J Antimicrob Chemother 2011; **66**: 1383–1391
doi:10.1093/jac/dkr116 Advance Access publication 31 March 2011

Journal of Antimicrobial Chemotherapy

Antibiotic use and the risk of carbapenem-resistant extended-spectrum-β-lactamase-producing *Klebsiella pneumoniae* infection in hospitalized patients: results of a double case–control study

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Received 26 November 2010; returned 29 December 2010; revised 20 February 2011; accepted 26 February 2011

Objectives: To identify the roles of various antibiotics as risk factors for carbapenem-resistant extended-spectrum β-lactamase (ESBL)-producing *Klebsiella pneumoniae* (KP) infection (ESBL-KP infection).

Methods: Data were collected over 26 months in a tertiary care university hospital with established endemicity of carbapenem-resistant ESBL-KP (ESBL-CRKP). Using a case–case–control design, patients who presented an infection caused by carbapenem-susceptible ESBL-KP (ESBL-CSKP) and patients with ESBL-CRKP infection were compared with a common control group of hospitalized patients. Effects of treatment and duration of treatment with antibiotics were examined, adjusting for major non-antibiotic risk factors and controlling for confounding effects among the antibiotics via logistic regression models.

Results: Ninety-six ESBL-CRKP cases, 55 ESBL-CSKP cases and 151 controls were analysed. Multivariate analysis, adjusting for major non-antibiotic risk factors, showed that the risk of ESBL-CRKP infection rose with increasing duration of prior treatment with β-lactam/β-lactamase inhibitor combinations [odds ratio (OR) 1.15 per day increase; $P=0.001$] and revealed that increased duration of treatment with fluoroquinolones amplified the impact of exposure to carbapenems (and vice versa) on ESBL-CRKP infection risk (OR 1.02 for interaction term; $P=0.009$). Duration of prior treatment with fluoroquinolones was also associated with increased risk of ESBL-CSKP infection (OR 1.07 per day increase; $P=0.028$), while prior receipt of carbapenems presented a protective effect against ESBL-CSKP infection (OR 0.21; $P=0.003$).

Conclusions: This study highlights the major role of treatment and duration of treatment with β-lactam/β-lactamase inhibitor combinations and combinations of carbapenems with fluoroquinolones. Clinicians should counterweight the potential benefits of administering these antibiotics against the increased risk of ESBL-CRKP infection.

Keywords: multidrug resistance, risk factors, Enterobacteriaceae, healthcare-acquired infections

Introduction

Klebsiella pneumoniae (KP), one of the most common nosocomial pathogens, has caused universal concern in recent years because it is frequently isolated as a multidrug-resistant organism.[1] In many geographical regions, extended-spectrum β-lactamase (ESBL)-producing KP (ESBL-KP) has become endemic in hospitals at varying levels of intensity.[1–3] For infections caused by these strains, carbapenems have been the most reliable treatment option, but isolation of carbapenem-resistant KP (CRKP) has been increasingly reported.[1–3] These strains are difficult to control because they spread easily within and between hospitals,[4] and treatment options for infections caused by CRKP are limited.[2]

Identification of risk factors for infection with CRKP may help in the empirical therapeutic decision-making process and may assist in the early implementation of appropriate infection control measures.[5] However, information about risk factors for CRKP infection in hospitalized patients is scarce and inconsistent. Increased risk of colonization and/or infection with CRKP has been identified for: patients with poor functional status,[6] severe illness[7] and prolonged hospitalization;[5,8] patients admitted to intensive care units (ICUs);[6,9,10] and patients exposed to healthcare-associated risk factors such as organ or

stem-cell transplantation,[5] mechanical ventilation,[5,10] surgery,[10,11] transfer between units[11] and antecedent treatment with different antibiotics.[5–7,9,10,12]

Antibiotic selection pressure has been ascribed a potentially crucial role in the risk of CRKP infection, but findings implicating specific antibiotics have been diverse across published studies. Increased risk of colonization or infection has been most frequently, but inconsistently, associated with exposure to carbapenems,[5,6,9,12] fluoroquinolones,[6,7,9,10] cephalosporins[5,7,12] and β-lactam/β-lactamase inhibitor combinations.[10] In contrast, other researchers have noted no evidence of association between prior antibiotic exposure and CRKP acquisition,[4,11] while a protective effect of fluoroquinolones has also been reported.[12]

Most studies have employed the standard case–control study design to identify risk factors for CRKP.[5,7–10,12] Potential methodological shortcomings, mainly related to a suboptimal control group selection, may explain discrepant results in previous studies.[13,14] Moreover, previous studies did not examine interactions of several antibiotics and used qualitative (present/absent) antibiotic exposure variables during narrow exposure periods, thereby not accounting for potential dose dependencies or a possible cumulative effect of a lengthy period of antibiotic exposure.[14]

In this study we sought to identify risk factors for infection with carbapenem-resistant ESBL-producing KP (ESBL-CRKP), with emphasis on elucidating the role of antibiotics. For this purpose, we employed the case–case–control study design as suggested by Kaye *et al.*,[15] which enables more accurate identification of risk factors for antimicrobial-resistant pathogens than the standard case–control design. Using data collected over a 26 month period in a tertiary care university hospital with established endemicity of ESBL-CRKP, we conducted parallel analyses in which patients who presented an infection caused by carbapenem-susceptible ESBL-producing KP (ESBL-CSKP) and patients with ESBL-CRKP infection were compared with a common control group of uninfected hospitalized patients.

