Risk factors for bloodstream infections caused by extended-spectrum β-lactamase-producing *Escherichia coli* and *Klebsiella pneumoniae*

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ABSTRACT

The objective of this study was to identify risk factors for bacteremia by extended-spectrum β-lactamase (ESBL)-producing *Escherichia coli* and *Klebsiella pneumoniae*. Retrospective case-control study performed in a 450-bed acute care academic tertiary hospital in Barcelona, Spain. Cases included 53 patients with ESBL-producing *E. coli* or *K. pneumoniae* bacteremia, and 159 controls with non-ESBL-producing *E. coli* or *K. pneumoniae* bacteremia. Controls were matched in a 3:1 ratio to case patients according to species of infecting organism, age, and severity of illness in the 24-48h before blood sample collection for culture calculated by the Simplified Acute Physiology Score (SAPS II) system. Previous antimicrobials were more frequently administered to cases than to controls (56.5% *vs* 17%, $p < 0.001$). Binary logistic regression showed that the number (> 2) of different families of antimicrobials received within 90 days before bloodstream infection was the only predictor of ESBL-producing *E. coli* or *K. pneumoniae* in blood culture (OR = 2.29, 95% CI 1.35-3.88, $p = 0.002$). Conclusion: Previous use of different families of antimicrobials (more than two) in patients with bloodstream infection caused by *E. coli* or *K. pneumoniae* increased the risk for ESBL-producing strains.

Keywords: bacteremia; β-lactamases; risk factors.

An increase of extended-spectrum β-lactamase (ESBL)-producing organisms isolated from blood cultures has emerged in recent years.[1] Despite the fact that previous use of antimicrobial therapy has been frequently associated with the identification of ESBL-producing organisms, there is a large heterogeneity in the obtained results in different studies, which may be partially attributed to methodological differences. A retrospective case-control study in patients with bloodstream infections caused by ESBL-producing *Escherichia coli* and *Klebsiella pneumoniae* was carried out to identify risk factors associated with these ESBL -producing organisms.

The investigation was conducted at *Hospital Universitario del Mar*, a 450-bed acute care academic tertiary hospital located in Barcelona, Spain. A matched case-control study was designed. All case and control patients were retrospectively identified through the bloodstream infection database of the hospital Infection Control Program. All patients with bloodstream infection for whom culture results were positive for *E. coli* or *K. pneumoniae* from January 2000 to December 2006 were eligible for inclusion in the study. Designation as a case patient or a control patient was based solely on whether the infecting organism was found to demonstrate ESBL resistance or not.

Each patient was included as a case patient only once. If ESBL-producing *E. coli* or *K. pneumoniae* was isolated on multiple occasions, only the first episode of infection was considered. Controls were identified among hospitalized patients with bloodstream infection who were infected with the non-ESBL-producing *E. coli* or *K. pneumoniae* during the same period. Controls were matched in a 3:1 ratio to case patients according to the following variables: species of infecting organism, age, and severity of illness in the 24-48h before blood sample collection for culture calculated by the Simplified Acute Physiology Score (SAPS II) system.[2]

Submitted on: 02/08/2011
Approved on: 04/18/2011

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We declare no conflict of interest.

For missing values, a replacement was calculated using the mean value of the result preceding, and the result after, the missing one.[3]

In those patients in whom the sample was drawn for blood culture on hospital admission, the SAPS II score on admission was considered.

Nosocomial acquisition of infection was considered when the infection occurred > 48h after admission to the hospital, while community-acquired bacteremia when it occurred < 48 h after admission to the hospital. Acquisition of infection in the outpatient setting was also considered in patients with bloodstream infection secondary to diagnostic or therapeutic procedures carried out in the outpatient setting, outpatients who were carriers of urinary or intravenous catheters, bloodstream infections in chronic hemodialysis and peritoneal dialysis patients, and bacteremias occurring in patients admitted to nursing homes or long-term care facilities. Infection that occurred > 48h after admission to the intensive care unit (ICU) was defined as ICU-acquired bacteremia.

