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Intestinal carriage of carbapenem-resistant *Acinetobacter baumannii* among patients in the intensive care unit: risk factors and the impact on subsequent infection

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Abstract

Objectives: To assess the incidence and the impact of carbapenem-resistant *Acinetobacter baumannii* (CRAB) intestinal carriage on subsequent CRAB infection and to study risk factors of acquiring CRAB intestinal carriage among patients in intensive care unit (ICU).

Design: Observational study including a case control study and a retrospective cohort study.

Setting: A 50-bed general ICU of a university hospital, China.

Methods: From May 2017 to April 2018, an observational study was conducted in a 50-bed general ICU of a university hospital in China. Rectal swabs were collected from ICU patients on admission and thereafter weekly. Risk factors of acquiring CRAB intestinal carriage were analyzed using multiple logistic regression. Patients with CRAB intestinal carriage were then compared to those without for the incidence of subsequent CRAB infection using Kaplan–Meier survival and COX multivariate analyses.

Results: CRAB intestinal carriage was detected in 6.87% (66/961; 95% CI 5.27%–8.47%) of patients on ICU admission, whereas 11.97% (115/961; 95% CI 9.91%–14.02%) of patients acquired CRAB intestinal carriage during the ICU stay. Pancreatitis (OR 2.16, 95% CI 1.28–3.67), hematological disease (OR 2.26, 95% CI 1.42–3.58), gastric tube feeding (OR 3.35, 95% CI 2.03–5.51), and use of carbapenems (OR 1.84, 95% CI 1.11–3.07) were independent risk factors for acquiring CRAB intestinal carriage. The incidence of subsequent CRAB infection was 1.75-fold in patients with CRAB intestinal carriage compared to that in patients without (95% CI 1.16–2.62, P=0.007).

Conclusion: More patients acquired CRAB intestinal carriage during their ICU stay than had on admission. Severity of illness, acute pancreatitis, tube feeding, and use of carbapenems were independent risk factors of acquisition of CRAB intestinal carriage. Patients with CRAB intestinal

carriage are more likely to develop CRAB infection.

Strengths and limitations of this study

A case control study was performed to analyze risk factors of the acquisition of CRAB intestinal carriage in ICU.

A retrospective cohort study was performed to address whether intestinal CRAB carriage could lead to an increased likelihood of subsequent CRAB infection.

Most influencing factors were considered in the study.

Not all screened CRABs were confirmed using Vitek II or other methods.

Background

Acinetobacter baumannii is one of the most common nosocomial pathogens[1]. A systematic review has revealed that *A. baumannii* accounted for 11.28% of nosocomial infections in general hospitals in China, making it the third most common nosocomial pathogen[2]. Carbapenems such as meropenem and imipenem are a class of potent antimicrobial agents for treating severe infections caused by Gram-negative bacteria including *A. baumannii*. However, carbapenem-resistant *A. baumannii* (CRAB) has emerged worldwide. As early as 2013, the US Centers for Disease Control and Prevention listed multi-drug resistant *A. baumannii* (MDRAB) including CRAB as a serious threat[3], and the World Health Organization listed CRAB as one of the three most critical threats in a global drug-resistant warning in 2017[4]. The prevalence of *A. baumannii* and its resistance to carbapenems varies from country to country. For instance, the European Bacterial Resistance Surveillance Report shows that the rate of *Acinetobacter* resistant to carbapenem in Europe in 2017 was 33.4% (95% CI 32%–35%), but it was as high as 96.2% in Croatia (95% CI 92%–98%) [5]. In the US, 49.5% of *A. baumannii* is resistant to carbapeems, while in Singapore, India, and Pakistan, it is 50%, 85%, and 62-100%, respectively[6,7]. The prevalence of CRAB is also very high in China. The surveillance data released by CHINET (China Antimicrobial Surveillance Network; http://chinets.com/Chinet), a national network in China, have shown that *A. baumannii* isolates ranked the 5th in the number of microbial isolates from all types of clinical samples in 2018, with 77.1% and 78.1% of *A. baumannii* isolates resistant to imipenem and meropenem, respectively[8].

Infections caused by CRAB can lead to serious consequences. A previous study has demonstrated that patients with CRAB infection had longer average length of stay (LOS) in ICUs (13.1 vs. 10.5 days) and $11,359 higher average in-hospital costs than those with carbapenem-

susceptible *A. baumannii* (CSAB) infection[9]. Another previous study has found that the mortality rate of patients with CRAB infection is 2.22-fold that of patients with CSAB infection[10]. A case-control study conducted by our team have also showed that the 28-day survival rate of patients with bloodstream CRAB infection was 66.17%, lower than the 96.95% of those with bloodstream CSAB infection[11].

It is well known that *A. baumannii* including CRAB usually colonized in the respiratory tract of hospitalized patients, in particular those with mechanical ventilation[12,13]. The colonization of CRAB in the respiratory tract has been found as a major risk factor for subsequent CRAB infection[14]. However, ICU patients may carry CRAB in intestine on admission or acquire CRAB during the ICU stay[15]. Patients with intestinal carriage of multi-drug resistant organisms (MDRO), in particular carbapenem-resistant *Enterobacteriaceae* (CRE), may sever as a reservoir for further dissemination in ICU[16] and could be associated with be associated with an increased risk of subsequent MDRO infections[17]. Therefore, active screening the carriage of CRE, which is usually performed using rectal swabs, has been recommended as a core component of the infection control bundle[7]. However, by contrast to CRE, the prevalence of CRAB intestinal carriage among ICU patients is much less studied and the risk factors of acquisition of CRAB intestinal carriage remains largely unknown. In addition, it remains to be determined whether CRAB intestinal carriage leads to increased risks of subsequent CRAB infection. To address these questions, we therefore conducted this study.

Methods

Study settings

An observational study was conducted in a 50-bed general ICU of a 4,300-bed university

hospital in China. From May 2017 to April 2018, all patients admitted to the ICU were subjected to collecting a rectal swab within 48 h of admission and thereafter weekly. For patients hospitalized for less than 3 days, a rectal swab was collected only once within 48 h of admission.

Inclusion and exclusion criteria

Inclusion criteria: This study included all patients who were ≥ 18 years of age, admitted to the ICU, and underwent collection of rectal swabs.

Exclusion criteria: 1) patients who did not receive a rectal swab within 48 h of admission to ICU; or 2) patients who were eligible for weekly follow-up collection of rectal swabs but did not receive subsequent sampling; or 3) patients with CRAB infection on admission.

Patient and public involvement

Patients were involved in this study.

Definitions

Patients with CRAB intestinal carriage were defined as those with CRAB isolated from a rectal swab, while patient without CRAB intestinal carriage referred to those whose swabs were all negative for CRAB during the ICU stay. Patients with CRAB isolated from a rectal swab collected within 48 h of ICU admission were defined as those with CRAB intestinal carriage on ICU admission. The acquisition of CRAB intestinal carriage referred to a patient who had a CRAB-negative rectal swab collected within 48 h of ICU admission but had CRAB from a swab collected after 48 h. CRAB infection was defined as the growth of CRAB from clinical specimens in the presence of clinical manifestations of infection[18]. Subsequent CRAB infection referred to

CRAB infection developed after the collection of a CRAB-positive rectal swab for patients with CRAB intestinal carriage and CRAB infection developed after 48 h admission to the ICU for patients without CRAB intestinal carriage.

Screening for CRAB by rectal swabs

For collecting rectal swabs, ready-to-use transport medium swabs (HBPT004; Hopebio Biotechnology, Qingdao, China) was inserted about 2–3 cm into the patient's anus and then gently rotated. After sampling, the swab was inserted into the ready-to-use transport medium and transported to the laboratory within 2 h. Rectal swabs were inoculated onto modified CHROMagar *Acinetobacter* colorimetric plates (Chromagar; Paris, France) containing 2 mg/L meropenem using the partition-and-streaking method[19,20]. Plates were then cultured at 37°C for 18–24 h[20].

Data collection and statistical analysis

In this study, the patient's demographic data, underlying diseases, invasive procedures, medical orders, and use of antimicrobial agents were retrieved from the electronic medical record system. Two professional statisticians collaborated to clean the data.

We performed two types of comparison. First, a case control study was performed to analyze risk factors of the acquisition of CRAB intestinal carriage in ICU. Patients with ICU acquisition of CRAB intestinal carriage were assigned to the case group, while those without CRAB intestinal carriage during their ICU stay were assigned to the control group. All potential factors were initially subjected to the univariate analysis. Quantitative data were described by the median (interquartile range) and were then analyzed using a rank-sum test. Qualitative data were described by number of cases (composition ratio) and were then analyzed using the chi-square test or Fisher exact probability method when applied. All variables showing *P* value less than 0.2 in the

univariate analysis were then included into the multiple logistic regression using the forward selection stepwise regression method[21,22]. Odds ratio (OR) and 95% confidence interval (CI) were calculated. The Hosmer-Lemeshow method was used to test the goodness-of-fit of the multiple logistic model[23].

Second, a retrospective cohort study was performed to address whether intestinal CRAB carriage could lead to an increased likelihood of subsequent CRAB infection. In this cohort study, the exposed group comprised patients with CRAB intestinal carriage either detected on ICU admission or acquired during the ICU stay, while the non-exposed group consisted of those without CRAB intestinal carriage. As the impact of CRAB intestinal carriage on subsequent infection may also be influenced by other factors such as patient demographics, underlying diseases, antimicrobial use and medical operations, we included these factors for analysis instead of evaluating CRAB carriage alone. Survival curves (probability of CRAB infection) in patients with and without CRAB intestinal carriage were mapped using the Kaplan–Meier method[24,25]. After introducing the interaction term of time and each variable (X*ln (T)) into the COX model [24,25], the proportional hazards hypothesis was tested, and the results showed no statistical significance ($P < 0.05$). Therefore, the COX regression (proportional hazards model) was used for univariate and multivariate analyses. Hazard ratio (HR) and 95% CI were calculated to explore whether CRAB intestinal carriage was a risk factor for subsequent CRAB infection. The Omnibus method was used to test the goodness-of-fit of the multivariate COX model[26]. We also performed subgroup analyses to investigate whether CRAB intestinal carriage on ICU admission and that acquired in ICU had different impact on subsequent CRAB infection using the same statistical method as describe above. For the subgroup analysis, patients with CRAB intestinal carriage on ICU admission and those with ICU acquisition of CRAB intestinal carriage were assigned to two

exposed subgroups, respectively, while those without CRAB intestinal carriage were assigned to the non-exposed group.

All statistical analyses were performed using SPSS 21.0 (IBM–SPSS Inc; Armonk, NY, US) with a 0.05 two-sided test level.

Results

Some patients (6.87%) had CRAB intestinal carriage on ICU admission and more (12.85%) acquired in ICU

From May 1, 2017 to April 30, 2018, a total of 1,605 patients were admitted to the ICU, of which 382 (23.8%) were not screened during their hospital stay. Of which the 382 patients, 323 (84.55%) stayed in the ICU for no more than 2 days, while the other 59 (15.45%) patients were missed for sampling. In addition, 118 patients (118/1,605, 7.4%) were excluded due to inappropriate or incomplete sampling including 104 patients whose first rectal swab was collected 48 h after admission and 14 patients who were not screened weekly. A total of 144 (144/1,605, 8.97%) had CRAB infection on ICU admission and were therefore also excluded. Taken together, a total of 961 patients (620 males, 64.52% and 341 female 35.48%) were included in the analysis, with an average age of 54 (44–68) years (Figure 1).

Among the 961 patients, 66 (6.87%, 95% CI 5.27%–8.47%) had CRAB intestinal carriage on ICU admission. For the remaining 895 patients, 115 acquired (12.85%, 95% CI 10.66%–15.04%) CRAB intestinal carriage during their ICU stay with an average age of 51 (40–70) and a 1.61 male/female ratio (71 male and 44 female).

Multiple risks factors of acquiring CRAB intestinal carriage were identified

The univariate analysis showed that APACHE II score (the patient's disease severity), respiratory failure, renal dysfunction, hematological disease, acute pancreatitis, indwelling central venous catheter, gastric tube feeding, nebulization, and use of vancomycin, aminoglycosides, carbapenems, tigecycline, and antifungal agents are risk factors for the acquisition of CRAB intestinal carriage in the ICU. Multiple logistic regression including all variables with $P < 0.2$ in the univariate analysis showed that APACHE II score, pancreatitis, hematological diseases, gastric tube feeding, and use of carbapenems were independent risk factors for acquiring CRAB intestinal carriage during the ICU stay (Table 1). For APACHE II score, the model estimated that the increase of the score by 1 point would lead to a 4% increase of the risk of acquiring CRAB intestinal carriage in the ICU. Hosmer-Lemeshow test generated a 0.73 P value (χ^2=5.25, df=8), suggesting adequate goodness-of-fit of the multiple logistic model.

CRAB intestinal carriage led to increased risks of subsequent CRAB infection

During the study period, 112 of the 961 patients (11.65%, 95% CI 9.63%–13.68%) developed CRAB infections during the ICU stay. As for the infection type, lower respiratory tract infections were the most common (n=82, 73.21%), followed by bloodstream infections (n=9, 8.04%), surgical site infection (n=8, 7.14%), while 13 patients (11.61%) had infections at other sites. CRAB intestinal carriage was a risk factor for subsequent CRAB infection (HR 2.69, 95% CI 1.85–3.92; $P < 0.001$; Figure 2). The 90-day cumulative probability of no CRAB infection in patients with and without CRAB intestinal carriage was 68.0% (95% CI 60.3%–75.7%) and 24.6% (95% CI 12.2%–37.0%), respectively (P<0.001). In the univariate analysis, CRAB intestinal carriage, APACHE II score, respiratory failure, hepatic insufficiency, hematological disease, pancreatitis, mechanical ventilation, placement of a central venous catheter, gastric tube feeding,

and the use of carbapenems were identified as risk factors for subsequent CRAB infection. In the COX multivariate analysis, CRAB intestinal carriage was also found to be an independent risk factor for subsequent CRAB infection (HR 1.75, 95% CI 1.16–2.62; Table 2). Omnibus test showed a log likelihood difference of 79.82 and generated a less than 0.001 P value, suggesting adequate goodness-of-fit of the COX model.

To evaluate whether CRAB intestinal carriage on admission and that acquired during the ICU stay has different impact on subsequent CRAB, we performed subgroup analyses. In the subgroup COX multivariate analysis, both CRAB intestinal carriage on admission and that acquired during the ICU stay were an independent risk factor for subsequent CRAB infection (HR 2.08, 95% CI 1.17–3.68 for carriage on admission, Table S1 in the Supplementary file; HR 1.81, 95% CI 1.14–2.88 for acquired carriage, Table S2). Omnibus test showed log likelihood difference of 66.06 and 74.18, respectively, and generated a less than 0.001 P value in the subgroup analysis, suggesting adequate goodness-of-fit of the COX model.

In addition to CRAB intestinal carriage, the use of ventilator (HR 2.37, 95% CI 1.15–4.89), liver dysfunction (HR 2.23, 95% CI 1.29–3.85), and the use of carbapenems (HR 2.75, 95% CI 1.74–4.35), were also identified as independent risk factors of subsequent CRAB infection, while the use of cephalosporins (HR 0.44, 95% CI 0.27–0.73) and cephamycins (HR 0.49, 95% CI 0.28–0.84) were protective factors (Table 2).

Discussion

In this study, we found that in a region with a high CRAB prevalence, 6.87% of patients (83.3% of those patients were transferred from other hospitals and 25.8% of them were stayed in emergency ICU before admitted to the ICU) admitted to the ICU had CRAB intestinal carriage on

ICU admission, while an additional 11.97% of patients acquired CRAB intestinal carriage during the ICU stay. The overall CRAB intestinal carriage rate was therefore 18.84%. This rate was similar with a study conduct in Thailand, in which 5.45% (15/275) of patients had intestinal carriage on ICU admission and 13.59% (28/206) patients acquired CRAB during their ICU stay[15] and with another study in Italy[27], in which 18.92%(74/391) of patients carried CRAB during ICU stay. However, the rate was significantly higher than those in Turkey (7.22%, 55/762)[28], Brazil (13.23%, 43/325)[29], USA (13.46%, 49/364)[30], and South Korea (15.06%, 168/1,115)[14], although other sites such as respiratory secretions were also screened in these studies. This difference may be related to the local CRAB prevalence.

Interestingly, we found that gastric tube feeding is a risk factor for both acquiring CRAB intestinal carriage of CRAB in ICU, which is consistent with the findings of Kiddee et al[15], in which tube feeding was also a high-risk factor for carriage of Gram-negative bacilli. This may suggest an entry point of CRAB into human intestine. In this study, 73.0% (84/115) of patients who acquired CRAB intestinal carriage using tube feeding. During the study, we performed a one-day snapshot sampling of the feeding tubes (at the tube port), feeding contents and containers for preparing feeding contents in the ICU and found the presence of CRAB in the tube feeding content (24.0%, 6/25), at the tube port (33.3%, 3/9) and the tube feeding containers (7.1%, 1/14), indicating contamination. This may be a key point for intervention in the ICU.

We also found that patients with CRAB intestinal carriage were more likely to develop subsequent CRAB infection than those without carriage. The survival curve in this study showed that the cumulative infection rates in 90 days in patients with and without CRAB intestinal carriage were 75.4% and 32%, respectively, similar to those reported in other studies[30]. However, the HR was 1.75, which is much lower than those in previous studies[15,30,31]. This may be due to

the fact that healthcare associated infections in our ICU were mainly caused by lower respiratory infections, which accounting for more than 70% of infections, while we only screened the colonization of the intestines. Interestingly, we found that the use of cephalosporins and cephamycins led to lower risks of subsequent CRAB infection, while carbapenem use led to increased risks. The association between CRAB and carbapenem use has been documented before[30,32]. CRAB is usually resistant to cephalosporins and cephamycins. The use of cephalosporins and cephamycins may reflect the fact that patients did not receive carbapenems and could therefore result in reduced selection pressure for CRAB.

There are a few limitations in this study. First, this is a single center study and the findings may not be generalized. Second, we used a modified CHROMagar *Acinetobacter* chromogenic plate to screen CRAB from rectal swabs. Not all screened CRABs were confirmed using Vitek II or other methods and there may be false negative results. Nonetheless, at the beginning of this study, we confirmed that the 58 CRAB strains grown on the chromogenic medium were indeed all *A. baumannii* by MALDI-TOF-MS and were all non-susceptible to imipenem or meropenem as determined using the agar dilution method recommended by the Clinical and Laboratory Standards Institute (CLSI)[20]. Third, we only collected the patients' rectal swabs for investigating CRAB carriage. Studies have shown concurrent swab collection of skin, oropharyngeal, and airway secretions in addition to rectal swabs, may improve sensitivity. However, the sample sizes in these studies were small with only 21 and 34 cases, respectively[12,33]. Nonetheless, for practical reasons and the aim to study CRAB intestinal carriage, we only collected rectal swabs. Last, this study failed to collect for the first rectal swab specimen within 48 h of ICU admission from 23.8% of the patients. Nonetheless, 84.55% of these patients stayed in the ICU for less than 48 h.

