Risk factors for, and clinical relevance of, faecal extended-spectrum β-lactamase producing (ESBL-EC) carriage in neutropenic patients with haematological malignancies

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Risk Factors for, and Clinical Relevance of, Faecal Extended-Spectrum β-Lactamase producing *Escherichia coli* (ESBL-EC) Carriage in Neutropenic Patients with Haematological Malignancies.

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ABSTRACT

Purpose: To assess the risk factors for, and the clinical relevance of faecal carriage by extended-spectrum β-lactamase producing *Escherichia coli* (ESBL-EC) in neutropenic cancer patients (NCP).

Methods: An observational prospective multicentre cohort study was conducted during two years at two teaching hospitals. Patients with acute leukaemia or undergoing stem cell transplantation were included during neutropenia episodes. Rectal swabs were obtained at hospital admission and weekly thereafter until discharge or death. ESBL-EC colonized episodes were compared with non-colonized episodes. ESBL-EC strains were studied by PCR and isoelectric focusing, and molecular typing was performed by PFGE.

Results: Among 217 episodes of neutropenia, the prevalence of ESBL-EC faecal carriage was 29%, 14% at hospital admission. Multivariate analysis identified previous antibiotics as the only independent risk factor for ESBL-EC faecal colonization (OR: 5.38; 95% CI: 2.79-10.39). Analysis of ESBL-EC isolates revealed a polyclonal distribution with CTX-M predominance (81.3%). *E. coli* bacteraemia was mainly caused by non-ESBL producing strains and its rate was similar in both groups (13% vs 11%). We found no association between ESBL-EC carriage and an increased risk of ESBL-EC bacteremia or a negative influence on other clinical outcomes, including length of hospitalisation, early and overall mortality rates.

Conclusions: ESBL-EC faecal colonization is frequent in NCP but difficult to identify by epidemiological or clinical features on presentation. Prior antibiotic therapy is the major associated risk factor. In this setting colonization does not appear

to have a significant clinical relevance. Thus, routine testing for ESBL-EC faecal carriage does not seem to be beneficial.

INTRODUCTION

Infectious complications remain a major cause of morbidity and mortality in neutropenic cancer patients (NCP). *Escherichia coli* is one of the commonest causes of bacteraemia in this patient population, and so the development of antimicrobial resistance in *E. coli* is a cause of special concern in NCP. In the last decade, there has been a marked increase in colonization and infection due to quinolone-resistant *E. coli*, probably as a consequence of the widespread use of fluoroquinolone prophylaxis for febrile neutropenic episodes [1-4]. More recently, other resistance mechanisms in *E. coli*, such as extended-spectrum β-lactamase (ESBL) production, have been described in a range of epidemiological scenarios worldwide [5-9]. Recently we reported a high prevalence of ESBL-EC faecal colonization among neutropenic patients with haematological malignancies [10]. However, no prospective studies have investigated the associated risk factors and the clinical relevance of this carriage in these patients.

METHODS

Setting, patients, and study design

An observational prospective multicentre cohort study was conducted between 1 May 2006 and 31 December 2007 in Barcelona, Spain, at two teaching hospitals: Hospital Duran i Reynals (hospital A) and Hospital Germans Trias i Pujol (hospital B).

The study population comprised consecutive adult patients with acute leukaemia or undergoing haematopoietic stem cell transplantation, and who received chemotherapy and developed grade IV neutropenia. The same patient could be included more than

once for different neutropenia episodes, provided there was an interval of two months in between and no history of previous colonization or infection by ESBL-*E coli*. Information regarding baseline characteristics, clinical data, rectal swabs, empirical antibiotic therapy and outcomes was carefully recorded in a computerized database. Rectal swabs were obtained at hospital admission and weekly thereafter until discharge or death. To assess risk factors for intestinal colonization by ESBL-EC, we compared baseline and demographic characteristics of colonized and non-colonized episodes. We also compared these two groups in order to assess the clinical relevance of faecal colonization. Following our institutional guidelines, most febrile neutropenic episodes were empirically treated with the combination of a broad-spectrum cephalosporin or a carbapenem, plus an aminoglycoside for the first 48 hours. The initial empirical antibiotic treatment was not modified according to the results of the rectal swabs. No antibacterial prophylaxis was administered during the study period. This study was approved by the ethics committee of our institution.