Potential risk factors for ESBL-producing *E. coli* or *K. pneumoniae* infection were ascertained by abstracting medical records. Data obtained included age, sex, underlying illness, severity of illness on hospital admission and 24-48h before blood sampling for culture both calculated by the SAPS II score, salient features of the patient's care, invasive procedures performed within 30 days prior to the episode of bacteremia, organisms isolated within 90 days prior to the episode of bacteremia, site of acquisition of infection, site of infection, antimicrobial sensitivity pattern of strains isolated in blood culture, antimicrobials administered within 90 days prior to the episode of bacteremia, immunosuppressive therapy, empirical and definitive antimicrobials administered for the treatment of bacteremia, and crude mortality. Severe neutropenia was defined as absolute neutrophil count < 500 neutrophils/μL and absolute neutropenia as < 100 neutrophils/μL.[4] Immunosuppressive therapy included the administration of corticoids, cytostatic agents and/or immunosuppressants within 30 days prior to the episode of bacteremia. Antimicrobial treatment was defined as adequate when an active antimicrobial agent (as determined by *in vitro* susceptibility testing) was prescribed at appropriate doses, at appropriate dosing intervals, and by the correct route[5] and with the capacity to achieve adequate concentrations in the infectious focus. Antibiotic treatment was defined as inappropriate when the aforementioned definition was not met.

Identification of strains of *E. coli* and *K. pneumoniae* was determined by the Vitek system (bioMérieux). According to the Clinical and Laboratory Standards Institute (CLSI), sensitivity of the isolated strains against one of the following antimicrobials was assessed: cefotaxime, ceftriaxone, ceftazidime, cefpodoxime, or aztreonam was initially determined using a disk diffusion method or a broth microdilution assay based on the minimal inhibitory concentration (MIC).[6] If results of this first test were positive, a phenotypic confirmatory test with cefotaxime and ceftazidime alone and in combination with clavulanic acid using a disk diffusion method or a microdilution assay was performed.

The presence of ESBL-producing organisms was confirmed by an increase of > 5 mm in inhibition halo of cefotaxime or ceftazidime after the addition of clavulanic acid by the disk diffusion method, and by a greater than three-fold reduction in the MICs of either ceftazidime or cefotaxime in the microdilution assay.

Data were analyzed using the Statistical Package for the Social Sciences (SPSS, version 12.0). Variables related to ESBL-producing *E. coli* or *K. pneumoniae* in the univariate analysis for which a p-value < 0.15 was found in the comparison of cases and controls were considered for inclusion in a binary logistic regression model, with the exception of those presenting a high number of missing data. In addition, certain key variables based on a priori hypothesis or empirical knowledge believed to be related to the dependent variable were also included in the model. A backward stepwise selection procedure was used with the likelihood ratio test as the criterion for determining variables to be excluded (variation < 15%). The strength of the association between the explanatory variables in the model and the dependent variable was established by odds ratios (ORs) and the 95% confidence intervals (CIs). The hypothesis that the logistic model adequately fitted the data was tested by means of the goodness-of-fit χ^2 test and the predictive capacity by the receiver operating characteristics (ROC) curve. Survival curves in cases and controls from isolation of ESBL-producing and non-ESBL-producing *E. coli* or *K. pneumoniae* until death and from hospital admission until death were obtained by the Kaplan Meier method.

During the study period, 4,172 episodes of bloodstream infections were identified, 1,218 (29.2%) of which were caused by *E. coli* and 226 (5.4%) by *K. pneumoniae*. ESBL-producing *E. coli* or *K. pneumoniae* were identified in 42 and 11 case patients, respectively. Controls included 126 episodes of bloodstream infection caused by *E. coli* and 33 by *K. pneumoniae*. There were statistically significant differences between cases and controls in the percentage of males, patients with malignant hematologic diseases, urgent hospital admission, ICU admission, length of ICU stay, mechanical ventilation, invasive procedure, days with catheter in place, hemodialysis, use of corticoids and cytostatic agents, and length of hospital stay. Moreover, community-acquired bacteremia was significantly more frequent among controls and nosocomial bloodstream infection among cases (Table 1). Primary bacteremia was considered in 6 (11,3%) cases and in 9 (5.7%) controls, and secondary bacteremia in 47 (88.7%) cases and in 150 (94.3%) controls. Main sources