In conclusion, some patients had CRAB intestinal carriage but more acquired during their ICU stay. Severity of illness, acute pancreatitis, tube feeding, and use of carbapenems were independent risk factors of the acquisition of CRAB intestinal carriage. Patients with CRAB intestinal carriage were more likely to have subsequent CRAB infection than those without.

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Author contributions

Fu Qiao, Zhiyong Zong and Chuanmin Tao contributed to study conception and design. Shichao Zhu and Yan Kang contributed to acquisition of data. Lin Cai collected rectal swabs and transported to the laboratory. Fu Qiao, Wenzhi Huang and Shan Gao analyzed and interpreted data. Li Wei inoculated rectal swabs onto plates cultured for 18-24h. Fu Qiao, Zhiyong Zong and Chuanmin Tao drafted the manuscript. All authors revised the manuscript for important intellectual content. All authors read and approved the final manuscript.

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Competing interests

The authors declare that they have no competing interests.

Patient consent for publication

Not required.

Ethics approval

This project was approved by the Ethics Committee of West China Hospital of Sichuan University.

Availability of data and materials

The datasets during the current study available from the corresponding author on reasonable request.

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Figure legends

Figure 1. Patient selection flow algorithm

Figure 2. Survival curves of patients with and without CRAB intestinal carriage (cumulative probability of no CRAB infection). The solid line represents patients with CRAB intestinal carriage, while the dashed line represents those without.

Table 1 Risk factors for the acquisition of CRAB intestinal carriage during the ICU stay

Characteristics	Patients with acquiring CRAB intestinal carriage		Univariate analysis		Multivariate analysis	
	Yes (n=115)	No (n=780)	OR (95% CI)	P	OR (95% CI)	*P*
Demographics						
Sex, male	71 (61.74%)	502 (64.36%)	1.12 (0.75–1.68)	0.590		
Ethnicity, Han Chinese	108 (93.91%)	712 (91.28%)	1.47 (0.66–3.29)	0.338		
Age (median)	51 (40–70)	56 (45–68)	/	0.207		
Underlying disease						
Myocardial infarction	1 (0.87%)	4 (0.51%)	1.7 (0.19–15.36)	0.500		
Peripheral vascular disease	11 (9.57%)	62 (7.95%)	1.22 (0.62–2.40)	0.550		
Cerebrovascular disease	4 (3.48%)	36 (4.62%)	0.74 (0.26–2.13)	0.582		
Dementia	1 (0.87%)	0 (0%)	/	0.130		
Connective tissue disease	1 (0.87%)	12 (1.54%)	0.56 (0.07–4.36)	0.887		
Peptic Ulcer	5 (4.35%)	25 (3.21%)	1.37 (0.51–3.66)	0.720		
Hemiplegia	0 (0%)	1 (0.13%)	/	1.000		
Hypertension	36 (31.30%)	180 (23.08%)	1.52 (0.99–2.33)	0.054		
Tuberculosis	1 (0.87%)	12 (1.54%)	0.56 (0.07–4.36)	0.887		
COPD	10 (8.70%)	54 (6.92%)	1.28 (0.63–2.59)	0.490		

Respiratory failure	40 (34.78%)	163 (20.90%)	2.02 (1.33–3.07)	**0.001**		
Kidney failure	11 (9.57%)	31 (3.97%)	2.56 (1.25–5.24)	**0.010**		
Heart failure	7 (6.09%)	19 (2.44%)	2.99 (1.28–7.01)	0.060		
Diabetes	21 (18.26%)	102 (13.08%)	1.48 (0.89–2.49)	0.132		
Liver dysfunction	5 (4.35%)	37 (4.74%)	0.91 (0.35–2.37)	0.850		
Hematological disease	**71 (61.74%)**	**268 (34.36%)**	**3.08 (2.06–4.62)**	**<0.001**	**2.26 (1.42–3.58)**	**0.001**
Pancreatitis	**35 (30.43%)**	**77 (9.87%)**	**3.99 (2.52–6.34)**	**<0.001**	**2.16 (1.28–3.67)**	**0.004**
Medical operation						
Surgery	**82 (71.30%)**	**645 (82.69%)**	**0.52 (0.33–0.81)**	**0.004**	**0.40 (0.24–0.68)**	**0.001**
CVC	78 (67.83%)	424 (54.36%)	1.77 (1.17–2.68)	**0.010**		
Ventilator	101 (87.83%)	666 (85.38%)	1.23 (0.68–2.23)	0.490		
Indwelling catheter	110 (95.65%)	742 (95.13%)	1.13 (0.43–2.92)	0.810		
Tube feeding	**84 (73.04%)**	**280 (35.90%)**	**4.84 (3.13–7.49)**	**<0.001**	**3.35 (2.03–5.51)**	**<0.001**
Nebulizer fiberoptic	73 (63.48%)	368 (47.18%)	1.95 (1.30–2.92)	**0.001**		
Bronchoscope	1 (0.87%)	21 (2.69%)	0.32 (0.04–2.38)	0.390		
Antimicrobial use						
Cephalosporin	**35 (30.43%)**	**312 (40.00%)**	**0.66 (0.43–1.00)**	**0.049**	**0.59 (0.37–0.95)**	**0.029**
Vancomycin	13 (11.30%)	32 (4.10%)	2.98 (1.51–5.86)	**0.001**		
Aminoglycosides	12 (10.43%)	31 (3.97%)	2.81 (1.40–5.65)	**0.002**		
Carbapenems	**82 (71.30%)**	**295 (37.82%)**	**4.09 (2.66–6.27)**	**<0.001**	**1.84 (1.11–3.07)**	**0.018**

Fluoroquinolones	26 (22.61%)	137 (17.56%)	1.37 (0.85–2.20)	0.190		
Antifungal agents	49 (42.61%)	138 (17.69%)	3.45 (2.29–5.22)	**<0.001**		
Cephamycins	16 (13.91%)	253 (32.44%)	0.34 (0.19–0.58)	**<0.001**		
Lincomycin	3 (2.61%)	61 (7.82%)	0.32 (0.10–1.02)	**0.040**		
Tigecycline	19 (16.52%)	69 (8.85%)	2.04 (1.18–3.54)	**0.010**		
APACHE II	**21.5 (17–26)**	**17 (12–22)**	/	**<0.001**	**1.04 (1.01–1.07)**	**0.013**
Charlson score	2 (1–5)	3 (2–4)	/	0.063		
Sharing room with other patients with CRAB intestinal carriage	20 (17.39%)	153 (19.62%)	0.86 (0.52–1.44)	0.573		

COPD, chronic obstructive pulmonary disease. CVC, central venous catheter.

Variables with $P < 0.05$ in the multiple logistic analysis are highlighted in bold.

Table 2 Variables associated with developing subsequent CRAB infection during the ICU stay

Item	Subsequent CRAB infection during the ICU stay		Univariate analysis		Multivariate analysis	
	Yes (n=112)	No (n=849)	HR (95% CI)	P	HR (95% CI)	P
CRAB intestinal carriage	**51 (45.54%)**	**130 (15.31%)**	**2.69 (1.85–3.92)**	**<0.001**	**1.75 (1.16–2.62)**	**0.007**
Demographics						
Sex, male	72 (64.29%)	548 (64.55%)	1.01 (0.68–1.48)	0.979		
Ethnicity, Han Chinese	106 (94.64%)	774 (91.38%)	1.68 (0.74–3.83)	0.215		
Age (median)	53 (42–67)	55 (44–68)	1.00 (0.99–1.01)	0.940		
APACHE II	21 (17–26)	17 (12–22)	1.06 (1.03–1.08)	**<0.001**		
Charlson score	3 (1–5)	3 (1.5–4)	0.99 (0.90–1.09)	0.869		
Underlying disease						
Myocardial infarction	0 (0%)	6 (0.71%)	0.05 (0–6037.12)	0.615		
Peripheral vascular disease	13 (11.61%)	66 (7.77%)	1.27 (0.71–2.27)	0.418		
Cerebrovascular disease	4 (3.57%)	36 (4.24%)	1.02 (0.37–2.77)	0.971		
Dementia	0 (0%)	1 (0.12%)	0.05 (0–5419.76)	0.609		
Connective tissue disease	1 (0.89%)	12 (1.41%)	0.72 (0.10–5.12)	0.739		
Peptic ulcer	4 (3.57%)	27 (3.18%)	1.07 (0.39–2.91)	0.891		
Hemiplegia	1 (0.89%)	0 (0%)	5.24 (0.73–37.73)	0.100		
Hypertension	28 (25.00%)	199 (23.44%)	1.06 (0.69–1.63)	0.792		
Tuberculosis	2 (1.79%)	12 (1.41%)	1.12 (0.28–4.54)	0.874		
COPD	11 (9.82%)	55 (6.48%)	1.46 (0.79–2.73)	0.231		
Respiratory failure	47 (41.96%)	170 (20.02%)	2.14 (1.47–3.12)	**<0.001**		
Kidney failure	9 (8.04%)	42 (4.95%)	1.61 (0.81–3.18)	0.171		
Heart failure	4 (3.57%)	27 (3.18%)	1.28 (0.47–3.47)	0.631		
Diabetes	15 (13.39%)	118 (13.90%)	0.85 (0.50–1.47)	0.570		
Liver dysfunction	**17 (15.18%)**	**34 (4.00%)**	**3.07 (1.83–5.15)**	**<0.001**	**2.23 (1.29–3.85)**	**0.004**
Hematological disease	60 (53.57%)	314 (36.98%)	1.71 (1.18–2.49)	**0.005**		
Pancreatitis	29 (25.89%)	107 (12.60%)	1.85 (1.21–2.83)	**0.004**		
Medical operation						

Surgery	83 (74.11%)	711 (83.75%)	0.76 (0.49–1.16)	0.199		
CVC	83 (74.11%)	470 (55.36%)	2.03 (1.33–3.10)	**0.001**		
Ventilator	**103 (91.96%)**	**719 (84.69%)**	**2.15 (1.09–4.26)**	**0.027**	**2.37 (1.15–4.89)**	**0.019**
Indwelling catheter	109 (97.32%)	808 (95.17%)	1.91 (0.61–6.03)	0.269		
Tube feeding	80 (71.43%)	332 (39.10%)	2.40 (1.58–3.62)	**<0.001**		
Nebulizer fiberoptic	72 (64.29%)	413 (48.65%)	1.13 (0.76–1.67)	0.542		
Bronchoscope	5 (4.46%)	18 (2.12%)	1.31 (0.53–3.21)	0.561		
Antimicrobial use						
Cephalosporin	**20 (17.86%)**	**236 (27.80%)**	**0.51 (0.31–0.82)**	**0.006**	**0.44 (0.27–0.73)**	**0.001**
Vancomycin	3 (2.68%)	35 (4.12%)	0.67 (0.21–2.12)	0.496		
Aminoglycosides	1 (0.89%)	23 (2.71%)	0.25 (0.04–1.82)	0.173		
Carbapenems	**82 (73.21%)**	**351 (41.34%)**	**3.05 (2.01–4.64)**	**<0.001**	**2.75 (1.74–4.35)**	**<0.001**
Fluoroquinolones	32 (28.57%)	154 (18.14%)	1.00 (0.66–1.52)	0.986		
Antifungal agents	23 (20.54%)	157 (18.49%)	1.02 (0.64–1.61)	0.944		
Cephamycins	**16 (14.29%)**	**196 (23.09%)**	**0.48 (0.28–0.81)**	**0.006**	**0.49 (0.28–0.84)**	**0.010**
Lincomycin	5 (4.46%)	35 (4.12%)	0.94 (0.38–2.31)	0.897		
Tigecycline	13 (11.61%)	65 (7.66%)	1.40 (0.78–2.49)	0.259		

COPD, chronic obstructive pulmonary disease. CVC, central venous catheter.

Variables with $P < 0.05$ in the multivariate COX analysis are highlighted in bold.

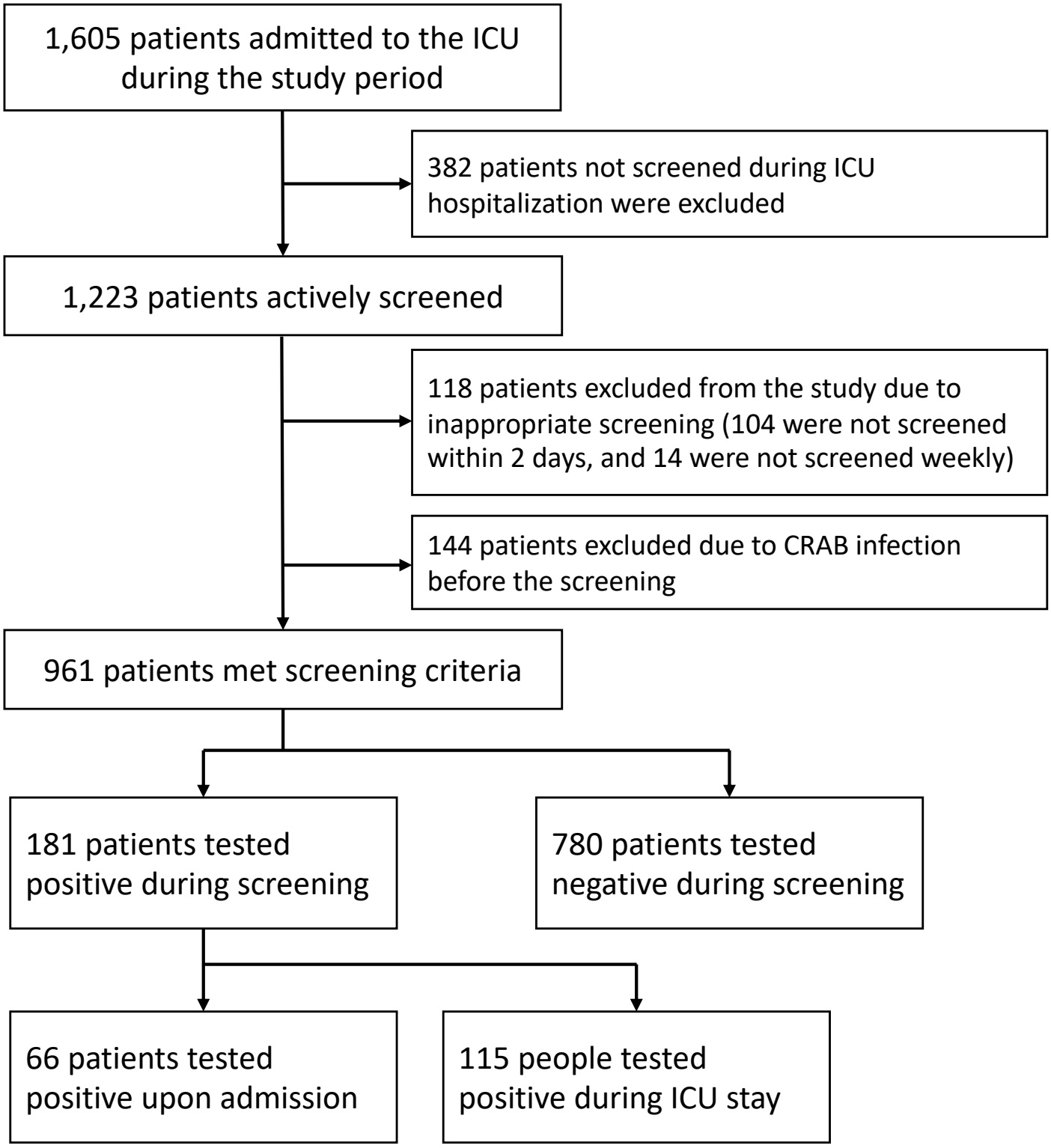

Figure 1. Patient selection flow algorithm

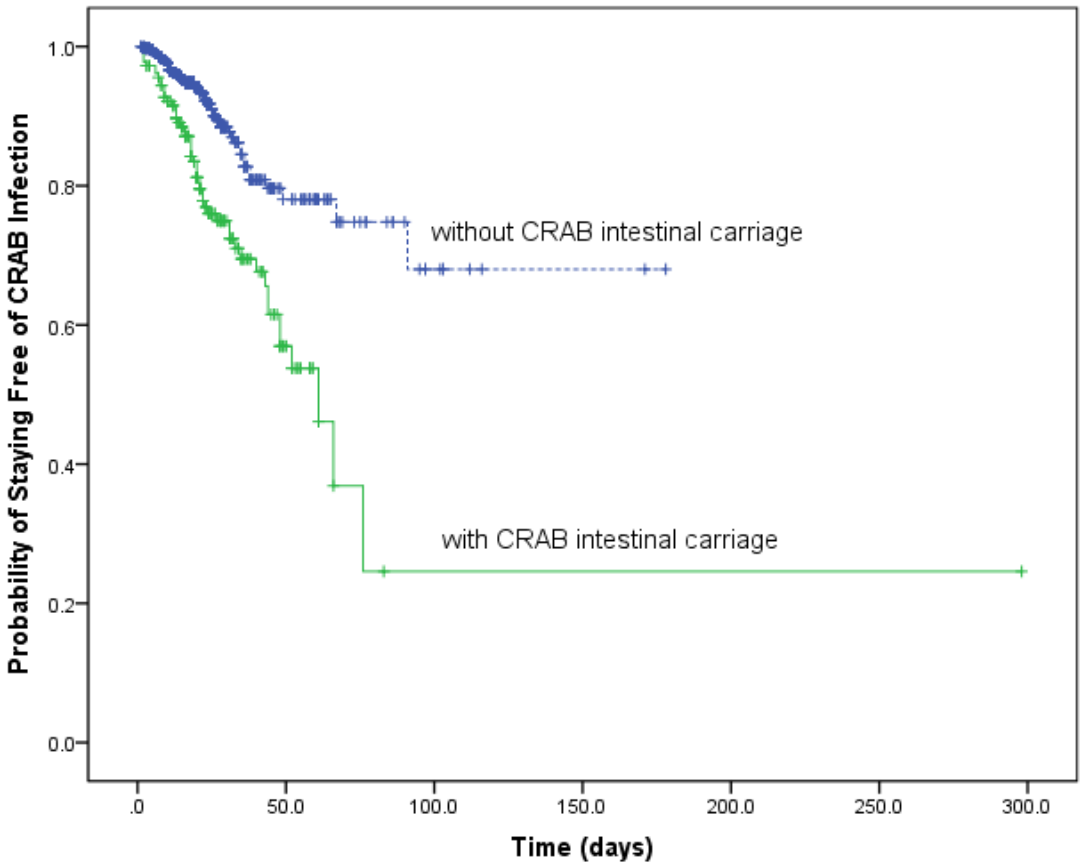

Figure 2. Survival curves of patients with and without CRAB intestinal carriage (cumulative probability of no CRAB infection). The solid line represents patients with CRAB intestinal carriage, while the dashed line represents those without.

