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Original Article

Risk factors for carbapenem-resistant *Pseudomonas aeruginosa* infection or colonization in a Chinese teaching hospital

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Abstract

Introduction: Carbapenem-resistant *Pseudomonas aeruginosa* (CRPA) is rapidly emerging as a life-threatening nosocomial infection. In this study, we aimed to identify risk factors, especially antibiotic use and co-carriage with other bacteria for CRPA infection or colonization.

Methodology: A retrospective study was conducted in the First Affiliated Hospital of Xi'an Jiaotong University, which involved a cohort of patients with *Pseudomonas aeruginosa* infection or colonization from January 2014 to June 2016. Univariate analysis and multivariate analysis were performed to estimate the risk factors of CRPA occurrence.

Results: Eight hundred and eighty-eight patients were included in the study. More than 50% of the risk factors were associated with CRPA infection or colonization according to univariate analysis ($P < 0.05$), such as invasive procedures, co-carriage with Gram-negative pathogens, and prior treatment with some antibiotics. However, only prior exposure to carbapenems (OR: 8.005; CI:4.507-14.217, $P<0.001$), the days of carbapenems treatment (OR: 1.190; CI: 1.073-1.272; $P < 0.001$), and co-carriage with *Escherichia coli* (OR: 1.824; CI: 1.005-3.310, $P = 0.048$) were considered independent risk factors by multivariate analysis. A higher mortality was found among patients with CRPA infection or colonization ($P < 0.05$).

Conclusions: Risk factors for CRPA infection or colonization were prior exposure to carbapenems, the days of carbapenems treatment, and co-carriage with *Escherichia coli*. The prevalence of CRPA could be influenced by Gram-negative pathogens, especially in *Escherichia coli*, and it need more researches. Moreover, restrictions in the clinical use of carbapenems should be taken into account.

Key words: *Pseudomonas aeruginosa*; carbapenem resistantance; risk factors; mortality.

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Introduction

Pseudomonas aeruginosa (PA) is a non-fermenter Gram-negative bacilli, which is one of the most common pathogens associated with nosocomial infections [1]. Carbapenems are commonly used for nosocomial infections, caused by Gram-negative pathogens including PA [2]. However, carbapenem-resistant *Pseudomonas aeruginosa* (CRPA) strains are emerging worldwide, and the rate of resistance in most countries ranges from 10% to 50% [1]. The rate of CRPA in Canada (3.3%) was the lowest of all countries. On the other hand, ratios in Brazil, Peru, Costa Rica, Russia, Greece, Poland, Iran, and Saudi Arabia were higher than 50% in all drugs of the carbapenem class ranging from 50% to 75.3%. Unfortunately, several surveillance studies from the USA and Europe have reported that the prevalence of CRPA isolates are on the rise [3,4]. These strains are difficult to control because they spread easily within and between hospitals, and treatment options for CRPA infection or colonization are limited [5,6].

Identifying risk factors for the development of CRPA acquisition is important for treatment options, and consequently, a number of risk factors for CRPA infection or colonization have been revealed in some retrospective clinical studies [7-14]. However, the conclusions were not always consistent among these studies. Antibiotic exposure was considered an independent factor for CRPA infection or colonization in some studies, especially carbapenem use [7-9], but not in all. Other independent factors were prior ICU stay [10], increased length of hospital stay [11-13], and urinary catheter insertion [14]. Co-carriage with other bacteria might be a significant predictor for isolating a strain of CRPA. However, these data were rare. This study assessed the incidence and risk factors of co-

carriage with other bacteria for CRPA. Overall, the goals of this study is to improve empirical therapy efficacy, and the risk factors by investigating CRPA infection or colonization and correlations.

Methodology

Subjects and study design

This retrospective cohort study was conducted in the First Affiliated Hospital of Xi'an Jiaotong University (FAHXJU) and involved a cohort of patients with PA infection or colonization (positive PA culture after admission) from January 2014 to June 2016 were included. The FAHXJU is a general tertiary-care teaching hospital and regional medical center having 2560 beds that is located in the northwest region of China. This hospital includes all major departments and services, including medicine, surgery, pediatrics, gynecology and obstetrics, hematology, oncology, and emergency department.