Methods

Setting and study design

The study was conducted in the University Hospital of Heraklion in Greece, a 750-bed tertiary care teaching hospital with approximately 55 000 admissions annually. Daily surveillance of clinical culture results to identify ESBL-producing isolates is part of the hospital's infection control programme. KP was the fourth most commonly isolated pathogen in our hospital, accounting for about 9% of all non-duplicate isolates recovered from inpatients in 2005. The ESBL phenotype in KP was endemic, accounting for 35% of all KP nosocomial isolates, with a hospital-wide incidence rate of 0.25 ESBL-KP isolates per 1000 patient-days. An increased frequency of ESBL-KP strains displaying reduced susceptibility to carbapenems was also noted during 2005. By the end of the year, endemic levels of carbapenem resistance had also been established, in about 37% of the ESBL-KP nosocomial isolates.

We therefore sought to identify risk factors for infection with ESBL-CRKP using a case–case–control study.[15] All adult patients (≥16 years of age) from whom a nosocomial ESBL-KP isolate had been recovered between February 2006 and March 2008 constituted the initial study cohort. Patients who had KP isolates recovered <48 h after hospital admission or in an outpatient setting were excluded from the study. The two case groups used in the double case–control study comprised patients who presented an infection caused by ESBL-CSKP and those infected by ESBL-CRKP. Infection was defined according to the CDC criteria.[16] Each case group was compared with a common control group of inpatients who had no clinical cultures positive for ESBL-KP during their hospitalization. For each case patient, one control patient was randomly selected from the same ward and during the same month that KP was isolated.

Data collection

Risk factor data were ascertained through review of medical records and direct interviews with the patients' attending physicians. The data were recorded within the period for which each patient was at risk, i.e. prior to KP isolation for case patients and prior to discharge for control patients. Demographic and clinical variables explored as possible risk factors included: age and sex; prior hospitalization; transfer from another healthcare facility; emergent admission; infection upon admission; severity of illness, as calculated by the Acute Physiology and Chronic Health Evaluation (APACHE) II score;[17] and underlying diseases and co-morbid conditions, in accordance with the weighted Charlson co-morbidity index.[18] Healthcare-associated exposures recorded were: length of hospital stay; admission and duration of stay in the ICU; surgery; exposure to invasive procedures (central vascular catheter, tracheostomy, mechanical ventilation and urinary catheterization); receipt of immunosuppressive therapy, including corticosteroids; renal replacement therapy; and treatment and duration of treatment with antibiotics. Severity of illness and co-morbidities were evaluated at the time of hospital admission. The time frame at risk was defined as 7 days for invasive procedures, 6 months for antibiotic use and surgery, and 12 months for prior hospitalization. All antibiotics that had been associated with CRKP risk in previous studies[5–7,9,10,12] were examined in this study. For purposes of analysis, these were grouped into carbapenems, β-lactam/β-lactamase inhibitor combinations, fluoroquinolones, second-generation cephalosporins, third- and fourth-generation cephalosporins, metronidazole and aminoglycosides.

Microbiological testing

Strain identification was performed with the API 20E system (bioMérieux SA, Marcy l'Étoile, France) or the Vitek 2 system (bioMérieux). Antimicrobial susceptibilities were determined by the disc diffusion method or the Vitek 2 system in accordance with the CLSI standards.[19] Identification of ESBL-producing strains was performed by phenotypic testing based on the synergy between clavulanic acid and extended-spectrum cephalosporins.[19]

Statistical analysis

To assess the impact of exposure of individual antibiotic groups on ESBL-CRKP and ESBL-CSKP infections and limit confounding by non-antimicrobial risk factors, we first calculated a prognostic score using non-antimicrobial variables and used this score in subsequent models to adjust for potential confounding. We used a logistic regression model for ESBL-KP infection risk to calculate the prognostic score as the predicted probability of infection for each patient, conditioned on that patient's specific non-antibiotic risk factors. In essence, prognostic scores represent a composite risk index summarizing the association of non-antibiotic risk factors with ESBL-KP infection risk and served as a summary confounder.[20,21]

Next, with ESBL-CSKP and ESBL-CRKP infection considered separately as outcomes in bivariate logistic regression models (in accordance with the case–case–control design), the effect of exposure to each antibiotic group was assessed, adjusting for non-antibiotic risk factors using the

prognostic score. Antibiotic exposures were examined as continuous variables (i.e. days of treatment with each antibiotic). Finally, because several antibiotics are often concomitantly or sequentially used to treat a patient, antibiotic groups significantly associated with each outcome were included in a multivariate logistic regression model adjusting for the prognostic score, again with either ESBL-CSKP or ESBL-CRKP infection as the outcome.

