Risk Factors for Colonization with Third-Generation Cephalosporin-Resistant Enterobacteriaceae

C. Wendt, D. Lin, H. von Baum

Abstract

Background and Method: Colonization and infections caused by Enterobacteriaceae resistant to third-generation cephalosporins (CRE) have been observed with increasing frequency in intensive care unit (ICU) patients. In contrast to outbreak investigations, information about risk factors for colonization in an endemic situation are rare. We studied risk factors for colonization with CRE in a case control study including 1,706 patients, admitted to any of the 15 ICUs of Heidelberg University Hospitals.

Results: 163 patients carried CRE with *Enterobacter* spp. representing the predominant species. Independent risk factors for CRE carriage in the multivariate logistic regression analysis were an age of under 2.5 years (OR 4.034), an indwelling central venous catheter (CVC) for more than 3 days (OR 2.640), treatment with second- or third-generation cephalosporin for longer than 3 days (OR 2.260) and any antibiotic therapy before admission to the ICU.

Conclusion: Apart from the well-recognized risk factor previous antibiotic treatment, the risk factors age and presence of a CVC might suggest that bacterial overgrowth of the gut either due to an increased susceptibility in younger age or as a consequence of parenteral nutrition is a relevant mechanism for acquiring carriage of CRE in a non-outbreak situation.

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Introduction

Colonization and infections caused by Enterobacteriaceae resistant to third-generation cephalosporins (CRE) have been observed with increasing frequency. Risk factors for acquiring CRE have been studied mainly in outbreak situations. The factors associated with acquisition of CRE in these circumstances included central venous catheter (CVC), previous antibiotic treatment, parenteral nutrition and previous treatment with third-generation cephalosporins and aminoglycosides [1, 2]. However, risk factors that have been identified in outbreak situations may differ from factors that are associated with acquisition for CRE in an endemic setting.

We had conducted a repetitive point prevalence study in 1,851 patients of 16 intensive care units (ICUs) and identified 186 carriers of Enterobacteriaceae resistant to third-generation cephalosporins. In the neonatal intensive care unit (NICU) we had verified an outbreak with a highly resistant *Enterobacter* spp. (reported elsewhere [3]). In the remaining 15 ICUs no outbreak was suspected; thus, risk factors for colonization with CRE in an endemic setting could be investigated.

Methods

Setting

The University of Heidelberg Hospitals is a 1,600 bed tertiary care center with approximately 50,000 admissions per year. All kinds of medical services, including solid organ and bone marrow transplantation are offered – in some cases in close cooperation with adjacent specialized hospitals, i.e. an orthopedic hospital and a hospital for pulmonary diseases. A maximum of 179 patients can be treated in 16 ICUs: eight surgical, five medical, and three pediatric units.

Study Population

For the prospective point prevalence study all patients admitted to one of the 16 ICUs of Heidelberg University Hospitals during a 26-week period, starting in October 2000, were eligible. Patients admitted to the NICU (n = 145) were excluded due to an outbreak during the study period caused by CRE in this department. Data and perirectal swabs to screen for CRE were collected once weekly and all patients who were treated in one of the units on these study days were included in the study, irrespective of their length of stay.

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Microbiology

CRE were defined as isolates of Enterobacteriaceae resistant to ceftazidime (MIC ≥ 32 mg/l) and cefpodoxime (MIC ≥ 8 mg/l). Colonization with CRE was present if CRE were isolated from at least one of the perirectal swabs of the patient.

Case Control Study

A case control study was performed to identify factors that were associated with colonization with CRE in our patient population. Cases were defined as patients who were colonized or infected with CRE, whereas all other patients served as controls. Data that were abstracted from the charts included demographic data, kind and severity of underlying diseases and cause of admission to the ICU, and available information about treatment before admission to the ICU. Data on antibiotic therapy and use of invasive devices were stratified according to the length of their usage into none, usage of less than or equal to 3 days and more than 3 days. Data that included time intervals were counted backwards from the day of first identification of CRE in cases or the day of the last negative investigation in controls.

Statistical analysis was performed using either the χ^2 test or Fisher's exact test, as appropriate, to evaluate the significance of differences between categorical variables. The differences between continuous variables were evaluated using the t-test. Alpha was set at 0.01 to control for multiple testing and all tests were performed two-tailed. A logistic regression model was calculated including all variables that were significantly associated with the dependent variable, i.e. infection or colonization with CRE. In a second model we investigated the influence of the underlying diseases by excluding them from the model and analyzing the changes that followed from the exclusion. The analyses were performed using stepwise forward inclusion of the independent variables. To estimate multivariate odds ratios (OR) for variables that were independently associated with colonization or infection with CRE the variables were simplified to binomial values and a nominal regression was calculated. The data analysis was performed on SPSS (SPSS Inc., Chicago, IL).