Supplementary files

Table S1 Variables associated with developing subsequent CRAB infection during the ICU stay (exposed group was those patients with intestinal carriage on ICU admission)

Item	Subsequent CRAB infection during the ICU stay		Univariate analysis		Multivariate analysis	
	Yes (n=80)	No (n=766)	*HR (95% CI)*	*P*	*HR (95% CI)*	*P*
CRAB intestinal carriage	**19 (23.75%)**	**47 (6.14%)**	**2.98 (1.78-5.00)**	**<0.001**	**2.08 (1.17-3.68)**	**0.012**
Demographics						
Sex, male	50 (62.50%)	499 (65.14%)	1.09 (0.70-1.72)	0.696		
Ethnicity, Han Chinese	77 (96.25%)	697 (90.99%)	2.66 (0.84-8.45)	0.096		
Age (median)	54 (42-68)	55 (44-68)	1.00 (0.99-1.02)	0.527		
APACHE II	**20 (15-26)**	**17 (12-22)**	**1.07 (1.04-1.10)**	**<0.001**	**1.04 (1.01-1.07)**	**0.020**
Charlson score	3 (1-5)	3 (2-4)	1.03 (0.91-1.15)	0.659		
Underlying disease						
Myocardial infarction	0 (0%)	5 (0.65%)	0.93 (0.23-3.80)	0.923		
Peripheral vascular disease	11 (13.75%)	57 (7.44%)	1.61 (0.85-3.04)	0.143		
Cerebrovascular disease	3 (3.75%)	33 (4.31%)	1.01 (0.32-3.21)	0.983		
Dementia	0 (0%)	0 (0%)	/	/		
Connective tissue disease	1 (1.25%)	11 (1.44%)	0.91 (0.13-6.52)	0.921		
Peptic ulcer	3 (3.75%)	23 (3.00%)	1.06 (0.33-3.36)	0.923		
Hemiplegia	1 (1.25%)	0 (0%)	4.30 (0.59-31.23)	0.149		
Hypertension	18 (22.50%)	173 (22.58%)	1.07 (0.63-1.80)	0.810		
Tuberculosis	1 (1.25%)	12 (1.57%)	0.65 (0.09-4.68)	0.669		
COPD	**11 (13.75%)**	**45 (5.87%)**	**2.19 (1.16-4.13)**	**0.016**	**2.42 (1.23-4.76)**	**0.011**

Respiratory failure	34 (42.50%)	143 (18.67%)	2.52 (1.61-3.93)	<0.001		
Kidney failure	5 (6.25%)	35 (4.57%)	1.22 (0.49-3.02)	0.667		
Heart failure	2 (2.50%)	21 (2.74%)	0.93 (0.23-3.80)	0.923		
Diabetes	11 (13.75%)	101 (13.19%)	1.00 (0.53-1.89)	0.998		
Liver dysfunction	**14 (17.50%)**	**32 (4.18%)**	**3.42 (1.92-6.11)**	**<0.001**	**2.12 (1.15-3.93)**	**0.016**
Hematological disease	42 (52.50%)	261 (34.07%)	2.05 (1.32-3.18)	0.001		
Pancreatitis	17 (21.25%)	84 (10.97%)	1.78 (1.04-3.05)	0.035		
Medical operation						
Surgery	62 (77.50%)	638 (83.29%)	0.94 (0.56-1.60)	0.830		
CVC	61 (76.25%)	414 (54.05%)	2.18 (1.30-3.65)	0.003		
Ventilator	72 (90.00%)	648 (84.60%)	1.80 (0.87-3.74)	0.115		
Indwelling catheter	78 (97.50%)	729 (95.17%)	2.03 (0.50-8.28)	0.325		
Tube feeding	54 (67.50%)	265 (34.60%)	2.34 (1.46-3.75)	<0.001		
Nebulizer fiberoptic	51 (63.75%)	349 (45.56%)	1.34 (0.85-2.12)	0.213		
Bronchoscope	5 (6.25%)	17 (2.22%)	2.39 (0.97-5.93)	0.060		
Antimicrobial use						
Cephalosporin	15 (18.75%)	210 (27.42%)	0.57 (0.33-1.00)	0.051		
Vancomycin	2 (2.50%)	28 (3.66%)	0.70 (0.17-2.84)	0.615		
Aminoglycosides	1 (1.25%)	15 (1.96%)	0.46 (0.06-3.32)	0.443		
Carbapenems	**58 (72.50%)**	**284 (37.08%)**	**3.77 (2.30-6.16)**	**<0.001**	**2.70 (1.56-4.67)**	**<0.001**
Fluoroquinolones	21 (26.25%)	127 (16.58%)	1.29 (0.78-2.14)	0.316		
Antifungal agents	17 (21.25%)	123 (16.06%)	1.19 (0.70-2.04)	0.525		
Cephamycins	12 (15.00%)	179 (23.37%)	0.54 (0.29-1.00)	0.049		
Lincomycin	3 (3.75%)	32 (4.18%)	0.75 (0.24-2.39)	0.632		
Tigecycline	8 (10.00%)	53 (6.92%)	1.34 (0.64-2.77)	0.438		

COPD, chronic obstructive pulmonary disease. CVC, central venous catheter.

Variables with $P < 0.05$ in the multivariate COX analysis are highlighted in bold.

Table S2. Variables associated with developing subsequent CRAB infection during the ICU stay (exposed group was those patients with ICU acquisition of CRAB intestinal carriage)

Item	Subsequent CRAB infection during the ICU stay		Univariate analysis		Multivariate analysis	
	Yes (n=93)	No (n=802)	*HR (95% CI)*	*P*	*HR (95% CI)*	*P*
CRAB intestinal carriage	**32 (34.41%)**	**83 (10.35%)**	**3.02 (1.97-4.64)**	**<0.001**	**1.81 (1.14-2.88)**	**0.012**
Demographics						
Sex, male	60 (64.52%)	513 (63.97%)	0.99 (0.65-1.51)	0.954		
Ethnicity, Han Chinese	87 (93.55%)	731 (91.15%)	1.01 (0.99-1.02)	0.398		
Age (median)	54 (44-68)	55 (45-68)	1.42 (0.62-3.24)	0.410		
APACHE II	**21 (18-26)**	**17 (12-22)**	**1.07 (1.05-1.10)**	**<0.001**	**1.04 (1.01-1.07)**	**0.016**
Charlson score	3 (1-5)	3 (2-4)	1.04 (0.93-1.15)	0.490		
Underlying disease						
Myocardial infarction	0 (0%)	5 (0.62%)	0.05 (0-28290)	0.656		
Peripheral vascular disease	11 (11.83%)	62 (7.73%)	1.36 (0.72-2.54)	0.344		
Cerebrovascular disease	4 (4.30%)	36 (4.49%)	1.11 (0.41-3.03)	0.835		
Dementia	0 (0%)	1 (0.12%)	0.05 (0-24060)	0.651		
Connective tissue disease	1 (1.08%)	12 (1.50%)	0.75 (0.10-5.35)	0.770		
Peptic ulcer	4 (4.30%)	26 (3.24%)	1.31 (0.48-3.58)	0.597		
Hemiplegia	1 (1.08%)	0 (0%)	3.99 (0.55-28.87)	0.17		
Hypertension	25 (26.88%)	191 (23.82%)	1.22 (0.77-1.92)	0.405		
Tuberculosis	2 (2.15%)	11 (1.37%)	1.19 (0.29-4.85)	0.805		

COPD	10 (10.75%)	54 (6.73%)	1.44 (0.75-2.78)	0.275		
Respiratory failure	**44 (47.31%)**	**159 (19.83%)**	**2.70 (1.79-4.07)**	**<0.001**	**1.99 (1.29-3.06)**	**0.002**
Kidney failure	7 (7.53%)	35 (4.36%)	1.92 (0.89-4.15)	0.097		
Heart failure	3 (3.23%)	24 (2.99%)	1.52 (0.48-4.83)	0.475		
Diabetes	11 (11.83%)	112 (13.97%)	0.74 (0.40-1.39)	0.351		
Liver dysfunction	13 (13.98%)	29 (3.62%)	3.11 (1.73-5.61)	<0.001		
Hematological disease	49 (52.69%)	290 (36.16%)	1.91 (1.27-2.87)	0.002		
Pancreatitis	20 (21.51%)	92 (11.47%)	1.81 (1.10-2.97)	0.019		
Medical operation						
Surgery	70 (75.27%)	666 (83.04%)	0.90 (0.56-1.45)	0.658		
CVC	68 (73.12%)	434 (54.11%)	1.86 (1.17-2.94)	0.008		
Ventilator	85 (91.40%)	683 (85.16%)	1.93 (0.94-4.00)	0.075		
Indwelling catheter	91 (97.85%)	761 (94.89%)	2.34 (0.58-9.52)	0.235		
Tube feeding	67 (72.04%)	300 (37.41%)	2.72 (1.72-4.29)	<0.001		
Nebulizer fiberoptic	64 (68.82%)	388 (48.38%)	1.56 (1.00-2.42)	0.050		
Bronchoscope	5 (5.38%)	18 (2.24%)	2.00 (0.81-4.93)	0.133		
Antimicrobial use						
Cephalosporin	18 (19.35%)	213 (26.56%)	0.66 (0.40-1.11)	0.114		
Vancomycin	2 (2.15%)	33 (4.11%)	0.52 (0.13-2.12)	0.363		
Aminoglycosides	0 (0%)	16 (2.00%)	0.05 (0.00-14.57)	0.297		
Carbapenems	**68 (73.12%)**	**319 (39.78%)**	**3.50 (2.21-5.54)**	**<0.001**	**2.17 (1.30-3.63)**	**0.003**
Fluoroquinolones	28 (30.11%)	144 (17.96%)	1.49 (0.96-2.33)	0.079		
Antifungal agents	16 (17.20%)	141 (17.58%)	0.92 (0.53-1.57)	0.749		
Cephamycins	15 (16.13%)	187 (23.32%)	0.57 (0.33-1.00)	0.049		
Lincomycin	4 (4.30%)	34 (4.24%)	0.82 (0.30-2.24)	0.703		

Tigecycline	7 (7.53%)	57 (7.11%)	1.03 (0.47-2.22)	0.947

COPD, chronic obstructive pulmonary disease. CVC, central venous catheter.

Variables with P < 0.05 in the multivariate COX analysis are highlighted in bold.

Reporting checklist for cohort study.

Based on the STROBE cohort guidelines.

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Complete this checklist by entering the page numbers from your manuscript where readers will find each of the items listed below.

Your article may not currently address all the items on the checklist. Please modify your text to include the missing information. If you are certain that an item does not apply, please write "n/a" and provide a short explanation.

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		Reporting Item	Page Number
Title and abstract			
Title	#1a	Indicate the study's design with a commonly used term in the title or the abstract	2
Abstract	#1b	Provide in the abstract an informative and balanced summary of what was done and what	2

		was found	
Introduction			
Background / rationale	#2	Explain the scientific background and rationale for the investigation being reported	4-5
Objectives	#3	State specific objectives, including any prespecified hypotheses	5
Methods			
Study design	#4	Present key elements of study design early in the paper	7-8
Setting	#5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	5-6
Eligibility criteria	#6a	Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up.	6
Eligibility criteria	#6b	For matched studies, give matching criteria and number of exposed and unexposed	n/a Not matched studies
Variables	#7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	6-8
Data sources / measurement	#8	For each variable of interest give sources of data and details of methods of assessment	6-8

		(measurement). Describe comparability of assessment methods if there is more than one group. Give information separately for for exposed and unexposed groups if applicable.	
Bias	#9	Describe any efforts to address potential sources of bias	8
Study size	#10	Explain how the study size was arrived at	n/a Including all the patients admitted to the ICU in the study period.
Quantitative variables	#11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen, and why	7
Statistical methods	#12a	Describe all statistical methods, including those used to control for confounding	7-8
Statistical methods	#12b	Describe any methods used to examine subgroups and interactions	8
Statistical methods	#12c	Explain how missing data were addressed	n/a No missing data.
Statistical methods	#12d	If applicable, explain how loss to follow-up was addressed	n/a Not applicable

Statistical methods	#12e	Describe any sensitivity analyses	n/a Not done.
Results			
Participants	#13a	Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed. Give information separately for for exposed and unexposed groups if applicable.	9
Participants	#13b	Give reasons for non-participation at each stage	9
Participants	#13c	Consider use of a flow diagram	Figure 1
Descriptive data	#14a	Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders. Give information separately for exposed and unexposed groups if applicable.	19-23
Descriptive data	#14b	Indicate number of participants with missing data for each variable of interest	n/a No missing data.
Descriptive data	#14c	Summarise follow-up time (eg, average and total amount)	Figure 2
Outcome data	#15	Report numbers of outcome events or summary measures over time. Give information separately	19-23

		for exposed and unexposed groups if applicable.	
Main results	#16a	Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	19-23
Main results	#16b	Report category boundaries when continuous variables were categorized	n/a Continuous variables were not categorized.
Main results	#16c	If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	n/a Not applicable
Other analyses	#17	Report other analyses done—e.g., analyses of subgroups and interactions, and sensitivity analyses	Supplementary files
Discussion			
Key results	#18	Summarise key results with reference to study objectives	11-13
Limitations	#19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias.	13
Interpretation	#20	Give a cautious overall interpretation considering objectives, limitations, multiplicity of analyses,	11-13

		results from similar studies, and other relevant evidence.	
Generalisability	#21	Discuss the generalisability (external validity) of the study results	13
Other Information			
Funding	#22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	14

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Intestinal carriage of carbapenem-resistant Acinetobacter baumannii among patients in the intensive care unit: risk factors and the impact on subsequent infection

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Intestinal carriage of carbapenem-resistant *Acinetobacter baumannii* among patients in the intensive care unit: risk factors and the impact on subsequent infection

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Running title: intestinal carriage of CRAB in ICU

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Abstract

Objectives: To assess the incidence and the impact of carbapenem-resistant *Acinetobacter baumannii* (CRAB) intestinal carriage on subsequent CRAB infection and to study risk factors of acquiring CRAB intestinal carriage among patients in intensive care unit (ICU).

Design: Observational study including a case control study and a retrospective cohort study.

Setting: A 50-bed general ICU of a university hospital, China.

Methods: From May 2017 to April 2018, an observational study was conducted in a 50-bed general ICU of a university hospital in China. Rectal swabs were collected from ICU patients on admission and thereafter weekly. A case control study was performed to analyze risk factors of the acquisition of CRAB intestinal carriage in ICU using multiple logistic regression. A retrospective cohort study was performed to address whether intestinal CRAB carriage could lead to an increased likelihood of subsequent CRAB infection using sub-distribution hazard model regarding death in the ICU as a competing risk event.

Results: CRAB intestinal carriage was detected in 6.87% (66/961; 95% CI 5.27%–8.47%) of patients on ICU admission, whereas 11.97% (115/961; 95% CI 9.91%–14.02%) of patients acquired CRAB intestinal carriage during the ICU stay. Pancreatitis (OR 2.16, 95% CI 1.28–3.67), hematological disease (OR 2.26, 95% CI 1.42–3.58), gastric tube feeding (OR 3.35, 95% CI 2.03–5.51), and use of carbapenems (OR 1.84, 95% CI 1.11–3.07) were independent risk factors for acquiring CRAB intestinal carriage. The incidence of subsequent CRAB infection was 2.24-fold in patients with CRAB intestinal carriage compared to that in patients without (95% CI 1.48–3.39, P<0.001).

Conclusion: More patients acquired CRAB intestinal carriage during their ICU stay than had on admission. Severity of illness, acute pancreatitis, tube feeding, and use of carbapenems were

independent risk factors of acquisition of CRAB intestinal carriage. Patients with CRAB intestinal carriage are more likely to develop CRAB infection.

Strengths and limitations of this study

A case control study was performed to analyze risk factors of the acquisition of CRAB intestinal carriage in ICU.

A retrospective cohort study was performed to address whether intestinal CRAB carriage was associated with subsequent CRAB infection.

Most influencing factors were considered in the study.

Not all screened CRABs were confirmed using Vitek II or other methods.

Background

Acinetobacter baumannii is one of the most common nosocomial pathogens in Asia and South America[1]. A systematic review has revealed that *A. baumannii* accounted for 11.28% of nosocomial infections in general hospitals in China, making it the third most common nosocomial pathogen[2]. And carbapenem-resistant *A. baumannii* (CRAB) has emerged worldwide. As early as 2013, the US Centers for Disease Control and Prevention listed multi-drug resistant *A. baumannii* (MDRAB) including CRAB as a serious threat[3], and the World Health Organization listed CRAB as one of the three most critical threats in a global drug-resistant warning in 2017[4]. The prevalence of *A. baumannii* and its resistance to carbapenems varies from country to country. For instance, the European Bacterial Resistance Surveillance Report shows that the rate of *Acinetobacter* resistant to carbapenem in Europe in 2017 was 33.4% (95% CI 32%–35%), but it was as high as 96.2% in Croatia (95% CI 92%–98%) [5]. In the US, 49.5% of *A. baumannii* is resistant to carbapeems, while in Singapore, India, and Pakistan, it is 50%, 85%, and 62-100%, respectively[6,7]. The prevalence of CRAB is also very high in China. The surveillance data released by CHINET (China Antimicrobial Surveillance Network; http://chinets.com/Chinet), a national network in China, have shown that 77.1% and 78.1% of *A. baumannii* isolates resistant to imipenem and meropenem, respectively[8].

Infections caused by CRAB can lead to serious consequences. A previous study has demonstrated that patients with CRAB infection had longer average length of stay (LOS) in ICUs (13.1 vs. 10.5 days) and $11,359 higher average in-hospital costs than those with carbapenem-susceptible *A. baumannii* (CSAB) infection[9]. Another previous study has found that the mortality rate of patients with CRAB infection is 2.22-fold that of patients with CSAB infection[10]. A case-control study conducted by our team have also showed that the 28-day

survival rate of patients with bloodstream CRAB infection was 66.17%, lower than the 96.95% of those with bloodstream CSAB infection[11].

It is well known that *A. baumannii* including CRAB may colonized in the respiratory tract of hospitalized patients, in particular those with mechanical ventilation[12,13]. The colonization of CRAB in the respiratory tract has been found as a major risk factor for subsequent CRAB infection[14]. However, ICU patients may carry CRAB in intestine on admission or acquire CRAB during the ICU stay[15]. Patients with intestinal carriage of multi-drug resistant organisms (MDRO), in particular carbapenem-resistant *Enterobacteriaceae* (CRE), may sever as a reservoir for further dissemination in ICU[16] and could be associated with be associated with an increased risk of subsequent MDRO infections[17]. Therefore, active screening the carriage of CRE, which is usually performed using rectal swabs, has been recommended as a core component of the infection control bundle[7]. However, by contrast to CRE, the prevalence of CRAB intestinal carriage among ICU patients is much less studied and the risk factors of acquisition of CRAB intestinal carriage remains largely unknown. In addition, it remains to be determined whether CRAB intestinal carriage leads to increased risks of subsequent CRAB infection. To address these questions, we therefore conducted this study.

