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Original article

Risk factors for carbapenem-resistant *Klebsiella pneumoniae* infection/colonization: A case–case-control study

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ABSTRACT

Carbapenem-resistant *Klebsiella pneumoniae* (CRKP) is increasingly reported worldwide. The aim of the present study was to identify risk factors associated with the development of CRKP infections. A retrospective, case–case-control study was performed at the University Hospital of Heraklion, Greece. The study population included 83 patients from whom CRKP was isolated, 79 from whom carbapenem-sensitive *K. pneumoniae* (CSKP) was isolated and 161 (control group) from whom *K. pneumoniae* was not isolated. The median age of CRKP and CSKP patients was 79 (28–101) and 80 (39–97) years, respectively, while that of the controls was 75 (18–100) years. *K. pneumoniae* was isolated predominantly from urine in both case groups, followed by blood. Independent risk factors for CRKP infection/colonization were admission to ICU ($p = 0.004$), prior surgical procedure ($p = 0.036$) and presence of renal disease ($p = 0.037$), while for CSKP were neurological disease ($p = 0.007$), and older age ($p = 0.011$). No association between CRKP and prior antimicrobial exposure was found. Of the entire cohort 40 patients (12%) died; 22 (27%) in the CRKP, 12 (15%) in the CSKP and 6 (4%) in the control group. Isolation of any *K. pneumoniae* strain was associated with higher mortality compared to the control group (21% vs. 4%; $p < 0.005$). Mortality was not statistically different between those infected/colonized/with a CRKP or a CSKP strain ($p = 0.084$). According to these results prior ICU stay, prior surgical procedure and renal disease were independent risk factors for the development of a CRKP infection/colonization.

1. Introduction

Unfortunately, due to continuous exposure to carbapenems, *Klebsiella pneumoniae* strains resistant to carbapenems (Carbapenem-resistant *K. pneumoniae*; CRKP) have emerged [1–7], representing a serious clinical problem, since the antimicrobial treatment options are very limited. These resistant strains have been the source of hospital-acquired infections in severely ill patients, while mortality seems to be higher among those infected with such strains compared to patients infected with carbapenem susceptible *K. pneumoniae* (CSKP) [8]. What makes the situation even more difficult is that the gene that encodes carbapenemase, the enzyme mostly responsible for the carbapenem-resistance, resides on a transmissible plasmid that can be transferred to other Enterobacteriaceae [3,9].

As a result, identifying patients at risk of infections by CRKP can facilitate the choice and finally the efficacy of the empirical therapy. Additionally this knowledge is important for the design and the implementation of interventions aiming to reduce the spread of antimicrobial resistance [10].

The aim of the present study was to identify risk factors for the development of CRKP acquisition. For this goal, in order to overcome the limitations and potential biases associated with case control studies, as described in the literature [10–16] an alternative and reliable design was used, the case–case-control study [9,17–19].

2. Materials and methods

2.1. Hospital setting and study population

This study was performed in the University Hospital of Heraklion, the only tertiary hospital in the island of Crete, Greece. The microbiology laboratory database was searched to identify all

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K. pneumoniae positive culture samples obtained from patients admitted to the Department of Internal Medicine over three years, i.e., from January 2009 through December 2011.

2.2. Microbiology and susceptibility testing

The isolates were identified to species level by conventional methods and the API 20E system (BioMérieux, Marcy l' Etoile, France) or the Vitek 2 automated system (BioMérieux) [20]. Antimicrobial susceptibilities were determined by the disk diffusion method or the Vitek 2 automated system (BioMérieux), and the results were interpreted according to the Clinical and Laboratory Standards Institute (CLSI). Furthermore the new revised CLSI breakpoints for carbapenems to categorize *Klebsiella* isolates as susceptible or resistant have been applied [21]. *Escherichia coli* ATCC 25922 and *Escherichia coli* ATCC 35218 were used as control strains. Identification of ESBL production was performed by phenotypic testing based on the demonstration of synergy between clavulanic acid and extended-spectrum cephalosporins [22]. Additionally, the modified Hodge test [MHT] was used for confirming carbapenemase production [21].

Finally molecular analysis for carbapenemase gene detection was not performed. However, phenotypic tests applied using specific carbapenemase inhibitors revealed that KPC production is the dominant mechanism for carbapenem resistance in *Klebsiella pneumoniae* in our hospital. Similarly, high prevalence of KPC-2-producing *Klebsiella pneumoniae* has been reported in our region and in all over Greece as well [23,24].