Results

A total of 1,706 patients were included into the analysis: 163 patients carried CRE and were consequently considered as cases. Of the 163 patients, 101 patients were already colonized at the time of the first investigation, whereas 62 patients had previously negative swabs. Most patients were colonized with *Enterobacter* spp. (n = 121), followed by *Citrobacter* spp. (n = 25) and *Escherichia coli* (n = 7). Ten patients were colonized with other gram-negative bacteria (*Morganella* spp., *Hafnia* spp., *Serratia* spp. and *Proteus* spp.). Eight patients who were carrying extended-spectrum beta-lactamase (ESBL)-producing Enterobacteriaceae were not included in the study, because this group seemed to be too small for reliable analysis. Patients not carrying CRE (n = 1,543) were chosen as controls.

Table 1
Characteristics of the patient population (n = 1,706).

Age (mean, range)	55 years (0–95 years)
Gender male	58.3%
ICU surgical	61.5%
Medical	28.8%
Pediatric	9.7%
Previous surgery	55.2%
Admission from other unit	59.3%
Devices that were used	
CVC	62.1%
Mechanical ventilation	25.9%
Urinary catheter	53.7%
Antibiotic treatment	66.2%

The mean age of the patients was 55 years and 41.7% were female (Table 1). 61% of the study patients stayed in surgical ICUs; more than half of these patients were in the unit for perioperative supervision. 62.1% of all patients had indwelling CVCs; 25% were on mechanical ventilation. Only one-third of the patients did not receive antibiotic treatment.

Factors that were associated with colonization with CRE by univariate analysis are presented in table 2. Not significantly associated with CRE colonization were gender, MacCabe and Jackson score, suprapubic catheterization, and gastrostomy tubes. Underlying diseases that were not associated with CRE colonization included: cardiac diseases, vascular diseases, elevated blood pressure, endocrinological diseases, benign gastrointestinal lesions, terminal renal failure, viral infection, substance abuse, other cerebral diseases, complications in pregnancy, solid carcinomas, and leukemia or lymphoma.

Age of the patients was identified as a significant risk factor with younger patients having a higher risk for CRE carriership. This finding is reflected by the fact that preterm infants and children with congenital disorders had an increased risk for CRE carriage. Patients suffering from typical diseases of an older age-group like cerebral infarction or benign neoplasms, however, were significantly less likely to be CRE carriers. Further risk factors in the univariate analysis were indicators for a prolonged and complicated clinical course like median length of stay in the hospital and ICU, transferal and antibiotic therapy prior to ICU admission and presence of invasive devices like CVCs, Foley catheters or mechanical ventilation for longer than 72 h.

If considering previous antibiotic therapies administration of aminoglycosides and second- and third-generation cephalosporins were significant risk factors for colonization with CRE, whereas quinolones, cotrimoxazole, glycopeptides, imidazole, lincosamides, macrolides, penems and β-lactamase combinations were not associated with CRE colonization.

For multivariate analysis only risk factors with a significance level of < 0.01 were included (Table 3). Both analyses identified presence of a CVC and antibiotic therapy before admission to the ICU and antibiotic therapy with second- and third-generation cephalosporins as independent risk factors. In model 1 several underlying conditions like preterm infancy, presence of congenital heart disease as well as chronic obstructive pulmonary diseases, liver cirrhosis,

renal failure and previous bacterial infections were risk factors, whereas presence of a benign solid tumor was protective. Some of these conditions were strongly age related; thus these variables were excluded in the second model and, as expected, the second model was similar to the first, but age turned out to be an additional risk factor.

A third model was calculated to estimate the magnitude of the ORs associated with the risk factors. The highest OR compared to the basic case (patient older than 2.5 years without indwelling CVC, no or short treatment of less than 3 days with second- or third-generation cephalosporin and no antibiotic treatment before admission to ICU) was associated with an age of less than 2.5 years (CI_{95} 2.501 to 6.506), i.e. younger patients had a 4 times higher risk to be colonized or infected with CRE (Table 4).