Logistic regression models were constructed using purposeful selection of variables.[22] Initially, the likelihood ratio test was used to examine bivariate associations of each potential risk factor with the outcome. Factors related to outcome with a conservative significance level of 0.25, along with all variables of potential clinical importance, were then included in a multiple logistic regression model. Variables that did not retain statistical significance in the multivariate model at the usual significance level of 0.05 were tested for confounding by adding them one at a time to the model and examining their effects on the β coefficients. Those causing substantial confounding (change in model coefficient of >10%) were retained in the model. Collinearity was examined using tolerance statistics. The Box–Tidwell test was performed for each continuous covariate (including days of treatment with antibiotics) to assess linearity in the log(odds) as required by logistic regression (i.e. an interaction term between each covariate and its natural logarithm was added to the model). If linearity was not observed, categorical scales for the covariate were examined on the basis of quartiles and logit graphs. After including the categorical analogue of the covariate in the model, adjacent categories that had model coefficients of similar magnitude were combined to form the final categorical scale for the covariate. The final logistic regression model was developed after evaluating effect modification. All possible two-way interaction terms were examined by adding them, one at a time, in the main effects model and testing them for statistical significance (likelihood ratio test $P<0.05$).

Model validity was assessed by estimating its goodness of fit (the extent to which the model reflects the data) using the Hosmer–Lemeshow test and by estimating its discrimination ability (the extent to which the model distinguishes case patients from control patients) using the area under the receiver operating characteristic (ROC) curve.[22] All analyses were performed using the SPSS 15.0 software package (SPSS Inc., Chicago, IL, USA).

Results

Study population

A total of 287 patients who had an ESBL-KP strain recovered were identified over the 26 month study period. Excluding ineligible cases, 151 adult inpatients who presented a nosocomial infection with ESBL-KP constituted the study cohort; 55 (36.4%) cases in the CSKP group and 96 (63.6%) cases in the CRKP group. The most common sites of infection were the urinary tract (45 cases; 29.8%), the bloodstream (38 cases; 25.2%), the respiratory tract (37 cases; 24.5%) and the surgical site (18 cases; 11.9%). ESBL-KP pathogenic isolates, both those susceptible and those resistant to carbapenems, had similar levels of co-resistance to other antibiotics. All isolates were resistant to piperacillin/tazobactam, 98% to ciprofloxacin, 90% to gentamicin, 88% to trimethoprim/sulfamethoxazole and 17% to colistin.

One hundred and fifty-one randomly selected patients without ESBL-KP infection were included as controls. There were no major differences in baseline demographic and clinical characteristics between control patients and ESBL-KP-infected patients, but case patients were significantly more likely than controls to have had numerous exposures to healthcare-associated factors, including longer hospital stay before infection, prior admission to the ICU and longer stay in the ICU at risk, and prior exposures to invasive devices and surgery (Table 1). The proportion of patients who had been exposed to any antibiotic in the 6 months preceding infection or discharge was similar for case patients and control patients, but case patients had received a significantly higher number of antibiotics. Previous use of carbapenems, β-lactam/β-lactamase inhibitor combinations, fluoroquinolones and aminoglycosides was significantly more common among case patients than control patients (Table 1).

Non-antibiotic risk factors for ESBL-KP infection

Control for confounding and effect modification among the non-antibiotic risk factors included in Table 1 revealed that longer stay in the hospital at risk [odds ratio (OR) 1.05 per day increase; 95% confidence interval (CI) 1.03–1.08; $P<0.001$], higher Charlson co-morbidity index (OR 1.14 per unit increase; 95% CI 1.00–1.31; $P=0.052$), central vascular catheterization (OR 5.22; 95% CI 2.38–11.46; $P<0.001$), urinary catheterization (OR 7.53; 95% CI 3.49–16.26; $P<0.001$) and tracheostomy (OR 5.18; 95% CI 1.81–14.88; $P=0.002$) were significantly associated with increased risk of ESBL-KP infection. A significant interaction between receipt of mechanical ventilation and urinary catheterization was also observed (OR 0.19 for interaction term; 95% CI 0.06–0.65; $P=0.008$); the effect of urinary catheterization was lower for mechanically ventilated patients (OR 1.44; 95% CI 0.55–3.74) compared with non-ventilated patients. The Hosmer–Lemeshow test indicated that the fit of the model to the data was adequate [$\chi^2(8)=8.26$; $P=0.408$] and the area under the ROC curve (0.85; 95% CI 0.81–0.89) indicated a good ability of the model to discriminate between ESBL-KP-infected patients and control patients. Using this explanatory model for infection risk, we constructed a prognostic score predicting each patient's probability of being infected with ESBL-KP conditioned on that patient's specific non-antibiotic risk factors, and used this score as a summary confounder in subsequent modelling of the effect of antibiotics.