2.3. Case definition, control definition, and study design

A retrospective case–case-control study design was used. This approach involves the construction of 2 separate case-control studies by using two case groups and one control group. The first case group, the "resistant case group", consisted of patients from whom CRKP was isolated; the second case group, the "susceptible case group", consisted of patients from whom CSKP was isolated, and finally the control group consisted of patients from whom *K. pneumoniae* was never isolated during their hospitalization. We included only the initial culture yielding *Klebsiella pneumoniae* performed after the patient's admission in order to preserve the independence of the risk factors. Control patients were selected randomly from the source population admitted to the same Department during the same time period, after excluding patients from whom *K. pneumoniae* had been isolated. For each case patient, one control patient admitted to the same Department during the same month that *K. pneumoniae* was isolated was randomly selected.

2.4. Data collection

Data were extracted from the patients' medical records according to a standardized questionnaire. The following variables were collected: demographics (age and sex), comorbid conditions (cardiovascular, pulmonary, renal, hepatic, and central nervous system disease, diabetes mellitus, malignancy, an organ transplantation, and the overall number of comorbid conditions), recent ($\leq$7 days) invasive procedures (central venous, arterial, urinary catheterization and endotracheal intubation), recent ($\leq$1 month) surgical procedures, special treatments (corticosteroids, chemotherapy and blood products), intensive care unit (ICU) stay prior to *K. pneumoniae* isolation (for controls ICU stay at any time during their hospitalization), transfer from another hospital, source of the positive sample, and recent antimicrobial administration ($\leq$6 months). Time at risk was defined as the length of stay prior to a positive culture for the two case groups and the total length of hospitalization for the control group.

2.5. Statistical analysis

Statistical analysis was performed with SPSS 11.5 (SPPS Inc., Chicago, IL, USA). Bivariate analysis was conducted by chi-square test or Fisher's exact test, as appropriate, for categorical variables, and Student's *t*-test or Mann–Whitney *U* test (for not normally distributed data) for continuous variables. Variables with *p* value <0.1 on bivariate analysis were included in a stepwise logistic regression model for multivariable analysis. All tests were 2-tailed, and a *p* value of <0.05 was considered as statistically significant.

Two simultaneous multivariate models were produced from the data obtained. The first model compared the "resistant case group" with the control group. The second model compared the "susceptible case group" with the same control group. Qualitative analysis between the 2 models was then performed so as to determine specific risk factors for CRKP infection.

3. Results

During the study period, 83 patients with CRKP and 79 patients with CSKP were identified. As a control group, 161 patients admitted to the Department of Internal Medicine during the same study period with no cultures growing *K. pneumoniae* were randomly selected.

K. pneumoniae was isolated in both case groups predominantly from urine (59% in the CRKP group vs. 77% in the CSKP group, $p = 0.2$), followed by blood (23% in the CRKP vs. 10% in the CSKP group, $p = 0.04$) (Table 1).

3.1. Risk factor analysis: resistant case group vs. control

Characteristics of patients infected/colonized by CRKP and CSKP compared with the control group are shown in Tables 2 and 3.

In bivariate analysis, factors associated with CRKP acquisition were: older age, presence of chronic renal disease, autoimmune disease, urinary, or central venous catheter, endotracheal tube, mechanical ventilation prior surgical procedure, prior hospital admission, ICU admission, and prior use of antipseudomonal penicillins.

A multivariable analysis including all the above mentioned factors was performed. Due to the fact that the days at risk were more for cases than controls and the *p*-value for this comparison was below the threshold for inclusion in the multivariable model (mean 10.9 vs. 7.8 days, $p = 0.065$), the multivariable analysis was time adjusted and showed that factors independently predictive of CRKP infection/colonization were: admission to ICU (odds ratio (OR): 12.19, 95% confidence interval (CI): 2.19–66.67, $p = 0.004$), prior surgical procedure (OR: 4.05, 95% CI: 1.09–14.93, $p = 0.036$), and presence of renal disease (OR: 2.21, 95% CI: 1.05–4.68, $p = 0.037$) (Table 4).

Table 1
Source of *Klebsiella pneumoniae* isolation.

Source	[a]CRKP $n = 83$ (%)	[b]CSKP $n = 78$ (%)
Urine	49 (59)	60 (77)
Blood	19 (23)	8 (10)
Wound/Skin/Soft tissue	8 (10)	2 (3)
Respiratory	7 (8)	7 (9)
Unknown	0 (0)	1 (1)

[a] CRKP: Carbapenem-resistant *Klebsiella pneumoniae*..
[b] CSKP: Carbapenem-sensitive *Klebsiella pneumoniae*.