Definitions

Grade IV neutropenia was defined as an absolute neutrophil count <500/mm³. Prior antibiotic therapy was defined as the receipt of any systemic antibiotic within one month before colonization, or one month before admission in non-colonized patients. Early mortality was defined as death for any cause within 7 days of admission and overall mortality as death by any cause during hospitalisation.

Microbiological studies

Rectal swabs were cultured on three plates: MacConkey agar alone and supplemented with cefotaxime (2μg/ml) and ceftazidime (4μg/ml) [11]. *E. coli* ATCC 25922 (non-ESBL strain) and *K. pneumoniae* ATCC 700603 (ESBL-strains) were used as controls. Identification of *E. coli* strains and their antibiotic susceptibility testing were performed using commercial panels from Microscan® system (SIEMENS). Susceptibility or resistance to antimicrobial agents was defined according to CLSI criteria, and ESBL production screening was detected by double-disk synergy test [12]. The genetic relatedness of *E. coli* strains was tested by pulsed field gel electrophoresis (PFGE). The whole DNA was digested with *XbaI*. Strains that differed in three o fewer bands were considered as belonging to the same cluster [13]. ESBLs were characterized by a multiplex PCR [14]. One strain per neutropenia episode was studied.

Statistical analysis

The prevalence of intestinal colonization by ESBL-EC was calculated as the percentage of carriers among the total number of episodes included. Colonized and non-colonized episodes were compared using univariate analysis with the chi-square test for categorical variables and Student's t-test or the Mann-Whitney U-test, as appropriate, for continuous variables, and multivariate logistic regression analysis was performed to determine the independent risk factors related to intestinal colonization. Multivariable conditional logistic-regression analysis of factors potentially associated with ESBL-EC acquisition and mortality included all statistically significant variables in univariate analysis, gender and age, and all clinically important variables, whether

they were statistically significant or not [15]. The analysis was performed with the stepwise logistic-regression model of the SPSS software package (SPSS).

RESULTS

During the study period, 217 episodes of neutropenia (130 hospital A and 87 hospital B) from 162 patients were studied (78 hospital A and 84 hospital B). The median number of episodes per patient was 1.3 (1-5). The mean number of stool samples obtained from each group (colonized and non-colonized episodes) was similar (4.55 ± 1.73 vs. 4.62 ± 1.91 samples). Overall, ESBL-EC strains were isolated from faeces of 63 (29%) of the 217 episodes studied, 29 (13%) of them on hospital admission. Table 1 shows the main baseline and demographic characteristics of all episodes compared by groups. No significant differences were found regarding the majority of characteristics analysed. Univariate analysis identified prior antibiotics (62% in the colonized group vs. 25% in the non-colonized group; $p< 0.001$) as the only factor associated with ESBL-EC colonization. An unconditional logistic regression model with ESBL-EC colonization as the dependent variable and adjusted for age, gender, presence of central venous catheter (CVC) and previous antibiotic therapy, also identified previous antibiotic therapy as an independent risk-factor (OR: 5.38; 95% CI: 2.79-10.39; $p < 0.001$) (Table 2).

Sixty of the 63 ESBL-EC strains were available for typing and ESBL characterization. The ESBLS identified among the 60 available strains in order of frequency were: CTX-M-9 group (55%, 33/60), CTX-M-1 (26.7%, 16/60), SHV (15%, 9/60), and TEM (3.3%, 2/60). Four isolates carried simultaneously an ESBL enzyme and an OXA type β-lactamase [(3 strains harboured simultaneously a CTX-

M-1 ESBL and OXA, and 1 strain harboured a TEM ESBL and OXA). Fifty-three different PFGE patterns were found among the 60 neutropenia episodes. Five patients had two different neutropenia episodes, and only two of them were colonized by an ESBL-EC strain of the same cluster in the two episodes. The remaining three patients with two different neutropenia episodes were colonized by ESBL-EC strains of different PFGE patterns. The spread of three clones (EC1, EC2 and EC3) carrying $bla_{\text{CTX-M14}}$ ESBL was detected among six patients of hospital B: EC1 in four patients, EC2 in two patients and EC3 in two patients (Figure 1). Resistance rates to non-β-lactam antibiotics were as follows: quinolones 75%, trimethoprim-sulfamethoxazole 66.7%, gentamicin 30%, and tobramycin 33.3%. All strains remained fully susceptible to amikacin and carbapenems. The 16.7% of the strains were resistant to amoxicillin-clavulanic and 3.4% to piperacillin-tazobactam. Table 3 shows the antibiotic resistance rates, the resistance patterns, and PFGE patterns of the 60 available ESBL-EC strains, according to ESBL type.