Case–control study 1: ESBL-CSKP infection group versus controls

The unadjusted effects and the effects adjusted for prognostic score of being treated with each antibiotic are summarized in Table 2. Univariate (unadjusted) analysis disclosed that ESBL-CSKP case patients were more likely than control patients to have had longer treatment with carbapenems, β-lactam/β-lactamase inhibitor combinations, fluoroquinolones, second-generation cephalosporins and aminoglycosides. After adjusting for the prognostic score model to control for differences in non-antibiotic risk factors between the two patient groups, only exposure to carbapenems and duration of treatment with fluoroquinolones remained significantly associated with ESBL-CSKP infection. In our final model, which also examined potential confounding effects by other antibiotics, fluoroquinolones and carbapenems were significantly and independently associated with ESBL-CSKP infection. Fluoroquinolones exhibited a significant linear positive relationship between duration of exposure and risk of CSKP infection (OR 1.07 per day increase in treatment; $P=0.028$). Carbapenems had a threshold-type negative association with ESBL-CSKP infection risk (OR 0.21; $P=0.003$), with the risk for infection in patients treated with

Table 1. Demographic and clinical characteristics, exposures to healthcare-associated risk factors and exposures to antibiotics in patients infected with ESBL-KP (case patients) compared with uninfected control patients

Variable[a]	Case patients ($n=151$)	Control patients ($n=151$)	OR	95% CI	*P*
Demographic and clinical characteristics[b]					
male sex	92 (60.9)	86 (57.0)	1.18	0.74–1.86	0.483
age, years	67.0 ± 16.7	67.6 ± 19.0	1.00[d]	0.99–1.01	0.776
prior hospitalization	79 (52.3)	87 (57.6)	0.81	0.51–1.27	0.355
transfer from other institution	20 (13.2)	10 (6.6)	2.15	0.97–4.77	0.052
emergent admission	128 (84.8)	134 (88.7)	0.71	0.36–1.38	0.308
infection on admission	57 (37.7)	71 (47.0)	0.68	0.43–1.08	0.103
APACHE II score	13.7 ± 6.2	12.3 ± 7.1	1.03[d]	1.00–1.07	0.079
Charlson co-morbidity index	2.6 ± 2.3	2.3 ± 2.1	1.08[d]	0.97–1.20	0.159
Prior healthcare-associated exposures[c]					
hospital length of stay, days	26.5 ± 24.0	11.7 ± 9.7	1.07[d]	1.05–1.09	<0.001
ICU stay	86 (57.0)	42 (27.8)	3.43	2.12–5.55	<0.001
ICU length of stay, days	8.5 ± 12.7	2.2 ± 5.2	1.11[d]	1.07–1.16	<0.001
central vascular catheter	73 (48.3)	17 (11.3)	7.38	4.06–13.40	<0.001
indwelling urinary catheter	108 (71.5)	37 (24.5)	7.74	4.64–12.92	<0.001
mechanical ventilation	73 (48.3)	45 (29.8)	2.20	1.37–3.54	0.001
tracheostomy	47 (31.1)	7 (4.6)	9.30	4.04–21.39	<0.001
renal replacement therapy	15 (9.9)	7 (4.6)	2.27	0.90–5.73	0.073
surgery	83 (55.0)	47 (31.1)	2.70	1.69–4.32	<0.001
immunosuppressive therapy or chemotherapy	16 (10.6)	12 (7.9)	1.37	0.63–3.01	0.427
chronic use of corticoids	12 (7.9)	12 (7.9)	1.00	0.43–2.30	0.999
Prior antibiotic exposures[c]					
use of any antibiotic	138 (91.4)	134 (88.7)	1.35	0.63–2.88	0.411
number of antibiotic groups	4.3 ± 2.8	2.6 ± 1.8	1.37[d]	1.23–1.53	<0.001
carbapenems	79 (52.3)	31 (20.5)	4.25	2.56–7.06	<0.001
β-lactam/β-lactamase inhibitor combinations	77 (51.0)	45 (29.8)	2.45	1.53–3.93	<0.001
fluoroquinolones	110 (72.8)	76 (50.3)	2.65	1.64–4.28	<0.001
cephalosporins, second generation	35 (23.2)	38 (25.2)	0.90	0.53–1.52	0.687
cephalosporins, third and fourth generations	30 (19.9)	19 (12.6)	1.72	0.92–3.22	0.085
aminoglycosides	26 (17.2)	10 (6.6)	2.93	1.36–6.32	0.004
metronidazole	39 (25.8)	32 (21.2)	1.29	0.76–2.21	0.342

[a]Data are number (%) of patients or mean ± SD.
[b]Assessed at time of hospital admission.
[c]Assessed prior to outcome of interest (KP isolation for case patients; discharge for control patients). Time frame at risk was 7 days for invasive devices and 6 months for surgery and antibiotic use.
[d]OR corresponds to a unit increase in the continuous scale of the variable.

carbapenems being decreased regardless of duration of therapy. No interaction between the two antibiotics was observed (likelihood ratio test, $P=0.155$). The Hosmer–Lemeshow goodness-of-fit test indicated that the model reflected the data well [$\chi^2(8)=8.11$; $P=0.422$] and the area under the ROC curve (0.86; 95% CI 0.80–0.91) indicated a good ability of the model to discriminate between ESBL-CSKP case patients and control patients.