Microbiologic analysis

The clinical microbiology laboratory in the FAHXJU provided data of bacterial identification and antibacterial susceptibility testing. All positive clinical specimens (blood, sputum, urine, wound, or normally sterile body fluid positive for PA) were included. Antimicrobial susceptibilities were determined by minimum inhibitory concentration (MIC) using the automated VITEK 2 automated system (bioMérieux, Lyons, France) and interpreted according to the criteria suggested by the Clinical and Laboratory Standards Institute document [15]. In this study, CRPA (Carbapenem-resistant *Pseudomonas aeruginosa*) was defined as an isolate with meropenem and/or imipenem MICs ≥ 8 mg/L. The other isolates with meropenem and/or imipenem MICs ≤ 2 mg/L were defined as carbapenem-susceptible *P. aeruginosa* (CSPA). Isolates with intermediate susceptibility were not included in the analysis. If PA was isolated from multiple cultures, only the first occurrence was included in order to preserve the independence of the risk factors. Exclusion criteria were age < 18 years old and patients from an outpatient setting.

Data collection

The following data of each patient were collected by reviewing medical and microbiological data records: where and when the strains were isolated, co-carriage with other bacteria (frequently isolated species including *Acinetobacter baumannii*, *Carbapenem-resistant Acinetobacter baumannii*, *Escherichia coli*, *Klebsiella pneumonia*, *Staphylococcus aureus*, *Enterococcus faecium*, *Enterococcus faecalis*, *Staphylococcus epidermidis*), patients age, gender, the reasons for admission, comorbidities, use of invasive procedures, hospital admission date, hospital discharge date, ICU admission date, ICU discharge date, clinical signs and laboratory data, previous exposure to various antibiotics, and duration of treatment with antibiotics. For infected patients, antibiotic use was collected up until the point of CRPA or CSPA detection.

Statistical analysis

In analysis of risk factors for CRPA infection or colonization, univariate logistic regression analysis was performed to search for potential risk factors. Continuous data were analyzed using the *t* test for parametric data or the Mann-Whitney *U* test for nonparametric data, and categorical variables were compared using the χ^2 test. To identify the independent risk factors, variables with $P < 0.05$ in the univariate analysis were included in multivariate logistic regression model and analyzed using backward stepwise regression. Odds ratio (OR) and 95% confidence interval (CI) were also calculated. For all statistical analyses, $P < 0.05$ indicated statistical significance.

Results

During the study period, a total of 888 patients with *P. aeruginosa* were involved. There were 264 patients with CRPA infection or colonization, and 624 patients with CSPA infection or colonization. Demographic and clinical characteristics of patients with *P. aeruginosa* are shown in Table 1. There was no significant difference in sex distribution and age between the both groups of patients ($P > 0.05$). Compared with the CSPA group, the CRPA group had the longer length of hospital stay before bacteremia onset, length of hospital stay, and length of ICU stay (12.07 ± 21.39 vs. 7.85 ± 11.57, 31.67 ± 33.82 vs. 22.33 ± 22.54, 12.85 ± 20.52 vs. 4.95 ± 12.41; $P < 0.05$, respectively). In CRPA group, a greater proportion of patients stayed in the ICU (64.39% vs. 32.53%, $P < 0.05$), and the fatality rates were higher (9.47% vs. 3.53%, $P < 0.05$). Other factors also were found to be associated with CRPA infection or colonization, including the invasive procedures, and some comorbidities, such as neurological disorders and hypoalbuminemia ($P < 0.05$).