Methods

Study settings

An observational study was conducted in a 50-bed general ICU of a 4,300-bed university hospital in China. From May 2017 to April 2018, all patients admitted to the ICU were subjected to collecting a rectal swab within 48 h of admission and thereafter weekly. For patients hospitalized for less than 3 days, a rectal swab was collected only once within 48 h of admission.

Inclusion and exclusion criteria

Inclusion criteria: This study included all patients who were ≥ 18 years of age, admitted to the ICU, and underwent collection of rectal swabs.

Exclusion criteria: 1) patients who did not receive a rectal swab within 48 h of admission to ICU; or 2) patients who were eligible for weekly follow-up collection of rectal swabs but did not receive subsequent sampling; or 3) patients with CRAB infection on admission.

Definitions

Patients with CRAB intestinal carriage were defined as those with CRAB isolated from a rectal swab, while patient without CRAB intestinal carriage referred to those whose swabs were all negative for CRAB during the ICU stay. Patients with CRAB isolated from a rectal swab collected within 48 h of ICU admission were defined as those with CRAB intestinal carriage on ICU admission. The acquisition of CRAB intestinal carriage referred to a patient who had a CRAB-negative rectal swab collected within 48 h of ICU admission but had CRAB from a swab collected after 48 h. CRAB infection was defined as the growth of CRAB from clinical specimens in the presence of clinical manifestations of infection[18]. Subsequent CRAB infection referred to CRAB infection developed after the collection of a CRAB-positive rectal swab for patients with CRAB intestinal carriage and CRAB infection developed after 48 h admission to the ICU for patients without CRAB intestinal carriage.

Screening for CRAB by rectal swabs

For collecting rectal swabs, ready-to-use transport medium swabs (HBPT004; Hopebio

Biotechnology, Qingdao, China) was inserted about 2–3 cm into the patient's anus and then gently rotated. After sampling, the swab was inserted into the ready-to-use transport medium and transported to the laboratory within 2 h. Rectal swabs were inoculated onto modified CHROMagar *Acinetobacter* colorimetric plates (Chromagar; Paris, France) containing 2 mg/L meropenem using the partition-and-streaking method[19,20]. Plates were then cultured at 37°C for 18–24 h[20].

Data collection and statistical analysis

In this study, the patient's demographic data, underlying diseases, invasive procedures, medical orders, and use of antimicrobial agents were retrieved from the electronic medical record system. Two professional statisticians collaborated to clean the data.

We performed two types of comparison. First, a case control study was performed to analyze risk factors of the acquisition of CRAB intestinal carriage in ICU. Patients with ICU acquisition of CRAB intestinal carriage were assigned to the case group, while those without CRAB intestinal carriage during their ICU stay were assigned to the control group. All potential factors were initially subjected to the univariate analysis. Quantitative data were described by the median (interquartile range) and were then analyzed using a rank-sum test. Qualitative data were described by number of cases (composition ratio) and were then analyzed using the chi-square test or Fisher exact probability method when applied. All variables showing P value less than 0.2 in the univariate analysis were then included into the multiple logistic regression using the forward selection stepwise regression method[21,22]. Odds ratio (OR) and 95% confidence interval (CI) were calculated. The Hosmer-Lemeshow method was used to test the goodness-of-fit of the multiple logistic model[23].

Second, a retrospective cohort study was performed to address whether intestinal CRAB

carriage could lead to an increased likelihood of subsequent CRAB infection. In this cohort study, the exposed group comprised patients with CRAB intestinal carriage either detected on ICU admission or acquired during the ICU stay, while the non-exposed group consisted of those without CRAB intestinal carriage. As the impact of CRAB intestinal carriage on subsequent infection may also be influenced by other factors such as patient demographics, underlying diseases, antimicrobial use and medical operations, we included these factors for analysis instead of evaluating CRAB carriage alone. Survival curves (probability of CRAB infection) in patients with and without CRAB intestinal carriage were mapped using the Fine and Gray model regarding death in the ICU as a competing risk event [24,25]. After introducing the interaction term of time and each variable (X*ln (T)) into the COX model [24,25], the proportional hazards hypothesis was tested, and the results showed no statistical significance ($P < 0.05$). Therefore, sub-distribution hazard model was used to obtain sub-distribution hazard ratios (SDHRs) and to explore whether CRAB intestinal carriage was a risk factor for subsequent CRAB infection for competing events (R package “cmprsk”)The Akaike information criteria (AIC) was used to select the multivariate model[26]. We also performed subgroup analyses to investigate whether CRAB intestinal carriage on ICU admission and that acquired in ICU had different impact on subsequent CRAB infection using the same statistical method as describe above. For the subgroup analysis, patients with CRAB intestinal carriage on ICU admission and those with ICU acquisition of CRAB intestinal carriage were assigned to two exposed subgroups, respectively, while those without CRAB intestinal carriage were assigned to the non-exposed group.

All statistical analyses were performed using SPSS 21.0 (IBM–SPSS Inc; Armonk, NY, US) and R version 3.5.3 with a 0.05 two-sided test level.

Patient and public involvement

Patients were not involved in this study.

Results

Some patients (6.87%) had CRAB intestinal carriage on ICU admission and more (12.85%) acquired in ICU

From May 1, 2017 to April 30, 2018, a total of 1,605 patients were admitted to the ICU, of which 382 (23.8%) were not screened during their hospital stay. Of which the 382 patients, 323 (84.55%) stayed in the ICU for no more than 2 days, while the other 59 (15.45%) patients were missed for sampling. In addition, 118 patients (118/1,605, 7.4%) were excluded due to inappropriate or incomplete sampling including 104 patients whose first rectal swab was collected 48 h after admission and 14 patients who were not screened weekly. A total of 144 (144/1,605, 8.97%) had CRAB infection on ICU admission and were therefore also excluded. Taken together, a total of 961 patients (620 males, 64.52% and 341 female 35.48%) were included in the analysis, with an average age of 54 (44–68) years (Figure 1).

Among the 961 patients, 66 (6.87%, 95% CI 5.27%–8.47%) had CRAB intestinal carriage on ICU admission. For the remaining 895 patients, 115 acquired (12.85%, 95% CI 10.66%–15.04%) CRAB intestinal carriage during their ICU stay with an average age of 51 (40–70) and a 1.61 male/female ratio (71 male and 44 female).

Multiple risks factors of acquiring CRAB intestinal carriage were identified

The univariate analysis showed that APACHE II score (the patient's disease severity), respiratory failure, renal dysfunction, hematological disease, acute pancreatitis, indwelling central

venous catheter, gastric tube feeding, nebulization, and use of vancomycin, aminoglycosides, carbapenems, tigecycline, and antifungal agents are risk factors for the acquisition of CRAB intestinal carriage in the ICU. Multiple logistic regression including all variables with $P < 0.2$ in the univariate analysis showed that APACHE II score, pancreatitis, hematological diseases, gastric tube feeding, and use of carbapenems were independent risk factors for acquiring CRAB intestinal carriage during the ICU stay (Table 1). For APACHE II score, the model estimated that the increase of the score by 1 point would lead to a 4% increase of the risk of acquiring CRAB intestinal carriage in the ICU. Hosmer-Lemeshow test generated a 0.73 P value (χ^2=5.25, df=8), suggesting adequate goodness-of-fit of the multiple logistic model.

CRAB intestinal carriage led to increased risks of subsequent CRAB infection

During the study period, 112 of the 961 patients (11.65%, 95% CI 9.63%–13.68%) developed CRAB infections during the ICU stay. As for the infection type, lower respiratory tract infections were the most common (n=82, 73.21%), followed by bloodstream infections (n=9, 8.04%), surgical site infection (n=8, 7.14%), while 13 patients (11.61%) had infections at other sites. CRAB intestinal carriage was a risk factor for subsequent CRAB infection (HR 2.82, 95% CI 1.94–4.09; $P < 0.001$; Figure 2). The 90-day cumulative probability of no CRAB infection in patients with and without CRAB intestinal carriage was 69.5.0% (95% CI 43.5%–95.5%) and 22.3% (95% CI 14.7%–29.9%), respectively (P<0.001). In the univariate analysis, CRAB intestinal carriage, APACHE II score, respiratory failure, liver dysfunction, hematological disease, pancreatitis, mechanical ventilation, placement of a central venous catheter, gastric tube feeding, and the use of carbapenems were identified as risk factors for subsequent CRAB infection. In the COX multivariate analysis, CRAB intestinal carriage was also found to be an independent risk

factor for subsequent CRAB infection (HR 2.24, 95% CI 1.48–3.39; Table 2). Omnibus test showed a log likelihood difference of 79.82 and generated a less than 0.001 P value, suggesting adequate goodness-of-fit of the COX model.

To evaluate whether CRAB intestinal carriage on admission and that acquired during the ICU stay has different impact on subsequent CRAB, we performed subgroup analyses. In the subgroup COX multivariate analysis, both CRAB intestinal carriage on admission and that acquired during the ICU stay were an independent risk factor for subsequent CRAB infection (HR 3.42, 95% CI 1.88–6.22 for carriage on admission, Table S1 in the Supplementary file; HR 1.81, 95% CI 1.15–2.86 for acquired carriage, Table S2). Omnibus test showed log likelihood difference of 66.06 and 74.18, respectively, and generated a less than 0.001 P value in the subgroup analysis, suggesting adequate goodness-of-fit of the COX model.

In addition to CRAB intestinal carriage, liver dysfunction (HR 2.33, 95% CI 1.30–4.17), and the use of carbapenems (HR 2.21, 95% CI 1.40–3.49), were also identified as independent risk factors of subsequent CRAB infection, while the use of cephalosporins (HR 0.45, 95% CI 0.28–0.73) and cephamycins (HR 0.53, 95% CI 0.31–0.90) were protective factors (Table 2).

Discussion

In this study, we found that in a region with a high CRAB prevalence, 6.87% of patients (83.3% of those patients were transferred from other hospitals and 25.8% of them were stayed in emergency ICU before admitted to the ICU) admitted to the ICU had CRAB intestinal carriage on ICU admission, while an additional 11.97% of patients acquired CRAB intestinal carriage during the ICU stay. The overall CRAB intestinal carriage rate was therefore 18.84%. This rate was similar with a study conduct in Thailand, in which 5.45% (15/275) of patients had intestinal

carriage on ICU admission and 13.59% (28/206) patients acquired CRAB during their ICU stay[15] and with another study in Italy[27], in which 18.92%(74/391) of patients carried CRAB during ICU stay. However, the rate was significantly higher than those in Turkey (7.22%, 55/762)[28], Brazil (13.23%, 43/325)[29], USA (13.46%, 49/364)[30], and South Korea (15.06%, 168/1,115)[14], although other sites such as respiratory secretions were also screened in these studies. This difference may be related to the local CRAB prevalence.

Interestingly, we found that gastric tube feeding is a risk factor for both acquiring CRAB intestinal carriage of CRAB in ICU, which is consistent with the findings of Kiddee et al[15], in which tube feeding was also a high-risk factor for carriage of Gram-negative bacilli. This may suggest an entry point of CRAB into human intestine. In this study, 73.0% (84/115) of patients who acquired CRAB intestinal carriage using tube feeding. During the study, we performed a one-day snapshot sampling of the feeding tubes (at the tube port), feeding contents and containers for preparing feeding contents in the ICU and found the presence of CRAB in the tube feeding content (24.0%, 6/25), at the tube port (33.3%, 3/9) and the tube feeding containers (7.1%, 1/14), indicating contamination. This may be a key point for intervention in the ICU.

We also found that patients with CRAB intestinal carriage were more likely to develop subsequent CRAB infection than those without carriage. The survival curve in this study showed that the cumulative infection rates in 90 days in patients with and without CRAB intestinal carriage were 69.5% and 22.3%, respectively, similar to those reported in other studies[30]. However, the HR was 2.24, which is much lower than those in previous studies[15,30,31]. This may be due to the fact that healthcare associated infections in our ICU were mainly caused by lower respiratory infections, which accounting for more than 70% of infections, while we only screened the colonization of the intestines. Interestingly, we found that the use of cephalosporins and

cephamycins led to lower risks of subsequent CRAB infection, while carbapenem use led to increased risks. The association between CRAB and carbapenem use has been documented before[30,32]. CRAB is usually resistant to cephalosporins and cephamycins. The use of cephalosporins and cephamycins may reflect the fact that patients did not receive carbapenems and could therefore result in reduced selection pressure for CRAB.

There are a few limitations in this study. First, this is a single center study and the findings may not be generalized. Second, we used a modified CHROMagar *Acinetobacter* chromogenic plate to screen CRAB from rectal swabs. Not all screened CRABs were confirmed using Vitek II or other methods and there may be false negative results. Nonetheless, at the beginning of this study, we confirmed that the 58 CRAB strains grown on the chromogenic medium were indeed all *A. baumannii* by MALDI-TOF-MS and were all non-susceptible to imipenem or meropenem as determined using the agar dilution method recommended by the Clinical and Laboratory Standards Institute (CLSI)[20]. Third, we only collected the patients' rectal swabs for investigating CRAB carriage. Studies have shown concurrent swab collection of skin, oropharyngeal, and airway secretions in addition to rectal swabs, may improve sensitivity. However, the sample sizes in these studies were small with only 21 and 34 cases, respectively[12,33]. Nonetheless, for practical reasons and the aim to study CRAB intestinal carriage, we only collected rectal swabs. Fourth, 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. Last, this study failed to collect for the first rectal swab specimen within 48 h of ICU admission from 23.8% of the patients. Nonetheless, 84.55% of these patients stayed in the ICU for less than 48 h.

In conclusion, some patients had CRAB intestinal carriage but more acquired during their ICU stay. Severity of illness, acute pancreatitis, tube feeding, and use of carbapenems were independent risk factors of the acquisition of CRAB intestinal carriage. Patients with CRAB intestinal carriage were more likely to have subsequent CRAB infection than those without.

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Author contributions

Fu Qiao, Zhiyong Zong and Chuanmin Tao contributed to study conception and design. Shichao Zhu and Yan Kang contributed to acquisition of data. Lin Cai collected rectal swabs and transported to the laboratory. Fu Qiao, Wenzhi Huang and Shan Gao analyzed and interpreted data. Li Wei inoculated rectal swabs onto plates cultured for 18-24h. Fu Qiao, Zhiyong Zong and Chuanmin Tao drafted the manuscript. All authors revised the manuscript for important intellectual content. All authors read and approved the final manuscript.

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Competing interests

The authors declare that they have no competing interests.

Patient consent for publication

Not required.

Ethics approval

This project was approved by the Ethics Committee of West China Hospital of Sichuan University. 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.

Availability of data and materials

The datasets during the current study available from the corresponding author on reasonable request.

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Figure legends

Figure 1. Patient selection flow algorithm

Figure 2. Survival curves of patients with and without CRAB intestinal carriage

Table 1 Risk factors for the acquisition of CRAB intestinal carriage during the ICU stay

Characteristics	Patients with acquiring CRAB intestinal carriage		Univariate analysis		Multivariate analysis	
	Yes (n=115)	No (n=780)	OR (95% CI)	P	OR (95% CI)	*P*
Demographics						
Sex, male	71 (61.74%)	502 (64.36%)	1.12 (0.75–1.68)	0.59		
Ethnicity, Han Chinese	108 (93.91%)	712 (91.28%)	1.47 (0.66–3.29)	0.34		
Age (median)	51 (40–70)	56 (45–68)	/	0.21		
Underlying disease						
Myocardial infarction	1 (0.87%)	4 (0.51%)	1.7 (0.19–15.36)	0.50		
Peripheral vascular disease	11 (9.57%)	62 (7.95%)	1.22 (0.62–2.40)	0.55		
Cerebrovascular disease	4 (3.48%)	36 (4.62%)	0.74 (0.26–2.13)	0.58		
Dementia	1 (0.87%)	0 (0%)	/	0.13		
Connective tissue disease	1 (0.87%)	12 (1.54%)	0.56 (0.07–4.36)	0.89		
Peptic Ulcer	5 (4.35%)	25 (3.21%)	1.37 (0.51–3.66)	0.72		
Hemiplegia	0 (0%)	1 (0.13%)	/	1.00		
Hypertension	36 (31.30%)	180 (23.08%)	1.52 (0.99–2.33)	0.05		
Tuberculosis	1 (0.87%)	12 (1.54%)	0.56 (0.07–4.36)	0.89		
COPD	10 (8.70%)	54 (6.92%)	1.28 (0.63–2.59)	0.49		
Respiratory failure	40 (34.78%)	163 (20.90%)	2.02 (1.33–3.07)	**0.001**		

Kidney failure	11 (9.57%)	31 (3.97%)	2.56 (1.25–5.24)	**0.01**		
Heart failure	7 (6.09%)	19 (2.44%)	2.99 (1.28–7.01)	0.06		
Diabetes	21 (18.26%)	102 (13.08%)	1.48 (0.89–2.49)	0.13		
Liver dysfunction	5 (4.35%)	37 (4.74%)	0.91 (0.35–2.37)	0.85		
Hematological disease	**71 (61.74%)**	**268 (34.36%)**	**3.08 (2.06–4.62)**	**<0.001**	**2.26 (1.42–3.58)**	**0.001**
Pancreatitis	**35 (30.43%)**	**77 (9.87%)**	**3.99 (2.52–6.34)**	**<0.001**	**2.16 (1.28–3.67)**	**0.004**
Medical operation						
Surgery	**82 (71.30%)**	**645 (82.69%)**	**0.52 (0.33–0.81)**	**0.004**	**0.40 (0.24–0.68)**	**0.001**
CVC	78 (67.83%)	424 (54.36%)	1.77 (1.17–2.68)	**0.01**		
Ventilator	101 (87.83%)	666 (85.38%)	1.23 (0.68–2.23)	0.49		
Indwelling catheter	110 (95.65%)	742 (95.13%)	1.13 (0.43–2.92)	0.81		
Tube feeding	**84 (73.04%)**	**280 (35.90%)**	**4.84 (3.13–7.49)**	**<0.001**	**3.35 (2.03–5.51)**	**<0.001**
Nebulizer fiberoptic	73 (63.48%)	368 (47.18%)	1.95 (1.30–2.92)	**0.001**		
Bronchoscope	1 (0.87%)	21 (2.69%)	0.32 (0.04–2.38)	0.39		
Antimicrobial use						
Cephalosporin	**35 (30.43%)**	**312 (40.00%)**	**0.66 (0.43–1.00)**	**0.05**	**0.59 (0.37–0.95)**	**0.03**
Vancomycin	13 (11.30%)	32 (4.10%)	2.98 (1.51–5.86)	**0.001**		
Aminoglycosides	12 (10.43%)	31 (3.97%)	2.81 (1.40–5.65)	**0.002**		
Carbapenems	**82 (71.30%)**	**295 (37.82%)**	**4.09 (2.66–6.27)**	**<0.001**	**1.84 (1.11–3.07)**	**0.02**

Fluoroquinolones	26 (22.61%)	137 (17.56%)	1.37 (0.85–2.20)	0.19		
Antifungal agents	49 (42.61%)	138 (17.69%)	3.45 (2.29–5.22)	**<0.001**		
Cephamycins	16 (13.91%)	253 (32.44%)	0.34 (0.19–0.58)	**<0.001**		
Lincomycin	3 (2.61%)	61 (7.82%)	0.32 (0.10–1.02)	**0.04**		
Tigecycline	19 (16.52%)	69 (8.85%)	2.04 (1.18–3.54)	**0.01**		
APACHE II	**21.5 (17–26)**	**17 (12–22)**	/	**<0.001**	**1.04 (1.01–1.07)**	**0.01**
Charlson score	2 (1–5)	3 (2–4)	/	0.06		
Sharing room with other patients with CRAB intestinal carriage	20 (17.39%)	153 (19.62%)	0.86 (0.52–1.44)	0.57		

COPD, chronic obstructive pulmonary disease. CVC, central venous catheter.