Case–control study 2: ESBL-CRKP infection group versus controls

Antibiotic risk factor analysis for ESBL-CRKP infection is presented in Table 3. Univariate (unadjusted) analysis showed that case patients were more likely than control patients to have been exposed to carbapenems, β-lactam/β-lactamase inhibitor combinations, fluoroquinolones and aminoglycosides. Apart from aminoglycosides, these antibiotic groups also appeared to be significantly associated with increased ESBL-CRKP infection risk after controlling for differences in non-antibiotic risk factors between the two patient groups and for potential confounding effects among the antibiotics. In our final model, days of exposure to β-lactam/β-lactamase inhibitor combinations showed a positive relationship with ESBL-CRKP infection risk (OR 1.15 per day increase; $P=0.001$). Exposures to carbapenems and fluoroquinolones presented a significant interaction effect (OR 1.02 for interaction term; $P=0.009$). Longer exposures to both carbapenems and fluoroquinolones were related to increased risk of ESBL-CRKP infection, such that increased exposure to one antibiotic group boosted the impact of exposure

Table 2. Effect of antibiotic treatment as a risk factor for ESBL-CSKP infection

Prior treatment with antibiotics	ESBL-CSKP case patients ($n=55$)	Control patients ($n=151$)	Unadjusted effect		Adjusted for prognostic score		Adjusted for prognostic score and other antibiotics	
			OR (95% CI)	*P*	OR (95% CI)	*P*	OR (95% CI)	*P*
Carbapenems	13 (23.6)	31 (20.5)	1.20 (0.57–2.50)	0.633	0.25 (0.09–0.70)	0.005	0.21 (0.07–0.64)	0.003
days of treatment (mean ± SD)	14.5 ± 6.3	8.6 ± 5.5	1.06 (1.00–1.12)	0.052	0.95 (0.88–1.02)	0.126		
β-Lactam/β-lactamase inhibitor combinations	20 (36.4)	45 (29.8)	1.35 (0.70–2.58)	0.374	0.87 (0.39–1.91)	0.727		
days of treatment (mean ± SD)	7.4 ± 4.9	5.5 ± 3.4	1.08 (0.99–1.17)	0.074	1.01 (0.92–1.11)	0.854		
Fluoroquinolones	34 (61.8)	76 (50.3)	1.60 (0.85–3.00)	0.142	0.98 (0.46–2.11)	0.968		
days of treatment (mean ± SD)	11.6 ± 7.6	7.6 ± 5.1	1.08 (1.03–1.13)	0.001	1.06 (1.00–1.12)	0.055	1.07 (1.00–1.14)	0.028
Cephalosporins, second generation	17 (30.9)	38 (25.2)	1.33 (0.67–2.63)	0.414	0.96 (0.42–2.20)	0.926		
days of treatment (mean ± SD)	10.0 ± 6.7	5.4 ± 4.3	1.09 (1.02–1.17)	0.012	1.07 (0.98–1.17)	0.111		
Cephalosporins, third and fourth generations	11 (20.0)	19 (12.6)	1.74 (0.77–3.93)	0.194	1.59 (0.59–4.29)	0.366		
days of treatment (mean ± SD)	8.7 ± 4.1	6.8 ± 6.3	1.07 (0.98–1.16)	0.116	1.07 (0.97–1.18)	0.189		
Aminoglycosides	10 (18.2)	10 (6.6)	3.13 (1.23–8.01)	0.019	1.97 (0.62–6.27)	0.251		
days of treatment (mean ± SD)	7.1 ± 3.6	6.1 ± 4.7	1.15 (1.02–1.31)	0.023	1.02 (0.88–1.18)	0.770		
Metronidazole	15 (27.3)	32 (21.2)	1.39 (0.69–2.84)	0.364	0.98 (0.42–2.30)	0.960		
days of treatment (mean ± SD)	8.5 ± 7.0	6.9 ± 5.4	1.04 (0.98–1.12)	0.214	1.01 (0.92–1.10)	0.899		

Case patients are patients with ESBL-CSKP infection. Control patients are patients who had no clinical cultures positive for ESBL-KP during their hospitalization. Data represent number (%) of patients who were treated with the antibiotic in the 6 months preceding KP isolation for case patients or discharge for control patients. Mean and SD for days of treatment were calculated for patients who were exposed to each antibiotic.

to the other antibiotic group on ESBL-CRKP infection risk (Figure 1). The Hosmer–Lemeshow goodness-of-fit statistic [$\chi^2(8)=10.13$; $P=0.256$] indicated a good fit of the final model. The area under the ROC curve was 0.92 (95% CI 0.89–0.96), indicating an excellent discrimination ability of the model.