Table 1. Demographic, clinical and microbiological characteristics of patients with bloodstream infection due to ESBL-producing* E. coli *or* K. pneumoniae *(cases) and non- ESBL-producing* E. coli *or* K. pneumoniae *(controls)

Data	Cases (n = 53)	Controls (n = 159)	p-value
Men	36 (67.9)*	71 (44.7)	0.004
Age, years, mean (95% CI)	69.1 (64.6-73.6)	71.1 (64.6-73.6)	0.416
Acquisition of infection			< 0.001
Hospital	25 (47.2)	35 (22.0)	
Community	19 (35.8)	117 (73.6)	
ICU	9 (17)	7 (4.4)	
Hospital admission in the previous 3 months	3 (5.7)	15 (9.4)	0.571
Clinical characteristics			
Solid tumor	14 (26.4)	39 (24.5)	0.855
Malignant hematologic disease	9 (17.0)	10 (6.3)	0.026
Hypertension	22 (41.5)	78 (49.1)	0.427
Dyslipidemia	4 (7.5)	27 (17)	0.117
Diabetes mellitus	16 (30.2)	34 (21.4)	0.196
Chronic obstructive pulmonary disease	8 (15.1)	20 (12.6)	0.643
Chronic heart failure	1 (1.9)	12 (7.5)	0.193
Renal failure	11 (20.8)	21 (13.2)	0.190
Liver dysfunction	3 (5.7)	4 (2.5)	0.370
Hepatitis C virus (HCV) infection	2 (3.8)	13 (8.2)	0.279
HIV infection	2 (3.8)	3 (1.9)	0.433
Renal transplantation	2 (3.8)	2 (1.3)	0.261
SAPS II score, mean (95% CI)			
On admission	30.5 (28.2-32.7)	28.8 (27.2-30.4)	0.153
24-48 h before blood sampling	32 (29.6-34.4)	31.2 (28.8-33.2)	0.318
Urgent hospital admission	40 (75.5)	148 (93.1)	0.002
Infection-related hospital admission	24 (45.3)	86 (54.1)	0.272
Clinical data before identification of causative pathogen in blood culture			
ICU admission	9 (17.0)	7 (4.4)	0.005
ICU stay, days, mean (95% CI)	28.1 (16.8-39.5)	13.4 (6.8-20.1)	0.020
Mechanical ventilation	13 (24.5)	10 (6.3)	0.001
Days on mechanical ventilation, mean (95% CI)	22.7 (10.8-34.5)	10.5 (2.3-18.7)	0.148
Days with catheter in place, mean (95% CI)	22.7 (10.8-34.5)	10.5 (2.3-18.7)	0.148
Carrier of urinary catheter	9 (17.0)	7 (4.4)	0.005
Carrier of feeding tube	28.1 (16.8-39.5)	13.4 (6.8-20.1)	0.020
Invasive procedure	13 (24.5)	10 (6.3)	0.001
Hemodialysis	22.7 (10.8-34.5)	10.5 (2.3-18.7)	0.148
Corticoids	22.7 (10.8-34.5)	10.5 (2.3-18.7)	0.148
Cytostatic agents	10 (18.9)	11 (6.9)	0.017
Immunosuppressants	2 (3.8)	3 (1.9)	0.601
Neutropenia ≤ 500 neutrophils/μL	5 (9.4)	9 (5.7)	0.346
Neutropenia ≤ 100 neutrophils/μL	5 (9.4)	8 (5.0)	0.319
Length hospital stay, days, mean (95% CI)	18.8 (12.8-24.8)	4.9 (3.6-6.1)	< 0.001
Microbiological data before identification of causative pathogen in blood culture			
Previous culture performed	36 (67.9)	26 (16.3)	< 0.001
ESBL-producing *E. coli* or *K. pneumoniae*	8/36 (22.2)	1/26 (3.8)	0.009

* Data as numbers and percentages in parenthesis unless otherwise stated.

of infection in secondary bacteraemia were urinary tract infection [19 (40.4%) *vs* 94 (62.7%)], gastrointestinal tract infection [6 (12.8%) *vs* 27 (18,0%)], intravascular device-related infection [9 (19.1%) *vs* 3 (3.0%)], pneumonia [3 (6.4%) *vs* 8 (5.3%)], surgical infection [4 (8.5%) *vs* 2 (1.3%)], respiratory infection (excluding pneumonia) [1 (2.1%) *vs* 4 (2.7%)], and skin and soft tissue infection [0 (0%) *vs* 1 (0.7%)].