Co-carriage with other bacteria as the potential risk factor was analyzed, which was presented in Table 1. Co-carriage with other bacteria was defined that the patients with infection or colonization of PA who had other bacteria at the time of hospitalization. Patients

with CRPA were more likely to be co-carried with the Gram-negative bacilli, for instance, *Acinetobacter baumannii*, Carbapenem-resistant *Acinetobacter baumannii*, *Escherichia coli*, and *Klebsiella pneumonia* (23.86% vs. 9.13%, 21.97% vs. 8.01%, 16.29% vs. 7.69%, 16.29% vs. 7.69%; $P < 0.05$, respectively). However, there were no significant differences between the two groups which co-carried with the Gram-positive bacilli (*Staphylococcus aureus,* or *Enterococcus faecalis,* or *Staphylococcus epidermidis*; $P > 0.05$, respectively), except for *Enterococcus faecium* (20.45% vs. 6.41%, $P < 0.05$).

Antibiotic risk factor analysis of CRPA infection or colonization is presented in Table 2. Analysis showed that CRPA patients had more exposure to carbapenems, glycopeptides, oxazolidinone, quinolones, imidazole derivatives, sulfonamides, tetracycline (68.94% vs. 10.90%, 16.67% vs. 6.73%, 9.09% vs. 2.24%, 19.32% vs. 9.46%, 12.88% vs. 7.05%, 4.17% vs. 1.44%, 5.68% vs. 0.96%; $P < 0.05$, respectively) than CSPA patients. Moreover, the days of exposure to these antibiotics were longer in CRPA group (5.13 ± 6.82 vs. 0.50 ± 1.97, 1.45 ± 4.87 vs. 0.45 ± 2.17, 0.63 ± 2.44 vs. 0.13 ± 0.99, 1.33 ± 3.71 vs. 0.51 ± 2.52, 1.07 ± 3.74 vs. 0.30 ± 1.52, 0.31 ± 1.81 vs. 0.10 ± 0.92, 0.41 ± 2.09 vs. 0.07 ± 0.89; $P < 0.05$, respectively). Difference of exposure to the other antibiotics or the days of previous use in both group were not statistically significant ($P > 0.05$).

Compared to patients with CSPA bacteremia, multivariate analysis in Table 3 showed that independent risk factors associated with CRPA bacteremia were co-carried with *Escherichia coli* (OR: 1.824; CI: 1.005-3.310, $P = 0.048$), previous use of carbapenems (OR: 8.005; CI:4.507-14.217, $P < 0.001$), and the days of previous use of carbapenems (OR: 1.190; CI: 1.073-1.272; $P < 0.001$). According to the result of the multivariate analysis, the previous use of carbapenems was investigated in some patients who were co-carried with both CRPA and other bacteria.

Table 1. Univariate analysis of risk factors associated with carbapenem-resistant *P. aeruginosa* infection or colonization.

Variable	CRPA (n = 264)	CSPA (n = 624)	*P*
Demographic and clinical characteristics			
Male, n (%)	188 (71.21)	425 (68.11)	0.383
Age (years)	61.42 ± 17.41	60.49 ± 17.51	0.611
Length of hospital stay before bacteraemia (days)	12.07 ± 21.39	7.85 ± 11.57	0.000
Length of hospital stay (days)	31.67 ± 33.82	22.33 ± 22.54	0.000
ICU stay, n (%)	170 (64.39)	203 (32.53)	0.000
Length of ICU stay (days)	12.85 ± 20.52	4.95 ± 12.41	0.000
Comorbidities, n (%)			
Neurological disorders	83 (31.44)	132 (21.15)	0.001
Diabetes	29 (10.98)	85 (13.62)	0.324
Lung disease	111 (42.05)	228 (36.54)	0.131
Malignancy	60 (22.73)	181 (29.01)	0.058
Renal dysfunction	52 (19.70)	102 (16.35)	0.245
Hypoalbuminaemia	88 (33.33)	134 (21.47)	0.000
Invasive procedures, n (%)			
Indwelling gastric tube	81 (30.68)	101 (16.19)	0.000
Indwelling urethral catheter	175 (66.29)	285 (45.67)	0.000
Tube drainage	159 (60.23)	246 (39.42)	0.000
Tracheostomy	61 (23.11)	70 (11.22)	0.000
Mechanical ventilation	113 (42.80)	132 (21.15)	0.000
Co-carriage, n (%)			
Acinetobacter baumannii	63 (23.86)	57 (9.13)	0.000
Carbapenem-resistant *Acinetobacter baumannii*	58 (21.97)	50 (8.01)	0.000
Escherichia coli	43 (16.29)	48 (7.69)	0.000
Klebsiella pneumoniae	43 (16.29)	48 (7.69)	0.000
Staphylococcus aureus	21 (7.95)	30 (4.81)	0.082
Enterococcus faecium	54 (20.45)	40 (6.41)	0.000
Enterococcus faecalis	11 (4.17)	17 (2.72)	0.294
Staphylococcus epidermidis	13 (4.92)	16 (2.59)	0.096
Outcomes, n (%)			
Hospital mortality [a]	25 (9.47)	22 (3.53)	0.000

