limits section.
Revised.

Reviewer: 3
Line 51: implies causality. Suggest replace with was associated with…
Revised.

Line 271: limitations: analyses of strain no carried out… were patients infected by their own strain?
Thanks, and this is indeed a limitation of our research. We have added this limitation in our article. "No molecular strain typing was performed. Though reasonable, it was not proven that CRAB isolated from intestinal colonization and site of nosocomial infection were identical." Unfortunately, we haven't retained the strains and cannot test this. However, Elaa Maamar [PMID: 29665444] used PFGE and MLST to test this and showed that colonization of CRAB is a major risk for nosocomial infection of CRAB.

Table 2: item 1: CRAB intestinal carriage… please specify whether on admission or at any point during stay
In patients with CRAB intestinal carriage, Day 0 corresponds to the day of the first positive rectal sample.

Reviewer: 4
- How did the authors account for the competing risks of death in ICU and subsequent infections in Cox model? Were patients censored if they died in the ICU prior to infection? If so, please specify that the study examined infections in survivors. If otherwise, please explain.

Death in ICU could indeed prevent us from observing the occurrence of the infection, and the two are competing events. As a result, traditional Cox proportional hazards model may falsely evaluate the

effects of covariates. Therefore, data are reanalyzed using sub-distribution hazard model considered death in ICU as a competing risk event. According to the result, we update the contents of Figure 2, Table 2 and supplementary tables.

VERSION 2 – REVIEW

REVIEWER	Axel Kola, MD Charité - University Medicine, Berlin, Germany
REVIEW RETURNED	25-May-2020

GENERAL COMMENTS	The authors responded to my first review, there are no further comments.

REVIEWER	Majdi Al-Hasan University of South Carolina School of Medicine, USA
REVIEW RETURNED	02-Apr-2020

GENERAL COMMENTS	I thank the authors for appropriately revising the manuscript based on prior comments.