On the other hand, isolation of ESBL-producing *E. coli* or *K. pneumoniae* within 90 days prior to the index episode was significantly more frequent among cases (22.2%, 8/36) than controls (3.8%, 1/26) (p = 0.009). Antimicrobials prior to isolation of the causative pathogen in blood cultures were more frequently administered to cases than controls (56.5% *vs* 17%, p < 0.001). Details of antimicrobial treatment are shown in Table 2.

In the binary logistic regression model adjusted by SAPS II on hospital admission, the number of different families of antimicrobials received within 90 days before the episode of bloodstream infection was the only significant factor associated with identification of ESBL-producing *E. coli* or *K. pneumoniae* in blood culture (OR = 2.29, 95% CI 1.35-3.88, p = 0.002). In addition, when the number of different families of antimicrobials was categorized as ≤ 2 and > 2 (the cut point that allowed a more homogeneous distribution of patients in the different categories), the use of > 2 different families of antimicrobials in the previous 90 days was the only variable independently associated with identification of ESBL-producing *E. coli* or *K. pneumoniae* in blood culture (OR = 12.50, 95% CI 2.50-62.42, p = 0.002). The goodness-of-fit test did not reach statistical significance (p = 0.390). The area under the ROC curve was 0.718 (95% CI 0.58-0.85, p = 0.005).

A total of 47 patients who did not receive empirical antimicrobials or in whom empirical treatment was inadequate, were then treated with adequate definitive antimicrobial therapy. Of these patients, 24 (51.1%) were cases and 23 (48.9%) were controls.

The mean number of hours until the use of adequate de-

Table 2. Antimicrobial treatment administered to patients with bloodstream infection due to ESBL-producing* E. coli *or* K. pneumoniae *(cases) and non-ESBL-producing* E. coli *or* K. pneumoniae *(controls) within 90 days before isolation of pathogen in blood culture

Data	Cases (n = 53)	Controls (n = 159)	p-value
All patients			
Types of antimicrobials, mean (95% CI)	1.9 (1.3-2.6)*	0.3 (0.2-0.4)	< 0.001
Different antimicrobial families, mean (95% CI)	1.7 (1.1-2.3)	0.3 (0.2-0.3)	< 0.001
Patients with previous antibiotic treatment			
Number of patients	30 (56.6)	27 (17)	< 0.001
Days on antimicrobial treatment, mean (95% CI)	38.9 (25.9-51.8)	13.7 (9.8-17.6)	0.002
Types of antimicrobials, mean (95% CI)	3.4 (2.5-4.3)	1.6 (1.3-1.8)	0.001
Antimicrobial families, mean (95% CI)	3.1 (2.3-3.8)	1.5 (1.2-1.8)	0.003
Different antimicrobial families, mean (95% CI)	3.0 (2.3-3.7)	1.4 (1.2-1.7)	0.002
Aminoglycosides	8 (26.7)	2 (7.4)	0.083
Carbapenems	4 (13.3)	3 (11.1)	1.000
Cephalosporins	16 (53.3)	7 (25.9)	0.058
Colistin	2 (6.7)	0	0.492
Glycopeptides	12 (40.0)	2 (7.4)	0.005
Lincosamides	0	1 (3.7)	0.474
Macrolides	3 (10.0)	0	0.239
Nitroimidazoles	5 (16.7)	1 (3.7)	0.197
Oxazolidinones	4 (13.3)	0	0.114
Penicillins	16 (53.3)	15 (55.6)	1.000
Quinolones	14 (46.7)	7 (25.9)	0.169
Sulfamides	4 (13.3)	1 (3.7)	0.356
Tuberculostatic agents	2 (6.7)	0	0.492

* Data as numbers and percentages in parenthesis unless otherwise stated.

finitive antimicrobial treatment was 46.08 (95% CI 25.92-66) in cases and 71.04 (95% CI 46.08-96.0) in controls (p = 0.111).