Discussion

In this study we sought to identify hospitalized patients who were at increased risk of infection with ESBL-CRKP, focusing on the role of antibiotic use as a risk factor. Our analysis showed that the risk of infection with ESBL-CRKP rose with increasing duration of prior treatment with β-lactam/β-lactamase inhibitor combinations, fluoroquinolones and carbapenems. An interaction effect between the latter two antibiotic groups was noted, revealing that increased exposure to fluoroquinolones amplified the impact of exposure to carbapenems (and vice versa) on ESBL-CRKP infection risk. Duration of prior treatment with fluoroquinolones was also associated with increased risk of infection with ESBL-CSKP in this study, while prior receipt of carbapenems presented a protective effect against ESBL-CSKP infection.

The antibiotics mentioned above are among those that have been most frequently, but inconsistently, implicated with increased risk of colonization or infection with CRKP in previous studies.[5–7,9,10,12] Diverse results produced by prior studies, as opposed to the findings of the present study, should be interpreted within the strengths and limitations of study design and data analysis. Appropriate control group selection, case definition, description of the extent of prior antibiotic exposure and adjustment for confounding factors are major issues that have been emphasized, debated and refined in methodological studies.[13–15,23–26]

Patients harbouring CSKP constituted the control group in most of the previously published case–control studies that assessed antibiotic use as a risk factor for CRKP.[5,7,9,10] However, such patients constitute a small proportion of the population giving rise to the cases and their exclusive use as control subjects may create selection bias that distorts (probably overestimates) the effects of antibiotics active against the susceptible, but not the resistant, form of the pathogen.[13–15,23] This limitation has been acknowledged in prior studies that identified the use of carbapenems as a risk factor for CRKP, and researchers were cautious or inconclusive regarding a potential role of these antibiotics.[5,9] By contrast, control patients in the present study consisted of patients potentially at risk of developing the infection who were selected from the same wards and with the same index time as case patients, to reduce both selection bias[13–15] and bias resulting from non-comparable ward environments.[27] Importantly, the case–case–control design protected our analysis from overestimating the effect of an antibiotic as a risk factor for CRKP infection solely because it may be protective against becoming part of the control group.[13–15] Indeed, our analyses showed that exposure to carbapenems was positively associated with ESBL-CRKP infection, but negatively associated with ESBL-CSKP infection. By distinguishing these effects, our approach offers further evidence that exposure to carbapenems is a risk factor for infection with ESBL-CRKP.

Table 3. Effect of antibiotic treatment as a risk factor for ESBL-CRKP infection

Prior treatment with antibiotics	ESBL-CRKP case patients ($n=96$)	Control patients ($n=151$)	Unadjusted effect		Adjusted for prognostic score		Adjusted for prognostic score and other antibiotics	
			OR (95% CI)	*P*	OR (95% CI)	*P*	OR (95% CI)	*P*
Carbapenems	66 (68.8)	31 (20.5)	8.52 (4.74–15.29)	<0.001	4.42 (2.15–9.11)	<0.001		
days of treatment (mean ± SD)	13.1 ± 9.6	8.6 ± 5.5	1.18 (1.12–1.25)	<0.001	1.10 (1.03–1.17)	0.001	0.96 (0.86–1.07)[a]	0.462
β-Lactam/β-lactamase combinations	57 (59.4)	45 (29.8)	3.44 (2.01–5.89)	<0.001	2.39 (1.19–4.84)	0.015		
days of treatment (mean ± SD)	10.5 ± 6.8	5.5 ± 3.4	1.22 (1.14–1.30)	<0.001	1.17 (1.08–1.27)	<0.001	1.15 (1.05–1.26)	0.001
Fluoroquinolones	76 (79.2)	76 (50.3)	3.75 (2.09–6.74)	<0.001	2.09 (0.99–4.42)	0.052		
days of treatment (mean ± SD)	11.4 ± 7.7	7.6 ± 5.1	1.13 (1.08–1.19)	<0.001	1.10 (1.04–1.17)	0.001	1.01 (0.94–1.08)[b]	0.830
Cephalosporins, second generation	18 (18.8)	38 (25.2)	0.69 (0.37–1.29)	0.236	0.41 (0.17–0.99)	0.043		
days of treatment (mean ± SD)	5.1 ± 2.8	5.4 ± 4.3	0.95 (0.86–1.04)	0.260	0.89 (0.78–1.03)	0.106		
Cephalosporins, third and fourth generations	19 (19.8)	19 (12.6)	1.71 (0.86–3.44)	0.130	2.11 (0.80–5.57)	0.132		
days of treatment (mean ± SD)	7.5 ± 4.9	6.8 ± 6.3	1.05 (0.98–1.14)	0.168	1.07 (0.97–1.18)	0.205		
Aminoglycosides	16 (16.7)	10 (6.6)	2.82 (1.22–6.51)	0.013	2.35 (0.77–7.18)	0.132		
days of treatment (mean ± SD)	7.8 ± 9.8	6.1 ± 4.7	1.11 (0.99–1.24)	0.081	1.00 (0.90–1.12)	0.936		
Metronidazole	24 (25.0)	32 (21.2)	1.24 (0.68–2.27)	0.488	1.09 (0.49–2.43)	0.824		
days of treatment (mean ± SD)	8.3 ± 6.6	6.9 ± 5.4	1.03 (0.97–1.10)	0.267	1.02 (0.93–1.11)	0.680		
Interaction between carbapenems and fluoroquinolones (days)							1.02 (1.00–1.04)	0.009