Variables with $P < 0.05$ in the multiple logistic analysis are highlighted in bold.

Table 2 Variables associated with developing subsequent CRAB infection during the ICU stay using sub-distribution hazard model

Item	Subsequent CRAB infection during the ICU stay		Univariate analysis		Multivariate analysis	
	Yes (n=112)	No (n=849)	SDHR (95% CI)	P	SDHR (95% CI)	P
CRAB intestinal carriage	**51 (45.54%)**	**130 (15.31%)**	2.82(1.94-4.09)	<0.001	**2.24 (1.48–3.39)**	**<0.001**
Demographics						
Sex, male	72 (64.29%)	548 (64.55%)	1.03(0.70-1.52)	0.87		
Ethnicity, Han Chinese	106 (94.64%)	774 (91.38%)	1.62(0.70-3.74)	0.26		
Age (median)	53 (42–67)	55 (44–68)	1.00(0.99-1.01)	0.71		
APACHE II	21 (17–26)	17 (12–22)	1.05(1.03-1.07)	<0.001		
Charlson score	3 (1–5)	3 (1.5–4)	0.98(0.88-1.08)	0.66		
Underlying disease						
Peripheral vascular disease	13 (11.61%)	66 (7.77%)	1.30(0.72-2.34)	0.38		
Cerebrovascular disease	4 (3.57%)	36 (4.24%)	0.89(0.34-2.33)	0.81		
Connective tissue disease	1 (0.89%)	12 (1.41%)	0.60(0.08-4.48)	0.62		
Peptic ulcer	4 (3.57%)	27 (3.18%)	1.11(0.46-2.71)	0.81		
Hypertension	28 (25.00%)	199 (23.44%)	1.02(0.67-1.57)	0.92		
Tuberculosis	2 (1.79%)	12 (1.41%)	1.22(0.28-5.27)	0.79		
COPD	11 (9.82%)	55 (6.48%)	1.35(0.70-2.59)	0.37		
Respiratory failure	47 (41.96%)	170 (20.02%)	2.02(1.38-2.96)	<0.001		
Kidney failure	9 (8.04%)	42 (4.95%)	1.42(0.73-2.75)	0.30		
Heart failure	4 (3.57%)	27 (3.18%)	1.22(0.48-3.11)	0.68		
Diabetes	15 (13.39%)	118 (13.90%)	0.87(0.50-1.49)	0.61		
Liver dysfunction	**17 (15.18%)**	**34 (4.00%)**	**3.15(1.86-5.35)**	<0.001	**2.33 (1.30–4.17)**	**0.005**
Hematological disease	60 (53.57%)	314 (36.98%)	1.61(1.11-2.34)	0.012		
Pancreatitis	29 (25.89%)	107 (12.60%)	1.94(1.29-2.92)	0.002		
Medical operation						

Surgery	83 (74.11%)	711 (83.75%)	0.70(0.45-1.07)	0.099		
CVC	83 (74.11%)	470 (55.36%)	1.85(1.21-2.81)	0.004		
Ventilator	**103 (91.96%)**	**719 (84.69%)**	**2.02(1.04-3.93)**	0.038		
Indwelling catheter	109 (97.32%)	808 (95.17%)	1.84(0.62-5.52)	0.27		
Tube feeding	80 (71.43%)	332 (39.10%)	2.44(1.62-3.69)	<0.001		
Nebulizer fiberoptic	72 (64.29%)	413 (48.65%)	1.18(0.80-1.73)	0.40		
Bronchoscope	5 (4.46%)	18 (2.12%)	1.44(0.59-3.52)	0.43		
Antimicrobial use						
Cephalosporin	**20 (17.86%)**	**236 (27.80%)**	**0.50(0.31-0.81)**	0.005	**0.45 (0.28–0.73)**	**0.001**
Vancomycin	3 (2.68%)	35 (4.12%)	0.68(0.21-2.15)	0.51		
Aminoglycosides	1 (0.89%)	23 (2.71%)	0.24(0.03-1.71)	0.15		
Carbapenems	**82 (73.21%)**	**351 (41.34%)**	**2.84(1.87-4.32)**	<0.001	**2.21(1.40–3.49)**	**<0.001**
Fluoroquinolones	32 (28.57%)	154 (18.14%)	1.04(0.69-1.56)	0.84		
Antifungal agents	23 (20.54%)	157 (18.49%)	0.96(0.61-1.5)	0.85		
Cephamycins	**16 (14.29%)**	**196 (23.09%)**	**0.51(0.30-0.86)**	0.011	**0.53 (0.31–0.90)**	**0.018**
Lincomycin	5 (4.46%)	35 (4.12%)	1.01(0.41-2.48)	0.99		
Tigecycline	13 (11.61%)	65 (7.66%)	1.33(0.76-2.34)	032		

COPD, chronic obstructive pulmonary disease. CVC, central venous catheter.

Variables with $P < 0.05$ in the multivariate COX analysis are highlighted in bold.

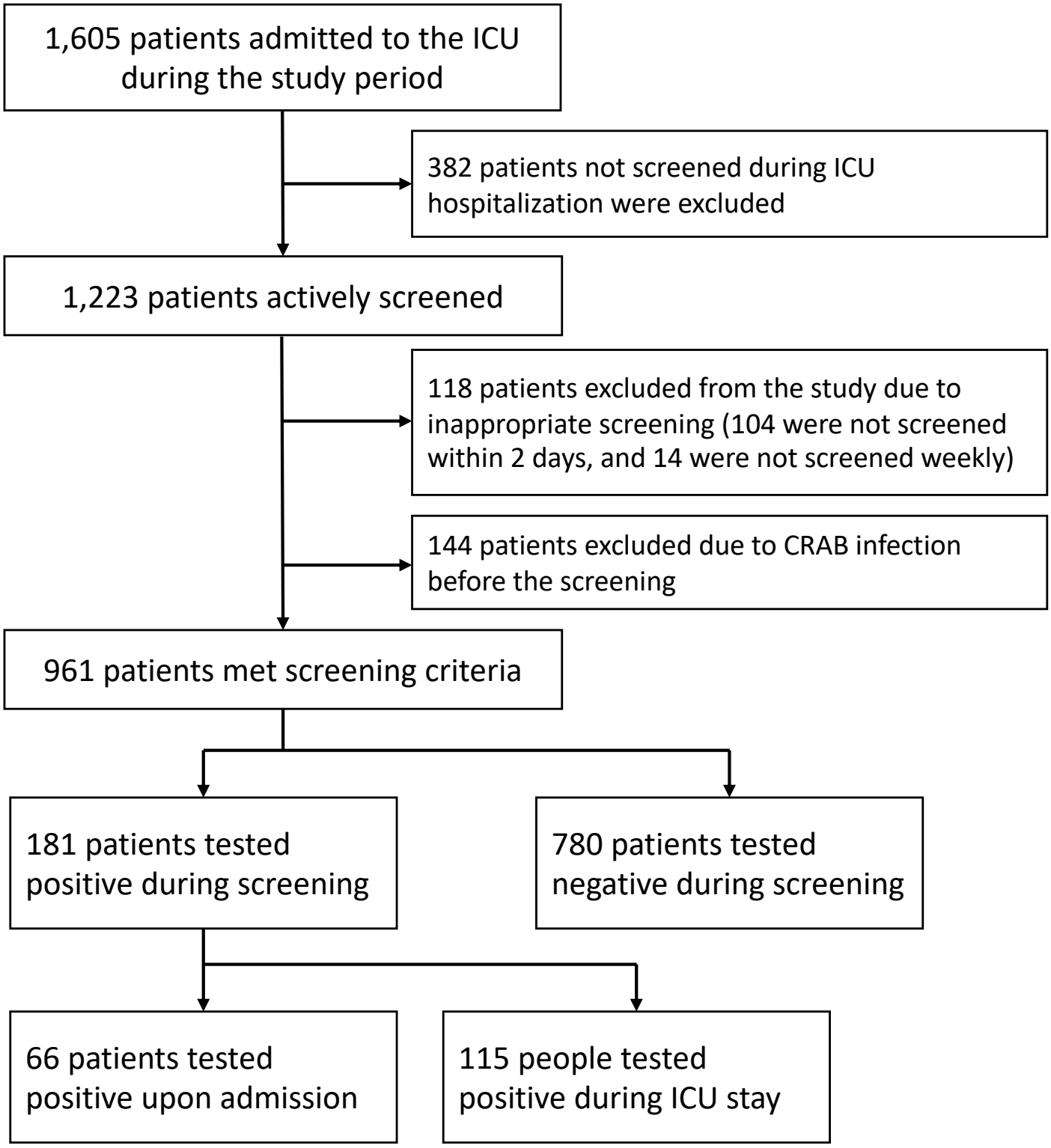

Figure 1. Patient selection flow algorithm

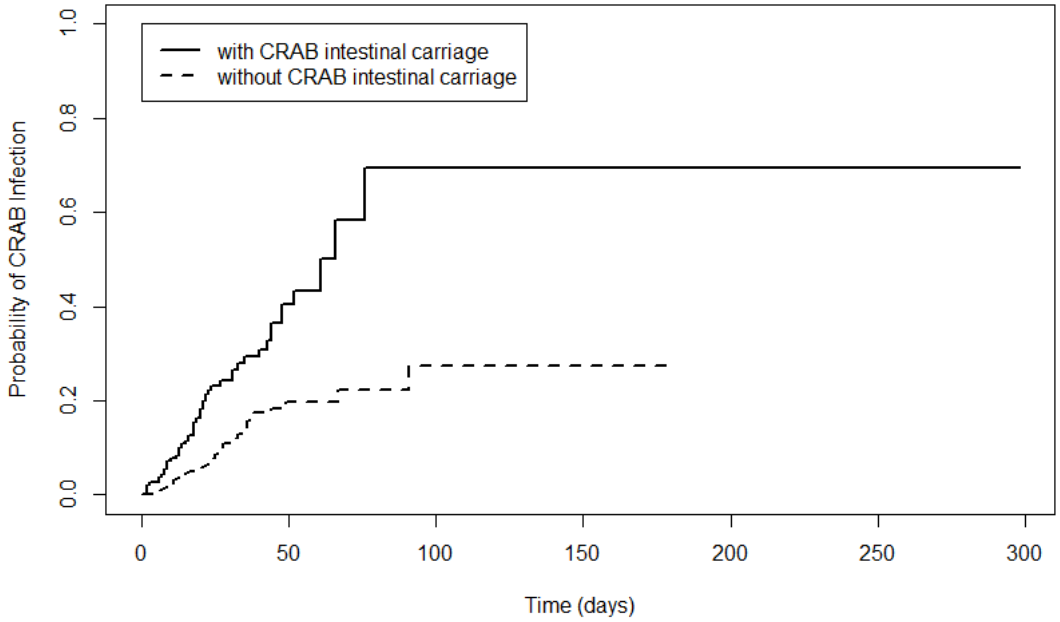

Figure 2. Survival curves of patients with and without CRAB intestinal carriage (cumulative probability of CRAB infection). Death in the ICU is considered as a competing event, not drawn in the figure. The solid line represents patients with CRAB intestinal carriage, while the dashed line represents those without. 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.

Supplementary files

Table S1 Variables associated with developing subsequent CRAB infection during the ICU stay using sub-distribution hazard model (exposed group was those patients with intestinal carriage on ICU admission)

Item	Subsequent CRAB infection during the ICU stay		Univariate analysis		Multivariate analysis	
	Yes (n=80)	No (n=766)	*SDHR (95% CI)*	*P*	*SDHR (95% CI)*	*P*
CRAB intestinal carriage	**19 (23.75%)**	**47 (6.14%)**	3.78(2.20-6.49)	**<0.001**	**3.42 (1.88-6.22)**	**<0.001**
Demographics						
Sex, male	50 (62.50%)	499 (65.14%)	1.15(0.73-1.80)	0.55		
Ethnicity, Han Chinese	77 (96.25%)	697 (90.99%)	2.54(0.78-8.24)	0.12		
Age (median)	54 (42-68)	55 (44-68)	1.00(0.99-1.02)	0.85		
APACHE II	**20 (15-26)**	**17 (12-22)**	**1.06(1.03-1.08)**	**<0.001**	**1.03 (1.00-1.05)**	**0.045**
Charlson score	3 (1-5)	3 (2-4)	1.00(0.88-1.13)	1.00		
Underlying disease						
Peripheral vascular disease	11 (13.75%)	57 (7.44%)	1.62(0.85-3.10)	0.14		
Cerebrovascular disease	3 (3.75%)	33 (4.31%)	0.87(0.29-2.66)	0.81		
Connective tissue disease	1 (1.25%)	11 (1.44%)	0.78(0.11-5.73)	0.80		
Peptic ulcer	3 (3.75%)	23 (3.00%)	1.19(0.41-3.46)	074		
Hypertension	18 (22.50%)	173 (22.58%)	0.97(0.58-1.64)	0.91		
Tuberculosis	1 (1.25%)	12 (1.57%)	0.72(0.10-5.37)	0.75		
COPD	**11 (13.75%)**	**45 (5.87%)**	**2.21(1.15-4.24)**	**0.017**	**2.71 (1.40-5.24)**	**0.003**
Respiratory failure	34 (42.50%)	143 (18.67%)	2.38(1.52-3.72)	**<0.001**		
Kidney failure	5 (6.25%)	35 (4.57%)	1.13(0.474-2.7)	0.78		
Heart failure	2 (2.50%)	21 (2.74%)	1.05(0.28-3.92)	0.95		

Diabetes	11 (13.75%)	101 (13.19%)	1.03(0.54-1.98)	0.92		
Liver dysfunction	**14 (17.50%)**	**32 (4.18%)**	**3.59(2.00-6.45)**	**<0.001**	**2.35 (1.30-4.25)**	**0.005**
Hematological disease	42 (52.50%)	261 (34.07%)	1.84(1.19-2.85)	**0.006**		
Pancreatitis	17 (21.25%)	84 (10.97%)	1.91(1.13-3.22)	**0.016**		
Medical operation						
Surgery	62 (77.50%)	638 (83.29%)	0.83(0.49-1.42)	0.50		
CVC	61 (76.25%)	414 (54.05%)	2.23(1.33-3.74)	**0.002**		
Ventilator	72 (90.00%)	648 (84.60%)	1.62(0.80-3.25)	0.18		
Indwelling catheter	78 (97.50%)	729 (95.17%)	1.91(0.53-6.94)	0.32		
Tube feeding	54 (67.50%)	265 (34.60%)	2.71(1.69-4.34)	**<0.001**		
Nebulizer fiberoptic	51 (63.75%)	349 (45.56%)	1.35(0.86-2.12)	0.19		
Bronchoscope	5 (6.25%)	17 (2.22%)	1.85(0.77-4.46)	0.17		
Antimicrobial use						
Cephalosporin	**15 (18.75%)**	**210 (27.42%)**	**0.54(0.31-0.95)**	**0.032**	**0.43(0.24-0.78)**	**0.006**
Vancomycin	2 (2.50%)	28 (3.66%)	0.70(0.16-2.98)	0.63		
Aminoglycosides	1 (1.25%)	15 (1.96%)	0.50(0.07-3.52)	0.48		
Carbapenems	**58 (72.50%)**	**284 (37.08%)**	**3.57(2.18-5.85)**	**<0.001**	**2.61(1.53-4.46)**	**<0.001**
Fluoroquinolones	21 (26.25%)	127 (16.58%)	1.07(0.66-1.74)	0.79		
Antifungal agents	17 (21.25%)	123 (16.06%)	1.26(0.74-2.12)	0.39		
Cephamycins	12 (15.00%)	179 (23.37%)	0.52(0.28-0.96)	**0.036**		
Lincomycin	3 (3.75%)	32 (4.18%)	0.84(0.26-2.68)	0.76		
Tigecycline	8 (10.00%)	53 (6.92%)	1.36(0.67-2.76)	0.39		

COPD, chronic obstructive pulmonary disease. CVC, central venous catheter.

Variables with $P < 0.05$ in the multivariate COX analysis are highlighted in bold.