The crude mortality rate was 50.9% (27/53) in cases and 13.2% (21/159) in controls (p < 0.001). The time elapsed from isolation of the causative pathogen in blood culture until death was statistically significant between cases (median 26 days, 95% CI 5.2-46.8) and controls (median 94 days, 95% CI 28.5-159.5) (p < 0.001) (Figure 1). There was a trend to a longer length of hospital stay from hospital admission to death in cases (median 53 days, 95% CI 30.0-76.0) in comparison to controls (median 118 days, 95% CI 49.7-186.3) (p = 0.073) (Figure 2).

The results of this study show that the administration of different families of antimicrobials during a period of 90 days before the episode of bloodstream infection was the only risk factor for the identification of ESBL-producing *E. coli* or *K. pneumoniae* in blood culture. In addition, those patients that previously received more than two different families of antimicrobials showed a risk of at least 2.5 times higher than patients treated with fewer antimicrobial families. Although the model showed good calibration, it had moderate discriminatory power [area under the ROC curve was 0.718 (95% CI 0.58-0.85, p = 0.005)].

Bloodstream infection caused by ESBL-producing *E. coli* or *K. pneumoniae* was not associated with previous antimicrobial treatment, use of individual agents of each antimicrobial family or a particular antimicrobial agent. Previous use of antimicrobial treatment has been one of the factors more extensively related to identification of ESBL-producing *E. coli* and *K. pneumoniae* in patients infected and/or colonized by these microorganisms.[7-9] Almost half of the case patients received delayed adequate antimicrobial treatment. Not considering the possibility of an ESBL-producing microorganisms isolation could be the cause of this delay.

Data reported in the literature regarding risk factors for ESBL-producing organisms are heterogeneous due, in part, to the inclusion of both infected and colonized patients in the different series. In order to minimize this bias, only patients with documented bloodstream infection were included in our study.

In contrast to our results, isolation of ESBL-producing strains in most studies has been associated with the use of specific types and classes of antimicrobial agents. Previous β-lactam antibiotics, mainly cephalosporins, have been identified as predictive factors for infection with ESBL-producing *E. coli* or *K. pneumoniae*.[10-12]

In a retrospective analysis of 2,172 episodes of healthcare-associated bacteremia diagnosed during a 3-year period in a teaching hospital, previous use of cephalosporins and carbapenems were significantly associated with ESBL-producing *E. coli* or *Klebsiella* spp.[13] In a prospective case-control study of bacteremia caused by *E. coli*, previous use of oxyimino-β-lactams or fluoroquinolones were independent risk factors among hospitalized patients for ESBL-producing strains.[14] Other studies, however, have found an association between previous exposure to antimicrobials in general and identification of ESBL-producing strains in patients with bacteremia caused by *K. pneumoniae*.[15]

In a retrospective case-control study in patients with *K. pneumoniae* bacteremia, for the addition of each antimicrobial agent, an increase of 1.55 in the risk of ESBL-producing *K. pneumoniae* strains was observed.[16]

Although a large number of studies included in their objectives to assess the association between use of antibiotics and the emergence of resistance, the systems used to define the prior use of antimicrobials have not been properly

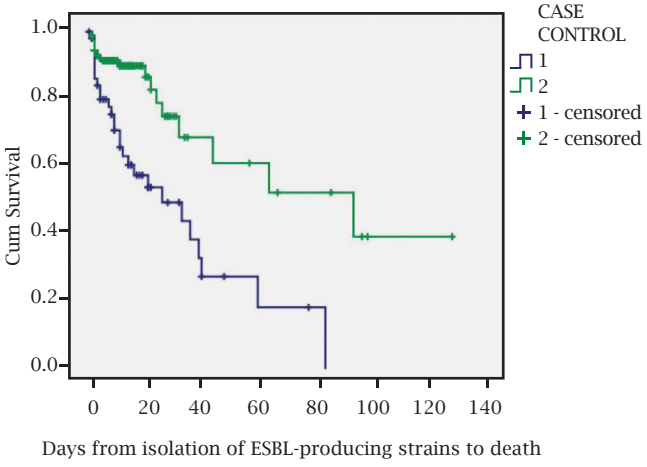

Figure 1: Actuarial curve for survival from isolation of ESBL-producing *E. coli* or *K. pneumoniae* to death.