Case patients are patients with ESBL-CRKP infection. Control patients are patients who had no clinical cultures positive for ESBL-KP during their hospitalization. Data represent number (%) of patients who were treated with the antibiotic in the 6 months preceding KP isolation for case patients or discharge for control patients. Mean and SD for days of treatment were calculated for patients who were exposed to each antibiotic.

[a]OR for a day increase in treatment with carbapenems in the absence of exposure to fluoroquinolones.

[b]OR for a day increase in treatment with fluoroquinolones in the absence of exposure to carbapenems.

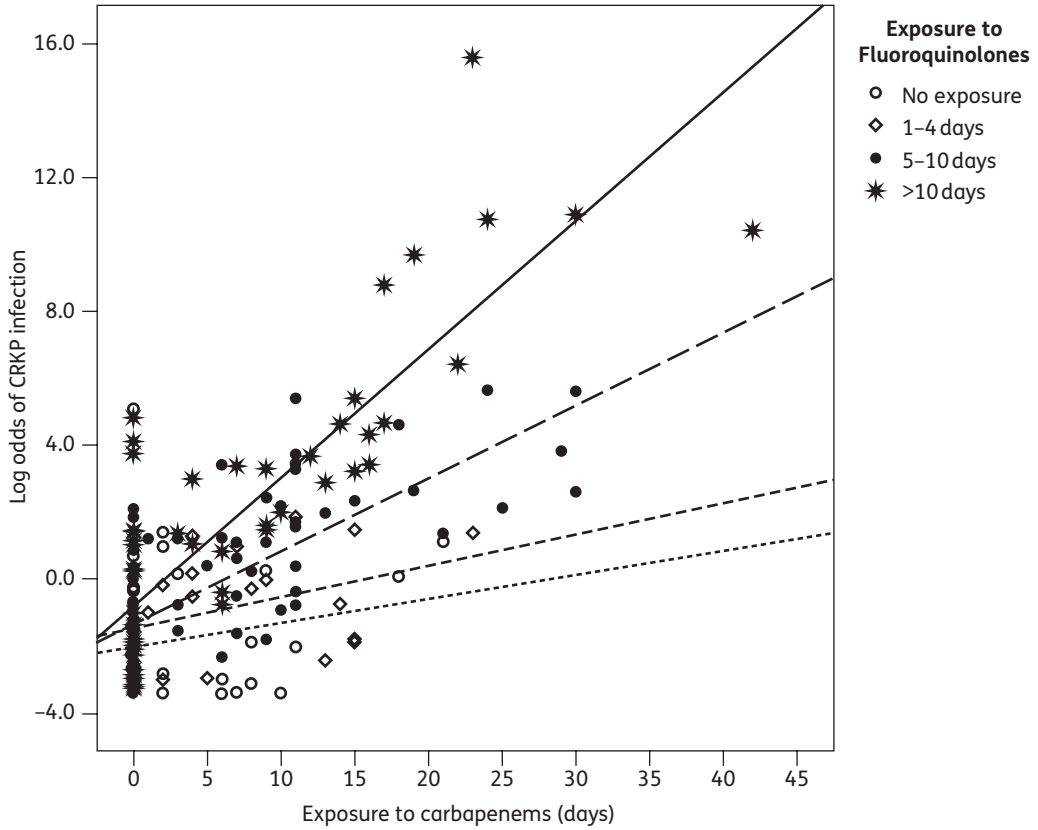

Figure 1. Scatter plot with fitted regression lines presenting the interaction effect of carbapenems and fluoroquinolones on the risk of ESBL-CRKP infection. Fitted regression lines represent the association between the risk of infection and exposure to carbapenems for various levels of exposure to fluoroquinolones. From bottom to top of the figure, the lines correspond to no exposure and 1–4, 5–10 and >10 days of exposure to fluoroquinolones.