Discussion

Resistant bacterial pathogens like CRE are increasingly prevalent in ICU populations and are associated with increased patient morbidity and mortality as well as prolonged hospital stays [4–6]. This development has a substantial impact on the choice of antibiotic therapies and the implementation of infection control measures. Several authors have observed Enterobacteriaceae that developed resistance while undergoing extensive antibiotic treatment [8]. *Muller* [9] et al. demonstrated a specific correlation

Table 2
Factors that were associated with colonization or infection with CRE in the univariate analysis.

	Controls (n = 1,543)	Cases (n = 163)	P-value	OR (95% CI)
Age (mean)	55.54	48.83	0.007	
Length of stay in the hospital (mean)	14.77	23.37	0.001	
Length of stay in the ICU (mean)	7.05	9.61	0.003	
Underlying disease				
Congenital cardiac malformation	3.6%	16.0%	< 0.001	5.134 (3.120–8.450)
COPD asthma	5.0%	11.0%	0.001	2.363 (1.376–4.059)
Liver cirrhosis	2.0%	5.5%	0.005	2.850 (1.333–6.098)
Bacterial infection	9.5%	17.2%	0.002	1.985 (1.277–3.085)
Cerebral infarction	7.8%	1.2%	0.002	0.147 (0.036–0.601)
Premature newborn	1.4%	4.9%	0.001	3.741 (1.630–8.586)
Congenital malformation	1.6%	6.7%	< 0.001	4.580 (2.201–9.532)
Solid neoplasms benign	3.8%	0.0%	0.001	0.901 (0.887–0.916)
Surgery none	46.8%	36.2%	0.006	
Elective	45.8%	58.9%		
Emergency	7.4%	4.9%		
Admission from other unit	57.7%	74.2%	< 0.001	2.111 (1.465–3.041)
Antibiotic treatment before admission to ICU	27.9%	45.2%	< 0.001	2.126 (1.519–2.976)
CVC none	43.5%	27.0%	< 0.001	
≤ 3 days	23.8%	12.3%		
> 3 days	32.7%	60.7%		
Catheterization transurethral none	43.6%	42.3%	< 0.001	
≤ 3 days	24.2%	9.8%		
> 3 days	32.3%	47.9%		
Intubation none	81.5%	68.7%	< 0.001	
≤ 3 days	6.4%	6.1%		
> 3 days	12.1%	25.2%		
Aminoglycoside none	92.9%	84.7%	0.001	
≤ 3 days	3.4%	6.1%		
> 3 days	3.7%	9.2%		
Cephalosporins I none	99.0%	99.4%	0.856	
≤ 3 days	1.0%	0.6%		
> 3 days	0.1%	0.0%		
Cephalosporins II none	80.9%	68.1%	< 0.001	
≤ 3 days	13.2%	10.4%		
> 3 days	6.0%	21.5%		
Cephalosporins III none	86.8%	73.0%	< 0.001	
≤ 3 days	5.2%	10.4%		
> 3 days	8.0%	16.6%		

COPD: chronic obstructive pulmonary disease

Table 3
Results of multivariate logistic regression.

Model 1		Model 2	
Factor	**Regression coefficient B**	**Factor**	**Regression coefficient B**
Congenital cardiac malformation	1.379		
COPD asthma	0.896		
Liver cirrhosis	1.197		
Bacterial infection	0.696		
Premature newborn	1.262		
Solid neoplasms benign	-18.620	Age	-0.008
Antibiotic treatment before admission to ICU		Antibiotic treatment before admission to ICU	0.499
CVC none		CVC none	
≤ 3 days	0.03	≤ 3 days	0.090
> 3 days	0.815	> 3 days	0.872
Cephalosporins II none		Cephalosporins II none	
≤ 3 days	-0.096	≤ 3 days	-2.01
> 3 days	1.230	> 3 days	1.213
Cephalosporins III none		Cephalosporins III none	
≤ 3 days	0.762	≤ 3 days	0.739
> 3 days	0.486	> 3 days	0.576

between ceftriaxone use and development of resistance in *Enterobacter cloacae* clinical isolates and *Kim* et al. [10] found that previous antibiotic treatment with extended-spectrum cephalosporins was independently associated with bacteremia due to resistant isolates of *Citrobacter freundii*. *Vollaard* et al. [11] propagated that cefotaxime may facilitate colonization with resistant Enterobacteriaceae. Other investigators showed that expanded-spectrum cephalosporins were more likely to select resistant strains of Enterobacteriaceae if compared to other β-lactams and that restriction of cephalosporin use reduced colonization with multiresistant bacteria in a neonatal unit [12–14]. In accordance with these observations, our study identified previous use of cephalosporins as an independent risk factor for colonization with CRE. However, the study made no differentiation between preexisting colonization at admission and new acquisition of CRE and as a consequence no information is available whether the treatment with cephalosporins led to selection of resistant isolates in patients who carried the pathogen endogenously. Alternatively the antibiotic pressure may have promoted an exogenous transmission route.