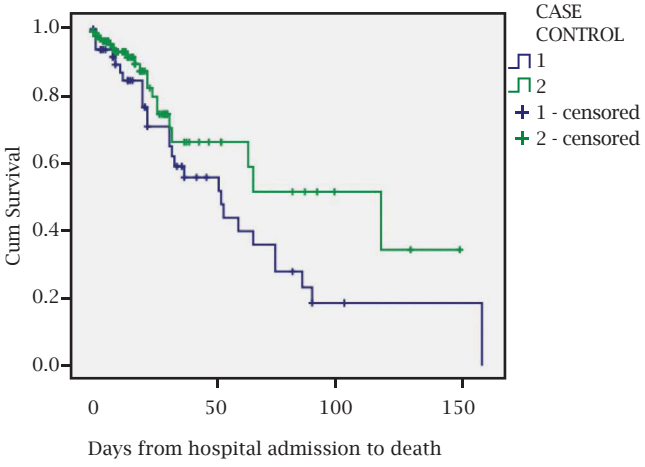

Figure 2: Actuarial curve for survival from hospital admission to death.

described. Thus, the selection of one or other method of categorization can lead to the identification of different risk factors.[17] For this reason, it is important to clarify these issues for the design of strategies aimed at reducing resistances.

Other variables associated with the identification of ESBL-producing strains in patients with bloodstream infection reported in the literature are the patient's age,[15] severe underlying diseases,[13] renal transplantation,[13] previous admission to the ICU,[18] duration of hospitalization before bacteremia,[19] prior exposure to urinary catheters,[10,14,16] invasive procedure within the previous 72 hours,[16] and the nosocomial origin of bacteremia.[10]

In a systematic review of studies evaluating the association between inappropriate antibiotic therapy and mortality among bacteremic patients, measurement of severity of illness without specified the time at which it was measured was considered a source of methodological heterogeneity that may explain conflicting findings.[20] Our study adhered to key recommendations of this review to reduce the effect of potential confounders by assessing severity of illness through the SAPS II score.[21]

The present study was designed as a retrospective case-control study in which controls were matched to cases according to three variables, including species of infecting organisms, patient's age and SAPS II score in the 24-48h before blood sampling for culture. Limitations of the study include the retrospective design, the sample size and the lack of inclusion of the bacteremic focus as a selection criterion for controls. In order to overcome this limitation, controls were matched in a 3:1 ratio to case patients.

The identification of patients with risk factors for ESBL-producing organisms is essential to target adequate early empirical antimicrobial therapy. Previous use of more than two different families of antimicrobials in patients with bloodstream infection caused by *E. coli* or *K. pneumoniae* increased the risk for ESBL-producing strains. This variable should be considered as a potential risk factor for bacteremia by ESBL-producing organisms.

ACKNOWLEDGEMENTS

Manuscript preparation: Marta Pulido, MD, provided assistance in preparing and editing the manuscript.