Table S2. Variables associated with developing subsequent CRAB infection during the ICU stay using sub-distribution hazard model (exposed group was those patients with ICU acquisition of CRAB intestinal carriage)

Item	Subsequent CRAB infection during the ICU stay		Univariate analysis		Multivariate analysis	
	Yes (n=93)	No (n=802)	*SDHR (95% CI)*	*P*	*SDHR (95% CI)*	*P*
CRAB intestinal carriage	**32 (34.41%)**	**83 (10.35%)**	**2.35(1.56-3.55)**	**<0.001**	**1.81 (1.15-2.86)**	**0.011**
Demographics						
Sex, male	60 (64.52%)	513 (63.97%)	1.01(0.66-1.55)	0.95		
Ethnicity, Han Chinese	87 (93.55%)	731 (91.15%)	1.32(0.56-3.08)	0.52		
Age (median)	54 (44-68)	55 (45-68)	1.00(0.99-1.01)	0.70		
APACHE II	21 (18-26)	17 (12-22)	1.05(1.03-1.07)	<0.001		
Charlson score	3 (1-5)	3 (2-4)	1.01(0.91-1.13)	0.80		
Underlying disease						
Peripheral vascular disease	11 (11.83%)	62 (7.73%)	1.34(0.71-2.53)	0.37		
Cerebrovascular disease	4 (4.30%)	36 (4.49%)	1.03(0.39-2.73)	0.96		
Connective tissue disease	1 (1.08%)	12 (1.50%)	0.68(0.09-5.07)	0.70		
Peptic ulcer	4 (4.30%)	26 (3.24%)	1.39(0.58-3.33)	0.46		
Hypertension	25 (26.88%)	191 (23.82%)	1.10(0.69-1.74)	0.70		
Tuberculosis	2 (2.15%)	11 (1.37%)	1.41(0.32-6.29)	0.65		
COPD	10 (10.75%)	54 (6.73%)	1.37(0.69-2.75)	0.37		
Respiratory failure	**44 (47.31%)**	**159 (19.83%)**	**2.42(1.59-3.69)**	**<0.001**	**1.84 (1.17-2.90)**	**0.009**
Kidney failure	7 (7.53%)	35 (4.36%)	1.56(0.75-3.28)	0.24		

Heart failure	3 (3.23%)	24 (2.99%)	1.34(0.47-3.82)	0.58		
Diabetes	11 (11.83%)	112 (13.97%)	0.75(0.40-1.39)	0.36		
Liver dysfunction	**13 (13.98%)**	**29 (3.62%)**	**3.18(1.74-5.81)**	**<0.001**	**2.13(1.05-4.32)**	**0.037**
Hematological disease	49 (52.69%)	290 (36.16%)	1.60(1.06-2.40)	0.025		
Pancreatitis	20 (21.51%)	92 (11.47%)	1.71(1.06-2.76)	0.028		
Medical operation						
Surgery	70 (75.27%)	666 (83.04%)	0.78(0.49-1.27)	0.32		
CVC	68 (73.12%)	434 (54.11%)	1.82(1.15-2.88)	0.011		
Ventilator	85 (91.40%)	683 (85.16%)	1.83(0.89-3.76)	0.10		
Indwelling catheter	91 (97.85%)	761 (94.89%)	2.37(0.58-9.63)	0.23		
Tube feeding	67 (72.04%)	300 (37.41%)	2.64(1.67-4.18)	<0.001		
Nebulizer fiberoptic	64 (68.82%)	388 (48.38%)	1.42(0.92-2.20)	0.11		
Bronchoscope	5 (5.38%)	18 (2.24%)	1.60(0.64-3.99)	0.31		
Antimicrobial use						
Cephalosporin	**18 (19.35%)**	**213 (26.56%)**	**0.60(0.36-1.00)**	**0.051**	**0.59(0.35-1.00)**	**0.048**
Vancomycin	2 (2.15%)	33 (4.11%)	0.53(0.13-2.15)	0.38		
Carbapenems	**68 (73.12%)**	**319 (39.78%)**	**2.92(1.84-4.64)**	**<0.001**	**2.11 (1.27-3.50)**	**0.004**
Fluoroquinolones	28 (30.11%)	144 (17.96%)	1.11(0.71-1.72)	0.65		
Antifungal agents	16 (17.20%)	141 (17.58%)	0.82(0.48-1.39)	0.46		
Cephamycins	15 (16.13%)	187 (23.32%)	0.56(0.33-0.98)	0.042		
Lincomycin	4 (4.30%)	34 (4.24%)	0.90(0.34-2.44)	0.84		
Tigecycline	7 (7.53%)	57 (7.11%)	0.91(0.43-1.96)	0.82		

COPD, chronic obstructive pulmonary disease. CVC, central venous catheter.

Variables with $P < 0.05$ in the multivariate COX analysis are highlighted in bold.

Reporting checklist for cohort study.

Based on the STROBE cohort guidelines.

Instructions to authors

Complete this checklist by entering the page numbers from your manuscript where readers will find each of the items listed below.

Your article may not currently address all the items on the checklist. Please modify your text to include the missing information. If you are certain that an item does not apply, please write "n/a" and provide a short explanation.

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In your methods section, say that you used the STROBE cohortreporting guidelines, and cite them as:

von Elm E, Altman DG, Egger M, Pocock SJ, Gotzsche PC, Vandenbroucke JP. The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) Statement: guidelines for reporting observational studies.

		Reporting Item	Page Number
Title and abstract			
Title	#1a	Indicate the study's design with a commonly used term in the title or the abstract	2
Abstract	#1b	Provide in the abstract an informative and balanced summary of what was done and what	2

		was found	
Introduction			
Background / rationale	#2	Explain the scientific background and rationale for the investigation being reported	4-5
Objectives	#3	State specific objectives, including any prespecified hypotheses	5
Methods			
Study design	#4	Present key elements of study design early in the paper	7-8
Setting	#5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	5-6
Eligibility criteria	#6a	Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up.	6
Eligibility criteria	#6b	For matched studies, give matching criteria and number of exposed and unexposed	n/a Not matched studies
Variables	#7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	6-8
Data sources / measurement	#8	For each variable of interest give sources of data and details of methods of assessment	6-8

		(measurement). Describe comparability of assessment methods if there is more than one group. Give information separately for for exposed and unexposed groups if applicable.	
Bias	#9	Describe any efforts to address potential sources of bias	8
Study size	#10	Explain how the study size was arrived at	n/a Including all the patients admitted to the ICU in the study period.
Quantitative variables	#11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen, and why	7
Statistical methods	#12a	Describe all statistical methods, including those used to control for confounding	7-8
Statistical methods	#12b	Describe any methods used to examine subgroups and interactions	8
Statistical methods	#12c	Explain how missing data were addressed	n/a No missing data.
Statistical methods	#12d	If applicable, explain how loss to follow-up was addressed	n/a Not applicable

Statistical methods	#12e	Describe any sensitivity analyses	n/a Not done.
Results			
Participants	#13a	Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed. Give information separately for for exposed and unexposed groups if applicable.	9
Participants	#13b	Give reasons for non-participation at each stage	9
Participants	#13c	Consider use of a flow diagram	Figure 1
Descriptive data	#14a	Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders. Give information separately for exposed and unexposed groups if applicable.	19-23
Descriptive data	#14b	Indicate number of participants with missing data for each variable of interest	n/a No missing data.
Descriptive data	#14c	Summarise follow-up time (eg, average and total amount)	Figure 2
Outcome data	#15	Report numbers of outcome events or summary measures over time. Give information separately	19-23

		for exposed and unexposed groups if applicable.	
Main results	#16a	Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	19-23
Main results	#16b	Report category boundaries when continuous variables were categorized	n/a Continuous variables were not categorized.
Main results	#16c	If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	n/a Not applicable
Other analyses	#17	Report other analyses done—e.g., analyses of subgroups and interactions, and sensitivity analyses	Supplementary files
Discussion			
Key results	#18	Summarise key results with reference to study objectives	11-13
Limitations	#19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias.	13
Interpretation	#20	Give a cautious overall interpretation considering objectives, limitations, multiplicity of analyses,	11-13

		results from similar studies, and other relevant evidence.	
Generalisability	#21	Discuss the generalisability (external validity) of the study results	13
Other Information			
Funding	#22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	14

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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

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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

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Running title: intestinal carriage of CRAB in ICU

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Abstract

Objectives: To assess the incidence and the impact of carbapenem-resistant *Acinetobacter baumannii* (CRAB) intestinal carriage on subsequent CRAB infection and to study risk factors of acquiring CRAB intestinal carriage among patients in intensive care unit (ICU).

Design: Observational study including a case control study and a retrospective cohort study.

Setting: A 50-bed general ICU of a university hospital, China.

Methods: From May 2017 to April 2018, an observational study was conducted in a 50-bed general ICU of a university hospital in China. Rectal swabs were collected from ICU patients on admission and thereafter weekly. A case control study was performed to analyze risk factors of the acquisition of CRAB intestinal carriage in ICU using multiple logistic regression. A retrospective cohort study was performed to address whether intestinal CRAB carriage could lead to an increased likelihood of subsequent CRAB infection using sub-distribution hazard model regarding death in the ICU as a competing risk event.

Results: CRAB intestinal carriage was detected in 6.87% (66/961; 95% CI 5.27%–8.47%) of patients on ICU admission, whereas 11.97% (115/961; 95% CI 9.91%–14.02%) of patients acquired CRAB intestinal carriage during the ICU stay. Pancreatitis (OR 2.16, 95% CI 1.28–3.67), hematological disease (OR 2.26, 95% CI 1.42–3.58), gastric tube feeding (OR 3.35, 95% CI 2.03–5.51), and use of carbapenems (OR 1.84, 95% CI 1.11–3.07) were independent risk factors for acquiring CRAB intestinal carriage. The incidence of subsequent CRAB infection was 2.24-fold in patients with CRAB intestinal carriage compared to that in patients without (95% CI 1.48–3.39, P<0.001).

Conclusion: More patients acquired CRAB intestinal carriage during their ICU stay than had on admission. Severity of illness, acute pancreatitis, tube feeding, and use of carbapenems were

independent risk factors of acquisition of CRAB intestinal carriage. Patients with CRAB intestinal carriage are more likely to develop CRAB infection.

Strengths and limitations of this study

This observational study contains a combination of a case control study for analyzing risk factors of the acquisition of CRAB intestinal carriage in ICU and a retrospective cohort study to address whether intestinal CRAB carriage was associated with subsequent CRAB infection.

The competing risk of death in ICU was considered using a well-established model.

This is a single-unit study and the findings may not be generalized.

A culture-based method to screen CRAB, which is less sensitive than PCR-based methods.

Only rectal swabs were collected for screening CRAB and some CRAB carriers might have been missed.

Background

Acinetobacter baumannii is one of the most common nosocomial pathogens in Asia and South America[1]. A systematic review has revealed that *A. baumannii* accounted for 11.28% of nosocomial infections in general hospitals in China, making it the third most common nosocomial pathogen[2]. And carbapenem-resistant *A. baumannii* (CRAB) has emerged worldwide. As early as 2013, the US Centers for Disease Control and Prevention listed multi-drug resistant *A. baumannii* (MDRAB) including CRAB as a serious threat[3], and the World Health Organization listed CRAB as one of the three most critical threats in a global drug-resistant warning in 2017[4]. The prevalence of *A. baumannii* and its resistance to carbapenems varies from country to country. For instance, the European Bacterial Resistance Surveillance Report shows that the rate of *Acinetobacter* resistant to carbapenem in Europe in 2017 was 33.4% (95% CI 32%–35%), but it was as high as 96.2% in Croatia (95% CI 92%–98%) [5]. In the US, 49.5% of *A. baumannii* is resistant to carbapeems, while in Singapore, India, and Pakistan, it is 50%, 85%, and 62-100%, respectively[6,7]. The prevalence of CRAB is also very high in China. The surveillance data released by CHINET (China Antimicrobial Surveillance Network; http://chinets.com/Chinet), a national network in China, have shown that 77.1% and 78.1% of *A. baumannii* isolates resistant to imipenem and meropenem, respectively[8].

Infections caused by CRAB can lead to serious consequences. A previous study has demonstrated that patients with CRAB infection had longer average length of stay (LOS) in ICUs (13.1 vs. 10.5 days) and $11,359 higher average in-hospital costs than those with carbapenem-susceptible *A. baumannii* (CSAB) infection[9]. Another previous study has found that the mortality rate of patients with CRAB infection is 2.22-fold that of patients with CSAB infection[10]. A case-control study conducted by our team have also showed that the 28-day

survival rate of patients with bloodstream CRAB infection was 66.17%, lower than the 96.95% of those with bloodstream CSAB infection[11].

It is well known that *A. baumannii* including CRAB may colonized in the respiratory tract of hospitalized patients, in particular those with mechanical ventilation[12,13]. The colonization of CRAB in the respiratory tract has been found as a major risk factor for subsequent CRAB infection[14]. However, ICU patients may carry CRAB in intestine on admission or acquire CRAB during the ICU stay[15]. Patients with intestinal carriage of multi-drug resistant organisms (MDRO), in particular carbapenem-resistant *Enterobacteriaceae* (CRE), may sever as a reservoir for further dissemination in ICU[16] and could be associated with be associated with an increased risk of subsequent MDRO infections[17]. Therefore, active screening the carriage of CRE, which is usually performed using rectal swabs, has been recommended as a core component of the infection control bundle[7]. However, by contrast to CRE, the prevalence of CRAB intestinal carriage among ICU patients is much less studied and the risk factors of acquisition of CRAB intestinal carriage remains largely unknown. In addition, it remains to be determined whether CRAB intestinal carriage leads to increased risks of subsequent CRAB infection. To address these questions, we therefore conducted this study.

Methods

Study settings

An observational study was conducted in a 50-bed general ICU of a 4,300-bed university hospital in China. From May 2017 to April 2018, all patients admitted to the ICU were subjected to collecting a rectal swab within 48 h of admission and thereafter weekly. For patients hospitalized for less than 3 days, a rectal swab was collected only once within 48 h of admission.

Inclusion and exclusion criteria

Inclusion criteria: This study included all patients who were ≥ 18 years of age, admitted to the ICU, and underwent collection of rectal swabs.

Exclusion criteria: 1) patients who did not receive a rectal swab within 48 h of admission to ICU; or 2) patients who were eligible for weekly follow-up collection of rectal swabs but did not receive subsequent sampling; or 3) patients with CRAB infection on admission.

Definitions

Patients with CRAB intestinal carriage were defined as those with CRAB isolated from a rectal swab, while patient without CRAB intestinal carriage referred to those whose swabs were all negative for CRAB during the ICU stay. Patients with CRAB isolated from a rectal swab collected within 48 h of ICU admission were defined as those with CRAB intestinal carriage on ICU admission. The acquisition of CRAB intestinal carriage referred to a patient who had a CRAB-negative rectal swab collected within 48 h of ICU admission but had CRAB from a swab collected after 48 h. CRAB infection was defined as the growth of CRAB from clinical specimens in the presence of clinical manifestations of infection[18]. Subsequent CRAB infection referred to CRAB infection developed after the collection of a CRAB-positive rectal swab for patients with CRAB intestinal carriage and CRAB infection developed after 48 h admission to the ICU for patients without CRAB intestinal carriage.

Screening for CRAB by rectal swabs

For collecting rectal swabs, ready-to-use transport medium swabs (HBPT004; Hopebio

Biotechnology, Qingdao, China) was inserted about 2–3 cm into the patient's anus and then gently rotated. After sampling, the swab was inserted into the ready-to-use transport medium and transported to the laboratory within 2 h. Rectal swabs were inoculated onto modified CHROMagar *Acinetobacter* colorimetric plates (Chromagar; Paris, France) containing 2 mg/L meropenem using the partition-and-streaking method[19,20]. Plates were then cultured at 37°C for 18–24 h[20].

Data collection and statistical analysis

In this study, the patient's demographic data, underlying diseases, invasive procedures, medical orders, and use of antimicrobial agents were retrieved from the electronic medical record system. Two professional statisticians collaborated to clean the data.

We performed two types of comparison. First, a case control study was performed to analyze risk factors of the acquisition of CRAB intestinal carriage in ICU. Patients with ICU acquisition of CRAB intestinal carriage were assigned to the case group, while those without CRAB intestinal carriage during their ICU stay were assigned to the control group. All potential factors were initially subjected to the univariate analysis. Quantitative data were described by the median (interquartile range) and were then analyzed using a rank-sum test. Qualitative data were described by number of cases (composition ratio) and were then analyzed using the chi-square test or Fisher exact probability method when applied. All variables showing P value less than 0.2 in the univariate analysis were then included into the multiple logistic regression using the forward selection stepwise regression method[21,22]. Odds ratio (OR) and 95% confidence interval (CI) were calculated. The Hosmer-Lemeshow method was used to test the goodness-of-fit of the multiple logistic model[23].

Second, a retrospective cohort study was performed to address whether intestinal CRAB

carriage could lead to an increased likelihood of subsequent CRAB infection. In this cohort study, the exposed group comprised patients with CRAB intestinal carriage either detected on ICU admission or acquired during the ICU stay, while the non-exposed group consisted of those without CRAB intestinal carriage. As the impact of CRAB intestinal carriage on subsequent infection may also be influenced by other factors such as patient demographics, underlying diseases, antimicrobial use and medical operations, we included these factors for analysis instead of evaluating CRAB carriage alone. Survival curves (probability of CRAB infection) in patients with and without CRAB intestinal carriage were mapped using the Fine and Gray model regarding death in the ICU as a competing risk event [24,25]. After introducing the interaction term of time and each variable (X*ln (T)) into the COX model [24,25], the proportional hazards hypothesis was tested, and the results showed no statistical significance ($P < 0.05$). Therefore, sub-distribution hazard model was used to obtain sub-distribution hazard ratios (SDHRs) and to explore whether CRAB intestinal carriage was a risk factor for subsequent CRAB infection for competing events (R package “cmprsk”)The Akaike information criteria (AIC) was used to select the multivariate model[26]. We also performed subgroup analyses to investigate whether CRAB intestinal carriage on ICU admission and that acquired in ICU had different impact on subsequent CRAB infection using the same statistical method as describe above. For the subgroup analysis, patients with CRAB intestinal carriage on ICU admission and those with ICU acquisition of CRAB intestinal carriage were assigned to two exposed subgroups, respectively, while those without CRAB intestinal carriage were assigned to the non-exposed group.

All statistical analyses were performed using SPSS 21.0 (IBM–SPSS Inc; Armonk, NY, US) and R version 3.5.3 with a 0.05 two-sided test level.

Patient and public involvement

Patients were not involved in this study.

Results

Some patients (6.87%) had CRAB intestinal carriage on ICU admission and more (12.85%) acquired in ICU

From May 1, 2017 to April 30, 2018, a total of 1,605 patients were admitted to the ICU, of which 382 (23.8%) were not screened during their hospital stay. Of which the 382 patients, 323 (84.55%) stayed in the ICU for no more than 2 days, while the other 59 (15.45%) patients were missed for sampling. In addition, 118 patients (118/1,605, 7.4%) were excluded due to inappropriate or incomplete sampling including 104 patients whose first rectal swab was collected 48 h after admission and 14 patients who were not screened weekly. A total of 144 (144/1,605, 8.97%) had CRAB infection on ICU admission and were therefore also excluded. Taken together, a total of 961 patients (620 males, 64.52% and 341 female 35.48%) were included in the analysis, with an average age of 54 (44–68) years (Figure 1).

Among the 961 patients, 66 (6.87%, 95% CI 5.27%–8.47%) had CRAB intestinal carriage on ICU admission. For the remaining 895 patients, 115 acquired (12.85%, 95% CI 10.66%–15.04%) CRAB intestinal carriage during their ICU stay with an average age of 51 (40–70) and a 1.61 male/female ratio (71 male and 44 female).