CRPA, Carbapenem-resistant *P. aeruginosa*; CSPA Carbapenem-susceptible *P. aeruginosa*; ICU Intensive Care Unit. The variables screened in univariate analysis with P < 0.05 were included in the multivariate logistic regression analysis. Hospital mortality was excluded in the multivariate analysis

Table 2. Effect of antibiotic treatment as a risk factor for carbapenem-resistant *P. aeruginosa* infection or colonization: prior antibiotic exposures and the days of treatment.

Prior treatment with antibiotics	CRPA (n = 264)	CSPA (n = 624)	*P*
Carbapenems, n (%)	182(68.94)	68(10.90)	0.000
Days of treatment (mean ± SD)	5.13 ± 6.82	0.50 ± 1.97	0.000
Penicillins and enzyme inhibitor, n (%)	34(12.88)	76(12.18)	0.824
Days of treatment (mean ± SD)	1.03 ± 3.69	0.52 ± 2.17	0.598
Cephalosporins, n (%)	108(40.91)	257(41.19)	1.000
Days of treatment (mean ± SD)	2.35 ± 4.78	2.19 ± 4.51	0.886
Monobactams, n (%)	2(0.76)	2(0.32)	0.587
Days of treatment (mean ± SD)	0.05 ± 0.66	0.04 ± 0.63	0.615
Glycopeptides, n (%)	44(16.67)	42(6.73)	0.000
Days of treatment (mean ± SD)	1.45 ± 4.87	0.45 ± 2.17	0.000
Oxazolidinone, n (%)	24(9.09)	14(2.24)	0.000
Days of treatment (mean ± SD)	0.63 ± 2.44	0.13 ± 0.99	0.000
Macrolides, lincosamides, and streptogramins, n (%)	0(0.00)	5(0.80)	0.329
Days of treatment (mean ± SD)	0.00 ± 0.00	0.06 ± 0.93	0.145
Quinolones, n (%)	51(19.32)	59(9.46)	0.000
Days of treatment (mean ± SD)	1.33 ± 3.71	0.51 ± 2.52	0.000
Aminoglycosides, n (%)	12(4.55)	23(3.69)	0.573
Days of treatment (mean ± SD)	0.38 ± 2.95	0.20 ± 1.77	0.519
Imidazole derivatives, n (%)	34(12.88)	44(7.05)	0.006
Days of treatment (mean ± SD)	1.07 ± 3.74	0.30 ± 1.52	0.003
Sulfonamides, n (%)	11(4.17)	9(1.44)	0.023
Days of treatment (mean ± SD)	0.31 ± 1.81	0.10 ± 0.92	0.012
Tetracycline, n (%)	15(5.68)	6(0.96)	0.000
Days of treatment (mean ± SD)	0.41 ± 2.09	0.07 ± 0.89	0.000

CRPA, Carbapenem-resistant *P. aeruginosa*; CSPA Carbapenem-susceptible *P. aeruginosa*. The variables screened in univariate analysis with P < 0.05 were included in the multivariate logistic regression analysis.

Table 3. Multivariate analysis of risk factors associated with carbapenem-resistant *P. aeruginosa* infection or colonization.