Outcomes of the study population according to the ESBL-EC faecal carriage are shown in Table 4. Out of the 217 episodes of neutropenia, 67 and 123 episodes of infection were documented in group 1 and group 2 respectively, either clinically or microbiologically, with no differences between groups. Among the microbiologically documented infections, non-ESBL-EC strains accounted for 15 infections in group 1 and 15 infections in group 2, of which 8 and 13 were bacteraemias and 7 and 2 were urinary tract infections respectively. ESBL-EC strains caused one infection (bacteraemia) in group 1 and two infections (bacteraemia and urinary tract infection) in group 2. One of the 2 patients with ESBL-EC bacteraemia received cefepime as the initial empirical antibiotic therapy, which was switched to imipenem 48 hours later; the patient died 4 days after the onset of bacteraemia due to cerebral haemorrhage.

The second patient was empirically treated with imipenem from the beginning, presenting a good clinical response. There were no statistical differences between groups regarding other outcomes during admission, including early and overall mortality.

DISCUSSION

In recent years, a significant increase in the incidence of ESBL-EC faecal carriage has been detected in a variety of settings, including hospitalised patients, outpatients and healthy individuals [16-20]. We recently reported a high prevalence of ESBL-EC colonization in NCP [10]. This figure, reaching a rate of 31.8%, was higher than that found by other investigators in non-neutropenic populations [20-22]. It would be interesting to know the corresponding data from other cancer centres.

Patients analysed in the present study can be considered representative of a homogeneous population of adults with haematological malignancies and neutropenia. There were no significant differences in their baseline characteristics or clinical features, between patients who presented ESBL-EC faecal carriage and those who did not. The only variable found to be an independent risk factor for ESBL-EC colonization was previous antibiotic exposure, which has been widely recognized as a risk factor for different ESBL-EC infections, especially bacteraemia and urinary tract infections, by several authors in non-neutropenic populations [5,7,9,23,24]. The use of prophylactic antibiotics, mostly quinolones, to prevent infection in febrile neutropenia is a controversial issue in the literature [25]. Taking into account that antibiotic exposure is a well defined risk factor for ESBL-EC colonization, it is reasonable to speculate that if our patients had received routine antibiotic prophylaxis with

quinolones for febrile neutropenic episodes, the subsequent ESBL-EC colonization rate would have been much higher. In our study, the presence of a CVC at admission was more frequently found in the colonised group. Nevertheless, we could not find a reasonable explanation for this finding, thus, we considered it irrelevant.

Molecular typing and characterization of the ESBL-EC strains showed a high clonal diversity among the isolates, with a clear predominance (81%) of ESBL type CTX-M. This finding is in good agreement with previous reports performed in the same geographic area and also in several parts of the world [19,24,26]. Antibiotic resistance by ESBL-EC strains to non β-lactam antibiotics was high, as previously reported [23,24].

As expected, infections caused by *E. coli* were frequent in our study, but we did not find a correlation between the detection of ESBL-EC faecal colonization and the risk of developing an ESBL-EC infection. In fact, the rate of non ESBL-EC bacteraemia was around 10% in both groups, whereas the rate of ESBL-EC bacteraemia was less than 2%. This may indicate that, in spite of the presence of ESBL-EC colonies in the selective plates, non ESBL-EC strains constitute the predominant *E. coli* population in the faecal flora. Moreover, the occurrence of two episodes of ESBL-EC infection (one episode of bacteraemia and one urinary tract infection) among the group of non-colonised patients is remarkable. One possible explanation is the potential lack of sensitivity of the screening test in the selective plates. Accordingly, our findings suggest that testing faecal carriage for the presence of ESBL-EC strains may not be cost effective.

On the other hand, as the number of persons colonized by ESBL-EC strains appears to be expanding in the community, one would expect clinical infections caused by these

252 strains to increase, albeit slowly. The therapeutic options for ESBL-EC infections are
253 limited, since these strains are considered to be resistant to all oxyiminobetalactams
254 and frequently present associated resistance to other antimicrobial families such as
255 aminoglycosides and quinolones [27]. This limitation is of special concern in NCP
256 and may lead to universal use of carbapenems as empirical therapy for the febrile
257 episodes. However, in view of our current knowledge, this practice should be
258 undertaken with caution and assessed in controlled trials.