Multiple risks factors of acquiring CRAB intestinal carriage were identified

The univariate analysis showed that APACHE II score (the patient's disease severity), respiratory failure, renal dysfunction, hematological disease, acute pancreatitis, indwelling central

venous catheter, gastric tube feeding, nebulization, and use of vancomycin, aminoglycosides, carbapenems, tigecycline, and antifungal agents are risk factors for the acquisition of CRAB intestinal carriage in the ICU. Multiple logistic regression including all variables with $P < 0.2$ in the univariate analysis showed that APACHE II score, pancreatitis, hematological diseases, gastric tube feeding, and use of carbapenems were independent risk factors for acquiring CRAB intestinal carriage during the ICU stay (Table 1). For APACHE II score, the model estimated that the increase of the score by 1 point would lead to a 4% increase of the risk of acquiring CRAB intestinal carriage in the ICU. Hosmer-Lemeshow test generated a 0.73 P value (χ^2=5.25, df=8), suggesting adequate goodness-of-fit of the multiple logistic model.

CRAB intestinal carriage led to increased risks of subsequent CRAB infection

During the study period, 112 of the 961 patients (11.65%, 95% CI 9.63%–13.68%) developed CRAB infections during the ICU stay. As for the infection type, lower respiratory tract infections were the most common (n=82, 73.21%), followed by bloodstream infections (n=9, 8.04%), surgical site infection (n=8, 7.14%), while 13 patients (11.61%) had infections at other sites. CRAB intestinal carriage was a risk factor for subsequent CRAB infection (HR 2.82, 95% CI 1.94–4.09; $P < 0.001$; Figure 2). The 90-day cumulative probability of no CRAB infection in patients with and without CRAB intestinal carriage was 69.5.0% (95% CI 43.5%–95.5%) and 22.3% (95% CI 14.7%–29.9%), respectively (P<0.001). In the univariate analysis, CRAB intestinal carriage, APACHE II score, respiratory failure, liver dysfunction, hematological disease, pancreatitis, mechanical ventilation, placement of a central venous catheter, gastric tube feeding, and the use of carbapenems were identified as risk factors for subsequent CRAB infection. In the COX multivariate analysis, CRAB intestinal carriage was also found to be an independent risk

factor for subsequent CRAB infection (HR 2.24, 95% CI 1.48–3.39; Table 2). Omnibus test showed a log likelihood difference of 79.82 and generated a less than 0.001 P value, suggesting adequate goodness-of-fit of the COX model.

To evaluate whether CRAB intestinal carriage on admission and that acquired during the ICU stay has different impact on subsequent CRAB, we performed subgroup analyses. In the subgroup COX multivariate analysis, both CRAB intestinal carriage on admission and that acquired during the ICU stay were an independent risk factor for subsequent CRAB infection (HR 3.42, 95% CI 1.88–6.22 for carriage on admission, Table S1 in the Supplementary file; HR 1.81, 95% CI 1.15–2.86 for acquired carriage, Table S2). Omnibus test showed log likelihood difference of 66.06 and 74.18, respectively, and generated a less than 0.001 P value in the subgroup analysis, suggesting adequate goodness-of-fit of the COX model.

In addition to CRAB intestinal carriage, liver dysfunction (HR 2.33, 95% CI 1.30–4.17), and the use of carbapenems (HR 2.21, 95% CI 1.40–3.49), were also identified as independent risk factors of subsequent CRAB infection, while the use of cephalosporins (HR 0.45, 95% CI 0.28–0.73) and cephamycins (HR 0.53, 95% CI 0.31–0.90) were protective factors (Table 2).

Discussion

In this study, we found that in a region with a high CRAB prevalence, 6.87% of patients (83.3% of those patients were transferred from other hospitals and 25.8% of them were stayed in emergency ICU before admitted to the ICU) admitted to the ICU had CRAB intestinal carriage on ICU admission, while an additional 11.97% of patients acquired CRAB intestinal carriage during the ICU stay. The overall CRAB intestinal carriage rate was therefore 18.84%. This rate was similar with a study conduct in Thailand, in which 5.45% (15/275) of patients had intestinal

carriage on ICU admission and 13.59% (28/206) patients acquired CRAB during their ICU stay[15] and with another study in Italy[27], in which 18.92%(74/391) of patients carried CRAB during ICU stay. However, the rate was significantly higher than those in Turkey (7.22%, 55/762)[28], Brazil (13.23%, 43/325)[29], USA (13.46%, 49/364)[30], and South Korea (15.06%, 168/1,115)[14], although other sites such as respiratory secretions were also screened in these studies. This difference may be related to the local CRAB prevalence.

Interestingly, we found that gastric tube feeding is a risk factor for both acquiring CRAB intestinal carriage of CRAB in ICU, which is consistent with the findings of Kiddee et al[15], in which tube feeding was also a high-risk factor for carriage of Gram-negative bacilli. This may suggest an entry point of CRAB into human intestine. In this study, 73.0% (84/115) of patients who acquired CRAB intestinal carriage using tube feeding. During the study, we performed a one-day snapshot sampling of the feeding tubes (at the tube port), feeding contents and containers for preparing feeding contents in the ICU and found the presence of CRAB in the tube feeding content (24.0%, 6/25), at the tube port (33.3%, 3/9) and the tube feeding containers (7.1%, 1/14), indicating contamination. This may be a key point for intervention in the ICU.

We also found that patients with CRAB intestinal carriage were more likely to develop subsequent CRAB infection than those without carriage. The survival curve in this study showed that the cumulative infection rates in 90 days in patients with and without CRAB intestinal carriage were 69.5% and 22.3%, respectively, similar to those reported in other studies[30]. However, the HR was 2.24, which is much lower than those in previous studies[15,30,31]. This may be due to the fact that healthcare associated infections in our ICU were mainly caused by lower respiratory infections, which accounting for more than 70% of infections, while we only screened the colonization of the intestines. Interestingly, we found that the use of cephalosporins and

cephamycins led to lower risks of subsequent CRAB infection, while carbapenem use led to increased risks. The association between CRAB and carbapenem use has been documented before[30,32]. CRAB is usually resistant to cephalosporins and cephamycins. The use of cephalosporins and cephamycins may reflect the fact that patients did not receive carbapenems and could therefore result in reduced selection pressure for CRAB.

There are a few limitations in this study. First, this is a single center study and the findings may not be generalized. Second, we used a modified CHROMagar *Acinetobacter* chromogenic plate to screen CRAB from rectal swabs. Not all screened CRABs were confirmed using Vitek II or other methods and there may be false negative results. Nonetheless, at the beginning of this study, we confirmed that the 58 CRAB strains grown on the chromogenic medium were indeed all *A. baumannii* by MALDI-TOF-MS and were all non-susceptible to imipenem or meropenem as determined using the agar dilution method recommended by the Clinical and Laboratory Standards Institute (CLSI)[20]. Third, we only collected the patients' rectal swabs for investigating CRAB carriage. Studies have shown concurrent swab collection of skin, oropharyngeal, and airway secretions in addition to rectal swabs, may improve sensitivity. However, the sample sizes in these studies were small with only 21 and 34 cases, respectively[12,33]. Nonetheless, for practical reasons and the aim to study CRAB intestinal carriage, we only collected rectal swabs. Fourth, 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. Last, this study failed to collect for the first rectal swab specimen within 48 h of ICU admission from 23.8% of the patients. Nonetheless, 84.55% of these patients stayed in the ICU for less than 48 h.

In conclusion, some patients had CRAB intestinal carriage but more acquired during their ICU stay. Severity of illness, acute pancreatitis, tube feeding, and use of carbapenems were independent risk factors of the acquisition of CRAB intestinal carriage. Patients with CRAB intestinal carriage were more likely to have subsequent CRAB infection than those without.

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Author contributions

Fu Qiao, Zhiyong Zong and Chuanmin Tao contributed to study conception and design. Shichao Zhu and Yan Kang contributed to acquisition of data. Lin Cai collected rectal swabs and transported to the laboratory. Fu Qiao, Wenzhi Huang and Shan Gao analyzed and interpreted data. Li Wei inoculated rectal swabs onto plates cultured for 18-24h. Fu Qiao, Zhiyong Zong and Chuanmin Tao drafted the manuscript. All authors revised the manuscript for important intellectual content. All authors read and approved the final manuscript.

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Competing interests

The authors declare that they have no competing interests.

Patient consent for publication

Not required.

Ethics approval

This project was approved by the Ethics Committee of West China Hospital of Sichuan University. 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.

Availability of data and materials

The datasets during the current study available from the corresponding author on reasonable request.

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Figure legends

Figure 1. Patient selection flow algorithm

Figure 2. Survival curves of patients with and without CRAB intestinal carriage

Table 1 Risk factors for the acquisition of CRAB intestinal carriage during the ICU stay

Characteristics	Patients with acquiring CRAB intestinal carriage		Univariate analysis		Multivariate analysis	
	Yes (n=115)	No (n=780)	OR (95% CI)	P	OR (95% CI)	*P*
Demographics						
Sex, male	71 (61.74%)	502 (64.36%)	1.12 (0.75–1.68)	0.59		
Ethnicity, Han Chinese	108 (93.91%)	712 (91.28%)	1.47 (0.66–3.29)	0.34		
Age (median)	51 (40–70)	56 (45–68)	/	0.21		
Underlying disease						
Myocardial infarction	1 (0.87%)	4 (0.51%)	1.7 (0.19–15.36)	0.50		
Peripheral vascular disease	11 (9.57%)	62 (7.95%)	1.22 (0.62–2.40)	0.55		
Cerebrovascular disease	4 (3.48%)	36 (4.62%)	0.74 (0.26–2.13)	0.58		
Dementia	1 (0.87%)	0 (0%)	/	0.13		
Connective tissue disease	1 (0.87%)	12 (1.54%)	0.56 (0.07–4.36)	0.89		
Peptic Ulcer	5 (4.35%)	25 (3.21%)	1.37 (0.51–3.66)	0.72		
Hemiplegia	0 (0%)	1 (0.13%)	/	1.00		
Hypertension	36 (31.30%)	180 (23.08%)	1.52 (0.99–2.33)	0.05		
Tuberculosis	1 (0.87%)	12 (1.54%)	0.56 (0.07–4.36)	0.89		
COPD	10 (8.70%)	54 (6.92%)	1.28 (0.63–2.59)	0.49		
Respiratory failure	40 (34.78%)	163 (20.90%)	2.02 (1.33–3.07)	**0.001**		

Kidney failure	11 (9.57%)	31 (3.97%)	2.56 (1.25–5.24)	**0.01**		
Heart failure	7 (6.09%)	19 (2.44%)	2.99 (1.28–7.01)	0.06		
Diabetes	21 (18.26%)	102 (13.08%)	1.48 (0.89–2.49)	0.13		
Liver dysfunction	5 (4.35%)	37 (4.74%)	0.91 (0.35–2.37)	0.85		
Hematological disease	**71 (61.74%)**	**268 (34.36%)**	**3.08 (2.06–4.62)**	**<0.001**	**2.26 (1.42–3.58)**	**0.001**
Pancreatitis	**35 (30.43%)**	**77 (9.87%)**	**3.99 (2.52–6.34)**	**<0.001**	**2.16 (1.28–3.67)**	**0.004**
Medical operation						
Surgery	**82 (71.30%)**	**645 (82.69%)**	**0.52 (0.33–0.81)**	**0.004**	**0.40 (0.24–0.68)**	**0.001**
CVC	78 (67.83%)	424 (54.36%)	1.77 (1.17–2.68)	**0.01**		
Ventilator	101 (87.83%)	666 (85.38%)	1.23 (0.68–2.23)	0.49		
Indwelling catheter	110 (95.65%)	742 (95.13%)	1.13 (0.43–2.92)	0.81		
Tube feeding	**84 (73.04%)**	**280 (35.90%)**	**4.84 (3.13–7.49)**	**<0.001**	**3.35 (2.03–5.51)**	**<0.001**
Nebulizer fiberoptic	73 (63.48%)	368 (47.18%)	1.95 (1.30–2.92)	**0.001**		
Bronchoscope	1 (0.87%)	21 (2.69%)	0.32 (0.04–2.38)	0.39		
Antimicrobial use						
Cephalosporin	**35 (30.43%)**	**312 (40.00%)**	**0.66 (0.43–1.00)**	**0.05**	**0.59 (0.37–0.95)**	**0.03**
Vancomycin	13 (11.30%)	32 (4.10%)	2.98 (1.51–5.86)	**0.001**		
Aminoglycosides	12 (10.43%)	31 (3.97%)	2.81 (1.40–5.65)	**0.002**		
Carbapenems	**82 (71.30%)**	**295 (37.82%)**	**4.09 (2.66–6.27)**	**<0.001**	**1.84 (1.11–3.07)**	**0.02**

Fluoroquinolones	26 (22.61%)	137 (17.56%)	1.37 (0.85–2.20)	0.19		
Antifungal agents	49 (42.61%)	138 (17.69%)	3.45 (2.29–5.22)	**<0.001**		
Cephamycins	16 (13.91%)	253 (32.44%)	0.34 (0.19–0.58)	**<0.001**		
Lincomycin	3 (2.61%)	61 (7.82%)	0.32 (0.10–1.02)	**0.04**		
Tigecycline	19 (16.52%)	69 (8.85%)	2.04 (1.18–3.54)	**0.01**		
APACHE II	**21.5 (17–26)**	**17 (12–22)**	/	**<0.001**	**1.04 (1.01–1.07)**	**0.01**
Charlson score	2 (1–5)	3 (2–4)	/	0.06		
Sharing room with other patients with CRAB intestinal carriage	20 (17.39%)	153 (19.62%)	0.86 (0.52–1.44)	0.57		

COPD, chronic obstructive pulmonary disease. CVC, central venous catheter.

Variables with $P < 0.05$ in the multiple logistic analysis are highlighted in bold.

Table 2 Variables associated with developing subsequent CRAB infection during the ICU stay using sub-distribution hazard model

Item	Subsequent CRAB infection during the ICU stay		Univariate analysis		Multivariate analysis	
	Yes (n=112)	No (n=849)	SDHR (95% CI)	P	SDHR (95% CI)	P
CRAB intestinal carriage	**51 (45.54%)**	**130 (15.31%)**	2.82(1.94-4.09)	<0.001	**2.24 (1.48–3.39)**	**<0.001**
Demographics						
Sex, male	72 (64.29%)	548 (64.55%)	1.03(0.70-1.52)	0.87		
Ethnicity, Han Chinese	106 (94.64%)	774 (91.38%)	1.62(0.70-3.74)	0.26		
Age (median)	53 (42–67)	55 (44–68)	1.00(0.99-1.01)	0.71		
APACHE II	21 (17–26)	17 (12–22)	1.05(1.03-1.07)	<0.001		
Charlson score	3 (1–5)	3 (1.5–4)	0.98(0.88-1.08)	0.66		
Underlying disease						
Peripheral vascular disease	13 (11.61%)	66 (7.77%)	1.30(0.72-2.34)	0.38		
Cerebrovascular disease	4 (3.57%)	36 (4.24%)	0.89(0.34-2.33)	0.81		
Connective tissue disease	1 (0.89%)	12 (1.41%)	0.60(0.08-4.48)	0.62		
Peptic ulcer	4 (3.57%)	27 (3.18%)	1.11(0.46-2.71)	0.81		
Hypertension	28 (25.00%)	199 (23.44%)	1.02(0.67-1.57)	0.92		
Tuberculosis	2 (1.79%)	12 (1.41%)	1.22(0.28-5.27)	0.79		
COPD	11 (9.82%)	55 (6.48%)	1.35(0.70-2.59)	0.37		
Respiratory failure	47 (41.96%)	170 (20.02%)	2.02(1.38-2.96)	<0.001		
Kidney failure	9 (8.04%)	42 (4.95%)	1.42(0.73-2.75)	0.30		
Heart failure	4 (3.57%)	27 (3.18%)	1.22(0.48-3.11)	0.68		
Diabetes	15 (13.39%)	118 (13.90%)	0.87(0.50-1.49)	0.61		
Liver dysfunction	**17 (15.18%)**	**34 (4.00%)**	**3.15(1.86-5.35)**	<0.001	**2.33 (1.30–4.17)**	**0.005**
Hematological disease	60 (53.57%)	314 (36.98%)	1.61(1.11-2.34)	0.012		
Pancreatitis	29 (25.89%)	107 (12.60%)	1.94(1.29-2.92)	0.002		
Medical operation						

Surgery	83 (74.11%)	711 (83.75%)	0.70(0.45-1.07)	0.099		
CVC	83 (74.11%)	470 (55.36%)	1.85(1.21-2.81)	0.004		
Ventilator	**103 (91.96%)**	**719 (84.69%)**	**2.02(1.04-3.93)**	0.038		
Indwelling catheter	109 (97.32%)	808 (95.17%)	1.84(0.62-5.52)	0.27		
Tube feeding	80 (71.43%)	332 (39.10%)	2.44(1.62-3.69)	<0.001		
Nebulizer fiberoptic	72 (64.29%)	413 (48.65%)	1.18(0.80-1.73)	0.40		
Bronchoscope	5 (4.46%)	18 (2.12%)	1.44(0.59-3.52)	0.43		
Antimicrobial use						
Cephalosporin	**20 (17.86%)**	**236 (27.80%)**	**0.50(0.31-0.81)**	0.005	**0.45 (0.28–0.73)**	**0.001**
Vancomycin	3 (2.68%)	35 (4.12%)	0.68(0.21-2.15)	0.51		
Aminoglycosides	1 (0.89%)	23 (2.71%)	0.24(0.03-1.71)	0.15		
Carbapenems	**82 (73.21%)**	**351 (41.34%)**	**2.84(1.87-4.32)**	<0.001	**2.21(1.40–3.49)**	**<0.001**
Fluoroquinolones	32 (28.57%)	154 (18.14%)	1.04(0.69-1.56)	0.84		
Antifungal agents	23 (20.54%)	157 (18.49%)	0.96(0.61-1.5)	0.85		
Cephamycins	**16 (14.29%)**	**196 (23.09%)**	**0.51(0.30-0.86)**	0.011	**0.53 (0.31–0.90)**	**0.018**
Lincomycin	5 (4.46%)	35 (4.12%)	1.01(0.41-2.48)	0.99		
Tigecycline	13 (11.61%)	65 (7.66%)	1.33(0.76-2.34)	032		

COPD, chronic obstructive pulmonary disease. CVC, central venous catheter.

Variables with $P < 0.05$ in the multivariate COX analysis are highlighted in bold.