Multivariate analysis	OR (95% CI)	*P*
Acinetobacter baumanni	2.175 (0.544-8.699)	0.272
Carbapenem-resistant *Acinetobacter baumannii*	0.523 (0.123-2.216)	0.379
Escherichia coli	1.824 (1.005-3.310)	0.048
Klebsiella pneumoniae	1.408 (0.748-2.648)	0.289
Enterococcus faecium	1.787 (0.970-3.290)	0.062
Carbapenems, n	8.005 (4.507-14.217)	0.000
Carbapenems, days	1.190 (1.073-1.272)	0.000
Glycopeptide, n (%)	0.819 (0.341-1.967)	0.655
Days of treatment (mean ± SD)	0.932 (0.85-1.022)	0.135
Oxazolidinone, n (%)	0.700 (0.133-3.681)	0.674

OR: odds ratio; CI: confidence interval. The variables screened in univariate analysis with P < 0.05 were included in the multivariate logistic regression analysis.

When the patients with infection or colonization of CRPA were co-carried with *Acinetobacter baumannii*, Carbapenem-resistant *Acinetobacter baumannii*, *Escherichia coli, Klebsiella pneumonia, Staphylococcus aureus*, *Enterococcus faecium*, *Enterococcus faecalis*, *or Staphylococcus epidermidis*, the percentage of carbapenems use were 79.37%, 81.03%, 79.07%, 74.42%, 95.24%, 87.04%, 72.73%, 92.31%, and the days of previous use of carbapenems were 7.62 ± 9.03, 8.09 ± 9.40, 6.93 ± 9.71, 7.63 ± 9.15, 7.48 ± 5.68, 7.00 ± 8.48, 8.91 ± 14.67, and 7.62 ± 7.61, respectively.

Discussion

Since the first cases on CRPA were reported, treatment of *P. aeruginosa* infection or colonization using carbapenems has been challenged. To improve empirical therapy efficacy, we studied the risk factors for CRPA infection or colonization. In this study, more than 50% of the risk factors were associated with infection or colonization by this microorganism according to univariate analysis. However, only prior exposure to carbapenems, the days of carbapenems treatment, and co-carriage with *Escherichia coli* were considered independent risk factors by multivariate analysis.

In accordance with previous prospective or retrospective studies, our results confirmed that prior exposure to carbapenems was an independent risk factor for CRPA infection or colonization [7-9]. However, in contrast with some other studies [13,16], we did not find that the use of fluoroquinolones, or aminoglycosides was associated with increased CRPA infection or colonization. These inconsistent findings might be due to differences in antibiotic prescribing practices in different countries [17]. Furthermore, the differences could also be explained that one of the constraints of regression analyses is that they show correlation, not necessarily cause and effect [18]. However, the association between prior carbapenems use and CRPA isolates has been well established in previous studies [11, 19]. Exposure to carbapenem could lead to the decreased levels of OprD porin and upregulation of the multidrug efflux pump, with subsequent resistance to carbapenems. Moreover, the risk associated with antibiotic exposure is probably cumulative [20]. Kritsotakis *et al*. [21] found that prior antibiotic exposures as continuous variables revealed dose-dependent effects of antibiotics on the risk of extended-spectrum β-lactamase (ESBL) carbapenem-resistant *Klebsiella pneumonia* (CRKP). Increasing duration of treatment with carbapenems, or fluoroquinolones, or β-lactam/β-lactamase inhibitor combination was associated with increased risk of ESBL-CRKP infection. The finding of Zhang *et al.* [11] that the days of antibiotic apply were identified as the independent predictors of infection with CRPA, however, the specific antibiotic was not analyzed. In this study, we found that increased duration of treatment with carbapenems remained significantly associated with CRPA infection or colonization. In 2011, China launched a specific national rectification scheme for the clinical use of antibiotics, and antibiotics were divided into 3 levels: non-restricted, restricted, and specialist antibiotic [22]. Carbapenems belong to the specialist antibiotic, which is the strictest and only physicians with senior specialized technical qualifications; for example, associate chief physicians and chief physicians, can prescribe them. From then, there were the significant reductions in antibiotic use in Chinese hospitals [23]. However, the overuse of carbapenems still is a serious condition after antimicrobial stewardship programme, despite the fact that National Health and Family Planning Commission of the People's Republic of China (NHFPC) has been paid more attentions on the rational use of carbapenems from February 2017 [24]. The clinical application of the carbapenems should be more restricted.