Age of the patients was identified as an additional significant risk factor with younger patients having a higher risk for CRE carriership. This finding is reflected by the fact that preterm infants and children with congenital disorders had an increased risk for CRE carriage. Patients suffering from typical diseases of an older age-group like cerebral infarction or benign tumors, however, were significantly less likely to be CRE carriers. Two aspects should be considered as explanations for these findings: first, an increased probability of nosocomial transmissions of CRE due to a more frequent and intensified contact between health-care workers (HCW) and younger pa-

Table 4
Estimation of the odds ratio for significant risk factors compared to the basic case: patient older than 2.5 years without indwelling CVC, no or treatment of less than 3 days with cephalosporin second- or third-generation and no antibiotic treatment before admission to ICU.

	Significance	**Exp(B) OR**	**95% CI for Exp(B)**	
Treatment with cephalosporin second- or third-generation for > 3 days	0.000	2.260	1.546	3.303
Indwelling CVC for > 3 days	0.000	2.640	1.851	3.765
Age less than 2.5 years	0.000	4.034	2.501	6.506
Antibiotic therapy before admission to ICU	0.014	1.544	1.092	2.182

tients [3]. Pulsed-field electrophoresis (PFGE) typing of the isolates showed that in pediatric ICUs small clusters of cross transmission occurred in the absence of an obvious outbreak [3]. However, many children were carrying an individual strain that was not shared with others. The second explanation may be the possibility of an age-related bacterial overgrowth of the gut with CRE. This assumption is supported by the work of several investigators who showed that the gut of young children is especially sensitive to bacterial overgrowth [15–17]. Our study included only patients in an ICU and many of these patients received mechanical ventilation and had Foley catheters and indwelling central lines. Thus, we were surprised to find that only presence of an indwelling CVC was an independent risk factor for CRE colonization. In outbreak situations presence of a CVC had been identified as a risk factor for isolation of resistant Enterobacteriaceae [1, 18]. However, in outbreak situations this can also be explained by handling failures of the devices. In an endemic setting, though, presence of a CVC might be regarded as a surrogate marker for patients receiving parenteral nutrition. We did not collect data on the kind of nutrition of the patients, but it can be assumed that especially many of the surgical patients were on parenteral nutrition. It has been suggested that parenteral nutrition promotes bacterial overgrowth with gram-negative bacilli [19]. This might be an explanation, why patients with previous antibiotic therapies plus parenteral nutrition seemed to be more likely to be colonized with CRE.

The generalizability of our results may be somewhat limited by the study design. We included only patients who were in the unit on the study days. Thus, patients with very short stays were underrepresented in our study population. To account for this, we refrained from calculating the relative risk, although that would have been possible because we collected our data prospectively from the cohort. Instead of this, we used the OR to describe associations between risk factor and colonization with CRE. For many variables the underrepresentation of patients with a short stay in the unit may have rather weakened the strength of the association.

Another limitation may have been the inclusion of patients already colonized with CRE at the time when the first swab was taken. In these patients it is almost impossible to differentiate between factors that were the reason for colonization and those which might have been a consequence of colonization. However, this misclassification might have only weakened the strength of association. A third limitation that arises from a similar problem is that we defined risk factors in cases as those that were present in the days before the first positive swab was taken, although in general the time between two swabs was 7 days. Thus, we cannot exclude that some risk factors in fact were present only after colonization with CRE had taken place. Therefore, we used two classes of presence – less than 4 days and more than 3 days – for factors that may have a time related association, i.e. duration of treatment. The analysis demonstrated that the association was usually stronger when the factor was present during a longer time period.

In conclusion, the results of this study, investigating a large population of ICU patients in an endemic setting, demonstrate that in addition to the accepted risk factor previous antibiotic treatment, age and an indwelling CVC are the main risk factors for colonization with CRE. Both factors might indicate that overgrowing of the gut could be a major mechanism for colonization with resistant gram-negative enterobacteria.

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