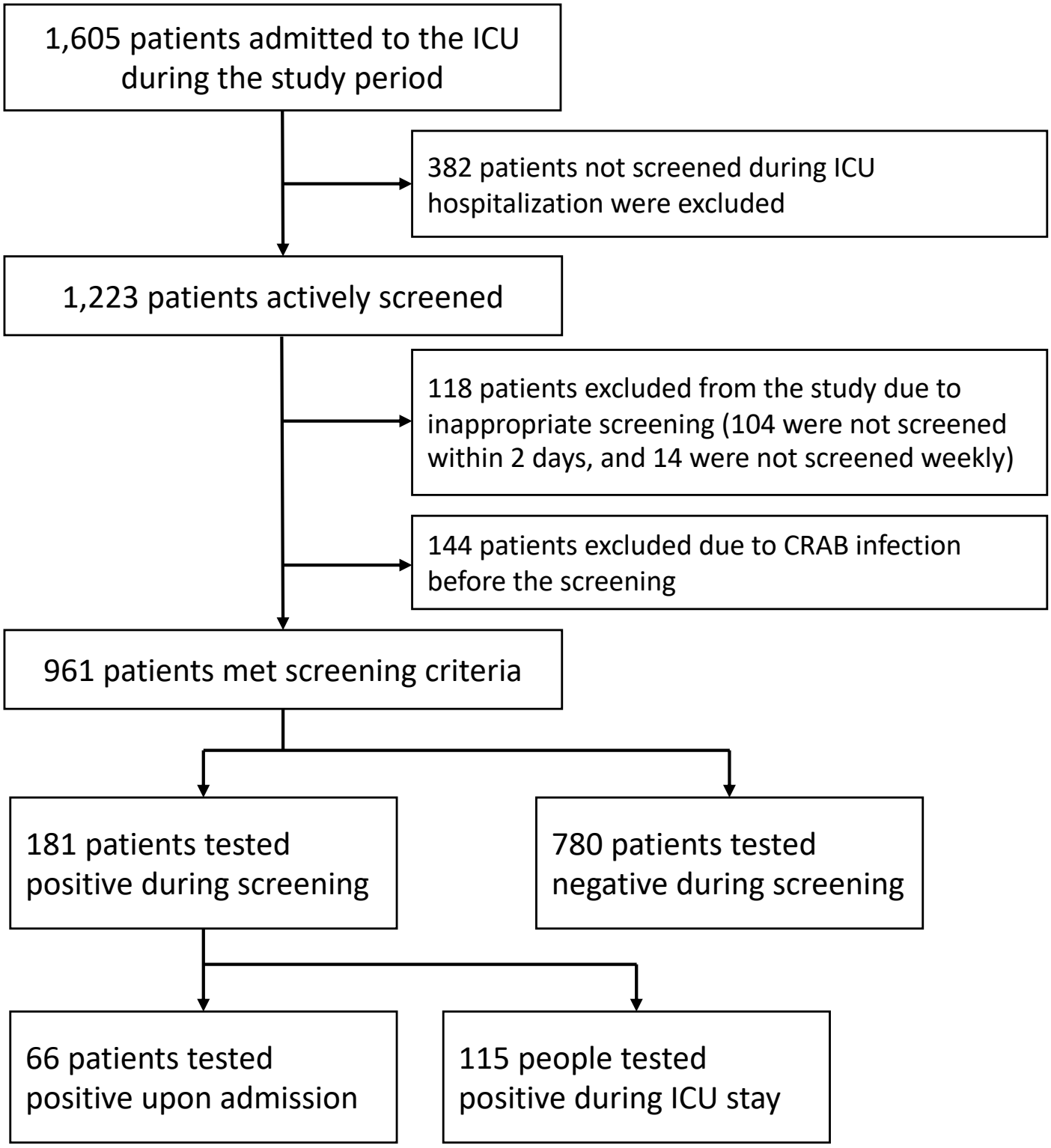

Figure 1. Patient selection flow algorithm

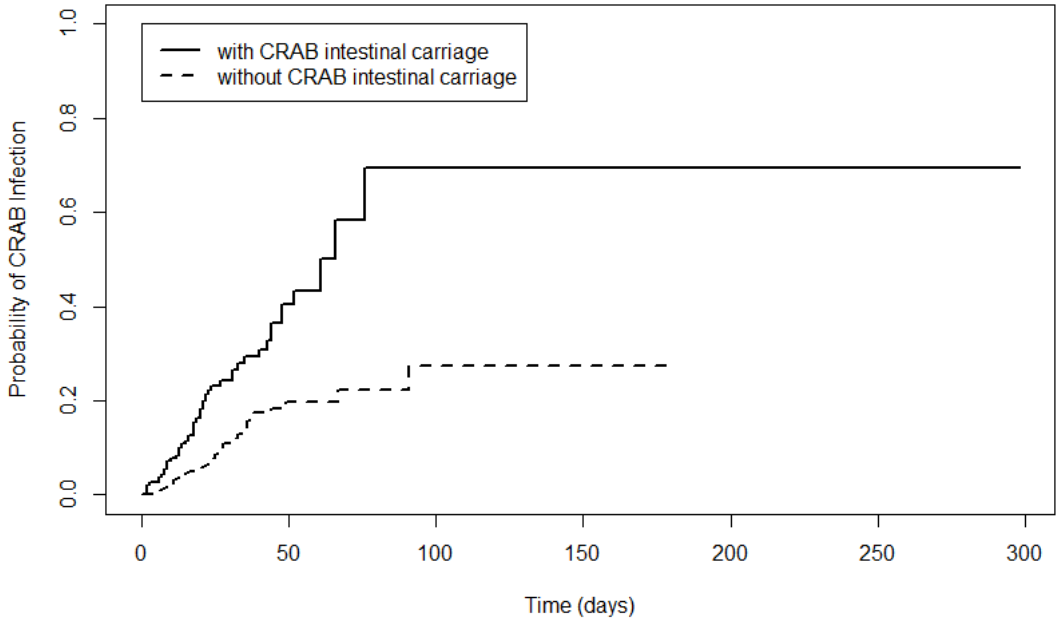

Figure 2. Survival curves of patients with and without CRAB intestinal carriage (cumulative probability of CRAB infection). Death in the ICU is considered as a competing event, not drawn in the figure. The solid line represents patients with CRAB intestinal carriage, while the dashed line represents those without. 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.

Supplementary files

Table S1 Variables associated with developing subsequent CRAB infection during the ICU stay using sub-distribution hazard model (exposed group was those patients with intestinal carriage on ICU admission)

Item	Subsequent CRAB infection during the ICU stay		Univariate analysis		Multivariate analysis	
	Yes (n=80)	No (n=766)	*SDHR (95% CI)*	*P*	*SDHR (95% CI)*	*P*
CRAB intestinal carriage	**19 (23.75%)**	**47 (6.14%)**	3.78(2.20-6.49)	**<0.001**	**3.42 (1.88-6.22)**	**<0.001**
Demographics						
Sex, male	50 (62.50%)	499 (65.14%)	1.15(0.73-1.80)	0.55		
Ethnicity, Han Chinese	77 (96.25%)	697 (90.99%)	2.54(0.78-8.24)	0.12		
Age (median)	54 (42-68)	55 (44-68)	1.00(0.99-1.02)	0.85		
APACHE II	**20 (15-26)**	**17 (12-22)**	**1.06(1.03-1.08)**	**<0.001**	**1.03 (1.00-1.05)**	**0.045**
Charlson score	3 (1-5)	3 (2-4)	1.00(0.88-1.13)	1.00		
Underlying disease						
Peripheral vascular disease	11 (13.75%)	57 (7.44%)	1.62(0.85-3.10)	0.14		
Cerebrovascular disease	3 (3.75%)	33 (4.31%)	0.87(0.29-2.66)	0.81		
Connective tissue disease	1 (1.25%)	11 (1.44%)	0.78(0.11-5.73)	0.80		
Peptic ulcer	3 (3.75%)	23 (3.00%)	1.19(0.41-3.46)	074		
Hypertension	18 (22.50%)	173 (22.58%)	0.97(0.58-1.64)	0.91		
Tuberculosis	1 (1.25%)	12 (1.57%)	0.72(0.10-5.37)	0.75		
COPD	**11 (13.75%)**	**45 (5.87%)**	**2.21(1.15-4.24)**	**0.017**	**2.71 (1.40-5.24)**	**0.003**
Respiratory failure	34 (42.50%)	143 (18.67%)	2.38(1.52-3.72)	**<0.001**		
Kidney failure	5 (6.25%)	35 (4.57%)	1.13(0.474-2.7)	0.78		
Heart failure	2 (2.50%)	21 (2.74%)	1.05(0.28-3.92)	0.95		

Diabetes	11 (13.75%)	101 (13.19%)	1.03(0.54-1.98)	0.92		
Liver dysfunction	**14 (17.50%)**	**32 (4.18%)**	**3.59(2.00-6.45)**	**<0.001**	**2.35 (1.30-4.25)**	**0.005**
Hematological disease	42 (52.50%)	261 (34.07%)	1.84(1.19-2.85)	**0.006**		
Pancreatitis	17 (21.25%)	84 (10.97%)	1.91(1.13-3.22)	**0.016**		
Medical operation						
Surgery	62 (77.50%)	638 (83.29%)	0.83(0.49-1.42)	0.50		
CVC	61 (76.25%)	414 (54.05%)	2.23(1.33-3.74)	**0.002**		
Ventilator	72 (90.00%)	648 (84.60%)	1.62(0.80-3.25)	0.18		
Indwelling catheter	78 (97.50%)	729 (95.17%)	1.91(0.53-6.94)	0.32		
Tube feeding	54 (67.50%)	265 (34.60%)	2.71(1.69-4.34)	**<0.001**		
Nebulizer fiberoptic	51 (63.75%)	349 (45.56%)	1.35(0.86-2.12)	0.19		
Bronchoscope	5 (6.25%)	17 (2.22%)	1.85(0.77-4.46)	0.17		
Antimicrobial use						
Cephalosporin	**15 (18.75%)**	**210 (27.42%)**	**0.54(0.31-0.95)**	**0.032**	**0.43(0.24-0.78)**	**0.006**
Vancomycin	2 (2.50%)	28 (3.66%)	0.70(0.16-2.98)	0.63		
Aminoglycosides	1 (1.25%)	15 (1.96%)	0.50(0.07-3.52)	0.48		
Carbapenems	**58 (72.50%)**	**284 (37.08%)**	**3.57(2.18-5.85)**	**<0.001**	**2.61(1.53-4.46)**	**<0.001**
Fluoroquinolones	21 (26.25%)	127 (16.58%)	1.07(0.66-1.74)	0.79		
Antifungal agents	17 (21.25%)	123 (16.06%)	1.26(0.74-2.12)	0.39		
Cephamycins	12 (15.00%)	179 (23.37%)	0.52(0.28-0.96)	**0.036**		
Lincomycin	3 (3.75%)	32 (4.18%)	0.84(0.26-2.68)	0.76		
Tigecycline	8 (10.00%)	53 (6.92%)	1.36(0.67-2.76)	0.39		

COPD, chronic obstructive pulmonary disease. CVC, central venous catheter.

Variables with $P < 0.05$ in the multivariate COX analysis are highlighted in bold.

Table S2. Variables associated with developing subsequent CRAB infection during the ICU stay using sub-distribution hazard model (exposed group was those patients with ICU acquisition of CRAB intestinal carriage)

Item	Subsequent CRAB infection during the ICU stay		Univariate analysis		Multivariate analysis	
	Yes (n=93)	No (n=802)	*SDHR (95% CI)*	*P*	*SDHR (95% CI)*	*P*
CRAB intestinal carriage	**32 (34.41%)**	**83 (10.35%)**	**2.35(1.56-3.55)**	**<0.001**	**1.81 (1.15-2.86)**	**0.011**
Demographics						
Sex, male	60 (64.52%)	513 (63.97%)	1.01(0.66-1.55)	0.95		
Ethnicity, Han Chinese	87 (93.55%)	731 (91.15%)	1.32(0.56-3.08)	0.52		
Age (median)	54 (44-68)	55 (45-68)	1.00(0.99-1.01)	0.70		
APACHE II	21 (18-26)	17 (12-22)	1.05(1.03-1.07)	<0.001		
Charlson score	3 (1-5)	3 (2-4)	1.01(0.91-1.13)	0.80		
Underlying disease						
Peripheral vascular disease	11 (11.83%)	62 (7.73%)	1.34(0.71-2.53)	0.37		
Cerebrovascular disease	4 (4.30%)	36 (4.49%)	1.03(0.39-2.73)	0.96		
Connective tissue disease	1 (1.08%)	12 (1.50%)	0.68(0.09-5.07)	0.70		
Peptic ulcer	4 (4.30%)	26 (3.24%)	1.39(0.58-3.33)	0.46		
Hypertension	25 (26.88%)	191 (23.82%)	1.10(0.69-1.74)	0.70		
Tuberculosis	2 (2.15%)	11 (1.37%)	1.41(0.32-6.29)	0.65		
COPD	10 (10.75%)	54 (6.73%)	1.37(0.69-2.75)	0.37		
Respiratory failure	**44 (47.31%)**	**159 (19.83%)**	**2.42(1.59-3.69)**	**<0.001**	**1.84 (1.17-2.90)**	**0.009**
Kidney failure	7 (7.53%)	35 (4.36%)	1.56(0.75-3.28)	0.24		

Heart failure	3 (3.23%)	24 (2.99%)	1.34(0.47-3.82)	0.58		
Diabetes	11 (11.83%)	112 (13.97%)	0.75(0.40-1.39)	0.36		
Liver dysfunction	**13 (13.98%)**	**29 (3.62%)**	**3.18(1.74-5.81)**	**<0.001**	**2.13(1.05-4.32)**	**0.037**
Hematological disease	49 (52.69%)	290 (36.16%)	1.60(1.06-2.40)	0.025		
Pancreatitis	20 (21.51%)	92 (11.47%)	1.71(1.06-2.76)	0.028		
Medical operation						
Surgery	70 (75.27%)	666 (83.04%)	0.78(0.49-1.27)	0.32		
CVC	68 (73.12%)	434 (54.11%)	1.82(1.15-2.88)	0.011		
Ventilator	85 (91.40%)	683 (85.16%)	1.83(0.89-3.76)	0.10		
Indwelling catheter	91 (97.85%)	761 (94.89%)	2.37(0.58-9.63)	0.23		
Tube feeding	67 (72.04%)	300 (37.41%)	2.64(1.67-4.18)	<0.001		
Nebulizer fiberoptic	64 (68.82%)	388 (48.38%)	1.42(0.92-2.20)	0.11		
Bronchoscope	5 (5.38%)	18 (2.24%)	1.60(0.64-3.99)	0.31		
Antimicrobial use						
Cephalosporin	**18 (19.35%)**	**213 (26.56%)**	**0.60(0.36-1.00)**	**0.051**	**0.59(0.35-1.00)**	**0.048**
Vancomycin	2 (2.15%)	33 (4.11%)	0.53(0.13-2.15)	0.38		
Carbapenems	**68 (73.12%)**	**319 (39.78%)**	**2.92(1.84-4.64)**	**<0.001**	**2.11 (1.27-3.50)**	**0.004**
Fluoroquinolones	28 (30.11%)	144 (17.96%)	1.11(0.71-1.72)	0.65		
Antifungal agents	16 (17.20%)	141 (17.58%)	0.82(0.48-1.39)	0.46		
Cephamycins	15 (16.13%)	187 (23.32%)	0.56(0.33-0.98)	0.042		
Lincomycin	4 (4.30%)	34 (4.24%)	0.90(0.34-2.44)	0.84		
Tigecycline	7 (7.53%)	57 (7.11%)	0.91(0.43-1.96)	0.82		

COPD, chronic obstructive pulmonary disease. CVC, central venous catheter.

Variables with $P < 0.05$ in the multivariate COX analysis are highlighted in bold.

Reporting checklist for cohort study.

Based on the STROBE cohort guidelines.

Instructions to authors

Complete this checklist by entering the page numbers from your manuscript where readers will find each of the items listed below.

Your article may not currently address all the items on the checklist. Please modify your text to include the missing information. If you are certain that an item does not apply, please write "n/a" and provide a short explanation.

Upload your completed checklist as an extra file when you submit to a journal.

In your methods section, say that you used the STROBE cohortreporting guidelines, and cite them as:

von Elm E, Altman DG, Egger M, Pocock SJ, Gotzsche PC, Vandenbroucke JP. The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) Statement: guidelines for reporting observational studies.

		Reporting Item	Page Number
Title and abstract			
Title	#1a	Indicate the study's design with a commonly used term in the title or the abstract	2
Abstract	#1b	Provide in the abstract an informative and balanced summary of what was done and what	2

		was found	
Introduction			
Background / rationale	#2	Explain the scientific background and rationale for the investigation being reported	4-5
Objectives	#3	State specific objectives, including any prespecified hypotheses	5
Methods			
Study design	#4	Present key elements of study design early in the paper	7-8
Setting	#5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	5-6
Eligibility criteria	#6a	Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up.	6
Eligibility criteria	#6b	For matched studies, give matching criteria and number of exposed and unexposed	n/a Not matched studies
Variables	#7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	6-8
Data sources / measurement	#8	For each variable of interest give sources of data and details of methods of assessment	6-8

		(measurement). Describe comparability of assessment methods if there is more than one group. Give information separately for for exposed and unexposed groups if applicable.	
Bias	#9	Describe any efforts to address potential sources of bias	8
Study size	#10	Explain how the study size was arrived at	n/a Including all the patients admitted to the ICU in the study period.
Quantitative variables	#11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen, and why	7
Statistical methods	#12a	Describe all statistical methods, including those used to control for confounding	7-8
Statistical methods	#12b	Describe any methods used to examine subgroups and interactions	8
Statistical methods	#12c	Explain how missing data were addressed	n/a No missing data.
Statistical methods	#12d	If applicable, explain how loss to follow-up was addressed	n/a Not applicable

Statistical methods	#12e	Describe any sensitivity analyses	n/a Not done.
Results			
Participants	#13a	Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed. Give information separately for for exposed and unexposed groups if applicable.	9
Participants	#13b	Give reasons for non-participation at each stage	9
Participants	#13c	Consider use of a flow diagram	Figure 1
Descriptive data	#14a	Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders. Give information separately for exposed and unexposed groups if applicable.	19-23
Descriptive data	#14b	Indicate number of participants with missing data for each variable of interest	n/a No missing data.
Descriptive data	#14c	Summarise follow-up time (eg, average and total amount)	Figure 2
Outcome data	#15	Report numbers of outcome events or summary measures over time. Give information separately	19-23

		for exposed and unexposed groups if applicable.	
Main results	#16a	Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	19-23
Main results	#16b	Report category boundaries when continuous variables were categorized	n/a Continuous variables were not categorized.
Main results	#16c	If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	n/a Not applicable
Other analyses	#17	Report other analyses done—e.g., analyses of subgroups and interactions, and sensitivity analyses	Supplementary files
Discussion			
Key results	#18	Summarise key results with reference to study objectives	11-13
Limitations	#19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias.	13
Interpretation	#20	Give a cautious overall interpretation considering objectives, limitations, multiplicity of analyses,	11-13

		results from similar studies, and other relevant evidence.	
Generalisability	#21	Discuss the generalisability (external validity) of the study results	13
Other Information			
Funding	#22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	14