REFERENCES

1. Biedenbach DJ, Moet GJ, Jones RN. Occurrence and antimicrobial resistance pattern comparisons among bloodstream infection isolates from the SENTRY Antimicrobial Surveillance Program (1997-2002). Diagn Microbiol Infect Dis 2004; 50:59-69.
2. Le Gall JR, Lemeshow S, Saulnier F. A new Simplified Acute Physiology Score (SAPS II) based on a European/North American multicenter study. JAMA 1993; 270:2957-63.
3. Marra AR, Bearman GML, Wenzel RP, Edmond MB. Comparison of severity of illness scoring system for patients with nosocomial bloodstream infection due to *Pseudomonas aeruginosa.* BMC Infect Dis 2006; 6:132.
4. Hutson PR. Hematology: red and white blood cell tests. In: Lee M, ed. Basic skills in interpreting laboratory data. Bethesda: American Society of Health-System Pharmacists 2004; 441-67.
5. Siempos I, Ioannidou E, Falagas M. The difference between adequate and appropriate antimicrobial treatment. Clin Infect Dis 2008; 46:642-4.
6. Performance standards for antimicrobial susceptibility testing: 20th informational supplement. Twentieth Informational Supplement. Clinical and Laboratory Standards Institute (CLSI); 2010: M100-S20.
7. Friedmann R, Raveh D, Zartzer E et al. Prospective evaluation of colonization with extended-spectrum β-lactamase (ESBL)-producing Enterobacteriaceae among patients at hospital admission and of subsequent colonization with ESBL-producing Enterobacteriaceae among patients during hospitalization. Infect Control Hosp Epidemiol 2009; 30:534-42.
8. Graffunder EM, Preston KE, Evans AM, Venezia RA. Risk factors associated with extended-spectrum β-lactamase-producing organisms at a tertiary care hospital. J Antimicrob Chemother 2005; 56:139-45.
9. Lautenbach E, Patel JB, Bilker WB, Edelstein PH, Fishman NO. Extended-spectrum β-lactamase-producing *Escherichia coli* and *Klebsiella pneumoniae*: risk factors for infection and impact of resistance on outcomes. Clin Infect Dis 2001; 32:1162-71.
10. Ortega M, Marco F, Soriano A et al. Analysis of 4758 *Escherichia coli* bacteraemia episodes: predictive factors for isolation of an antibiotic-resistant strain and their impact on the outcome. J Antimicrob Chemother 2009; 63:568-74.
11. Du B, Long Y, Liu H et al. Extended-spectrum β-lactamase-producing *Escherichia coli* and *Klebsiella pneumoniae* bloodstream infection: risk factors and clinical outcome. Intensive Care Med 2002; 28:1718-23.
12. Paterson DL, Ko WC, Von Gottberg A et al. International prospective study of *Klebsiella pneumoniae* bacteremia: implications of extended-spectrum β-lactamase production in nosocomial Infections. Ann Intern Med 2004; 140:26-32.
13. Martínez JA, Aguilar J, Almela M et al. Prior use of carbapenems may be a significant risk factor for extended-spectrum β-lactamase-producing *Escherichia coli* or *Klebsiella* spp. in patients with bacteraemia. J Antimicrob Chemother 2006; 58:1082-5.
14. Rodríguez-Baño J, Navarro MD, Romero L et al. Risk-factors for emerging bloodstream infections caused by extended-spectrum β-lactamase-producing *Escherichia coli*. Clin Microbiol Infect 2008; 14:180-3.
15. Tumbarello M, Spanu T, Sanguinetti M et al. Bloodstream infections caused by extended-spectrum-β-lactamase-producing *Klebsiella pneumoniae*: risk factors, molecular epidemiology, and clinical outcome. Antimicrob Agents Chemother 2006; 50:498-504.
16. Kang CI, Kim SH, Kim DM et al. Risk factors for and clinical outcomes of bloodstream infections caused by extended-spectrum β-lactamase-producing *Klebsiella pneumoniae*. Infect Control Hosp Epidemiol 2004; 25:860-7.
17. MacAdam H, Zaoutis TE, Gasink LB, Bilker WB, Lautenbach E. Investigating the association between antibiotic use and antibiotic resistance: impact of different methods of categorising prior antibiotic use. Int J Antimicrob Agents 2006; 28:325-32.

18. Mosqueda-Gómez JL, Montaño-Loza A, Rolón AL et al. Molecular epidemiology and risk factors of bloodstream infections caused by extended-spectrum β-lactamase-producing *Klebsiella pneumoniae*: A case-control study. Int J Infect Dis 2008; 12:653-9.
19. Serefhanoglu K, Turan H, Timurkaynak FE, Arslan H. Bloodstream infections caused by ESBL-producing *Escherichia coli* and *Klebsiella pneumoniae*: risk factors for multidrug-resistance. Braz J Infect Dis 2009; 13:403-7.
20. McGregor JC, Rich SE, Harris AD et al. A systematic review of the methods used to assess the association between appropriate antibiotic therapy and mortality in bacteremic patients. Clin Infect Dis 2007; 45:329-37.
21. Wunsch H, Linde-Zwirble WT, Angus DC. Methods to adjust for bias and confounding in critical care health services research involving observational data. J Crit Care 2006; 21:1-7.