The other interesting finding of this study was that co-carriage with *Escherichia coli* was an independent risk factor for CRPA. Co-infection with other pathogens is quite common [25]. Snyder *et al.* found the prevalence of co-colonization with ≥ 2 different multidrug-resistant Gram-negative bacteria (MDRGN) was substantial [26]. However, the phenomenon of co-colonization with multiple different species of bacteria have not been fully elucidated. Sidjabat *et al.* found the resistance gene could cross the interspecies barrier in the different bacteria [27]. Furthermore, the patients were co-carried with the Gram-negative bacilli who were more likely with CRPA, rather than the Gram-positive bacilli. In order to analyze the effect of carbapenems, the use of Carbapenems was investigated. However, the percentage of carbapenems use was largest in the patients who were co-carried with *Staphylococcus epidermidis*, and the day of previous use of carbapenems was longest in the patients who were co-carried with *Enterococcus faecalis*. Therefore, the usage of carbapenems could not explain the phenomenon. Marchaim found that among patients with colonization or infection due to Carbapenem-resistant *Enterobacteriaceae* (CRE) in metropolitan Detroit, co-carriage with a carbapenem-resistant *Acinetobacter baumannii* (CRAB) and/or CRPA was

found to be a significant predictor for isolating a strain of colistin-resistant CRE [28]. There might be something, which was commonly secreted by Gram-negative bacteria, especially in *E. coli*, and it could affect the development of CRPA. In recent years, a new regulating mechanism defined as quorum sensing was found to play an important role in biofilm formation [29] The signaling molecules of quorum sensing mainly contain two parts, oligopeptides are commonly secreted by Gram-positive bacteria, while N-acyl-L-homoserine lactones (AHLs) are commonly secreted by Gram-negative bacteria [30]. AHLs are one of the most well-characterized signaling molecules, and it was called the talking language in Gram-negative bacteria [31]. AHLs are involved in regulating virulence factor secretion, exoenzyme production and biofilm formation based on quorum sensing system [32]. There is a need to do further research to find the relationship between AHLs and CRPA.

Some reports suggested that CRPA infection might be associated with adverse outcomes, and the mortality caused by CRPA is increased [7, 10]. In our study, the mortality was 9.47% for CRPA infection or colonization, compared to 3.53% in patients with CSPA infection or colonization. There were no more independent risk factors in the study, however, the patients with CRPA infection or colonization had more ICU stay, length of ICU stay, length of hospital stay, and invasive procedures. There are some potential limitations in this study. Firstly, there was not an established criterion for differentiating infection from colonization of PA in the study. Second, this study was retrospective, and it was only conducted in one tertiary-care teaching hospital. Prospective, multicenter clinical trials are expected to be performed.

Conclusions

This retrospective study indicated that only prior exposure to carbapenems, the days of carbapenems treatment, and co-carriage with *Escherichia coli* were considered independent risk factors for CRPA. These findings suggested that the clinicians should place emphasis on the appropriate antibiotic use and invasive procedures. Futhermore, co-carriage with other pathogens needs to be studied.

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Authors' contributions

DZ and YD conceived the idea for the article and drafted the manuscript. KC, TW, YS, and CM collected the data and carried out the analysis. HD and WF participated in the design of the overall evaluation. All authors read and approved the final manuscript. None of the authors have any conflicts of interest to report.

Ethical Approval

The study was reviewed and approved by the hospital's Human Ethics Committee. The requirement for informed consent was waived because this was a retrospective study, and only de-identified data were used in the study.

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