Questions also arise regarding the various degrees of resistance and the phenotypes of resistant isolates included in previous studies of risk factors for CRKP. Prior studies did not report the complete susceptibility profiles of both CSKP and CRKP isolates,[5–7,10,11] or had significantly different co-resistance patterns in the two groups of isolates.[9] Therefore, it is unclear whether distinct co-resistance patterns were treated as a unique entity in previous studies, which would partly explain the discrepancies in study findings pertaining to which antibiotics are risk factors.[25] In the present study, we restricted our case definitions to include patients infected with KP strains that were ESBL producers, thereby observing similar levels of co-resistance to fluoroquinolones, aminoglycosides, trimethoprim/sulfamethoxazole and colistin in ESBL-CSKP and ESBL-CRKP pathogenic isolates. Thus, our analysis is unlikely to have been complicated by unidentified co-resistance patterns and pertains specifically to the carbapenem resistance phenotype.

The method of describing the extent of prior antibiotic exposure and the time interval prior to the positive culture result during which the exposure is assessed are two important, but neglected, issues in studies of antimicrobial resistance determinants.[26,27] Most prior studies investigating the impact of antibiotic exposures on CRKP risk assessed antibiotic use within narrow periods of 14–30 days prior to recovery of CRKP,[8,10] or limited data collection during the period of hospital stay.[7,11,12] However, the risk associated with antibiotic exposure is probably cumulative,[14] and there may be considerable variability in antibiotic consumption before hospitalization.[27] Moreover, previous studies have included only receipt of antibiotics, ignoring a potential impact of treatment duration. However, it has been noted that describing antibiotic use as a dichotomous variable reduces statistical power to detect associations and may result in data misinterpretation.[26] To provide a more robust characterization of antibiotic exposures in the present study, we recorded both treatment and duration of treatment with antibiotics in the 6 months preceding infection for case patients or discharge for control patients. This time window for data collection is broader compared with other studies, but the information was readily available either in the medical records or by directly interviewing the patients' attending physicians. Inclusion of prior antibiotic exposures as continuous variables in this study revealed dose–response effects of antibiotics on the risk of ESBL-CRKP infection, which was seen to increase with increasing duration of prior treatment with β-lactam/β-lactamase inhibitor combinations, fluoroquinolones and carbapenems. These findings are consistent with recent work that noted the benefit of short-duration high-dose courses as a means to limit unnecessary antibiotic exposure and thus reduce the emergence of resistance.[28] Moreover, the interaction effect between fluoroquinolones and carbapenems detected in this study emphasizes

that, given the multidrug-resistant nature of CRKP, reduction of the exposure to a particular class of antibiotics may not suffice to alter the selection pressure that allows these pathogens to thrive.

Time at risk, severity of illness and co-morbidity constitute the minimum set of confounders recommended to be taken into account in studies analysing risk factors for antibiotic-resistant pathogens.[13,14] We accounted for these confounders in our analyses, but we also found that several medical interventions and invasive devices were significantly more common among case patients than control patients, thereby also inducing potential confounding effects. Inclusion of a single numerical measure for the several non-antibiotic risk factors identified in this study and use of this score as a summary confounder in multivariable models enabled us to assess the independent effects of antibiotics while retaining an allowable level in the dimensionality of analysis.[20,21] Future studies may need to assess the usefulness and applicability of methods to address confounding by study design, such as the incidence density sampling approach to selecting control patients. The utility of summary confounder scores, such as propensity scores and disease risk scores,[21] may also need further study in the context of examining the effect of treatment duration for several antibiotics in case–case–control studies.

Despite our effort to improve upon several methodological and analytical issues in this study, several potential limitations remain. First, we relied on clinical culture results, rather than active surveillance screening, to detect case patients in this study. It is therefore possible that some control patients may actually have been ESBL-KP carriers. Second, inaccuracies in current automated susceptibility testing to detect carbapenem resistance[29] may have led to the misclassification of some patients between the ESBL-CSKP and ESBL-CRKP case groups in our study. Third, molecular analysis was not performed and details of the kind of carbapenemase were not available. The dominant mechanism for carbapenem resistance in KP in our region is the production of KPC-2,[30] but other mechanisms may also exist among isolates harboured by our study patients and it is possible that the effect of antibiotics may differ according to the mechanism of resistance. Moreover, we cannot conclude definitely whether any outbreak or clonal spread influenced our results, although we found no epidemiological evidence of spatial or temporal clustering of CRKP-infected patients in our institution during the study period.

In conclusion, this study highlights a major role of antibiotic usage in the risk of ESBL-CRKP infection in an endemic setting. Our analysis suggests that ESBL-CRKP infection risk rises with increasing duration of prior treatment with β-lactam/β-lactamase inhibitor combinations and combinations of carbapenems with fluoroquinolones. Clinicians should counterweight the potential benefits of administering these antibiotics against the increased risk of ESBL-CRKP infection.

Funding

This study was performed without external financial support. All data required for the study have been generated as part of the routine work of the institution at which the study was carried out.

Transparency declarations

None to declare.

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