259 Despite a number of strengths (prospective and multicentre study) our study has some
260 limitations that should be acknowledged. First, the study was conducted in a small
261 geographical area, which may not reflect the situation in different epidemiological
262 settings. And second, the small number of patients with ESBL-EC infections may
263 have not allowed us to find differences in outcomes between both groups.

264 In conclusion, in our study we found that ESBL-EC faecal colonization is frequent in
265 NCP but difficult to identify by epidemiological or clinical features on presentation.
266 Prior antibiotic therapy and is the major associated risk factor. Although *E. coli*
267 remains a frequent cause of infection in NCP, ESBL-EC bacteraemia is still rare.
268 Overall, the clinical relevance of this colonization appears to be low, and it does not
269 have a significant negative influence on the outcomes. In this setting, routine testing
270 for ESBL-EC faecal carriage does not seem to be beneficial.

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27. Paterson DL, Bonomo RA (2005) Extended-spectrum beta-lactamases: a clinical update. Clin Microbiol Rev 18:657-86.

396 Table 1. Baseline characteristics of the study population according to the extended-

397 spectrum β-lactamase *E. coli* faecal carriage.

Characteristics	**Total**	**ESBL-EC carriage**	**ESBL-EC non-carriage**	***p***
No. of episodes	217	63	154	-
Sex (M/F)	132 / 85	35 / 28	97 / 57	0.3
Mean age (yr, range)	51.39± 12.29	50.95± 11.92	51.57± 12.47	0.7
Underlying disease				
Acute leukaemia	141 (65%)	36 (57%)	105 (68%)	-
Malignant Lymphoma	31 (14%)	13 (21%)	18 (12%)	-
Multiple myeloma	34 (15%)	11 (17%)	23 (15%)	-
Myelodysplastic Syndrome	7 (3%)	3 (5%)	4 (3%)	-
Myeloproliferative disease	4 (3%)	0	4 (3%)	-
Haematopoietic stem cell transplant	99 (46%)	31 (49%)	68 (44%)	0.5
Previous chemotherapy (6 months)	148 (68%)	42 (67%)	106 (69%)	0.7
Health-care contact	172 (79%)	47 (75%)	125 (81%)	0.3
Central venous catheter at admission	99 (45%)	22 (35%)	76 (50%)	0.048
Urinary catheter at admission	5 (2%)	2 (3%)	3 (2%)	0.6
Previous antibiotic therapy	77 (36%)	39 (62%)	38 (25%)	< 0.001

398 NOTE: ESBL-EC: extended-spectrum β-lactamase *E. coli*

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400 Table 2. Independent risk factors for faecal extended-spectrum β-lactamase-producing

401 *E. coli* carriage.

Risk-factor	**Adjusted OR (95% CI)**	***p***
Age (years, mean)	0.99 (0.97-1.02)	0.73
Gender (female)	1.39 (0.72-2.67)	0.32
Central venous catheter	0.43 (0.22-0.85)	0.015
Previous antibiotic therapy	5.38 (2.79-10.39)	< 0.001

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413 Table 3. Antibiotic resistance, PFGE patterns and resistance patterns of 60 extended-

414 spectrum β-lactamase (ESBL) producing *E. coli* strains according to ESBL type.

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Antibiotic	CTX-M9 (n=33)	CTX-M1 (n=16)	SHV (n=9)	TEM (n=2)
	No of resistant strains			
Amoxicillin-clavulanic acid	2	4	3	1
Piperacillin-tazobactam	0	0	2	0
Ciprofloxacin	26	11	8	0
Gentamicin	14	4	0	0
Tobramycin	13	6	0	1
Amikacin	0	0	0	0
Cotrimoxazole	25	10	5	0
Nº different PFGE patterns	27	15	9	2
Resistance patterns (Nº of strains)	Cip, Gen , Tob, SxT (11) Cip, SxT (11) Cip (4) SxT (3) Cip, Gen, Tob (2) Cip, Gen (1) S (1)	Cip, SxT (3) Cip (3) Cip, Gen, Tob, SxT (2) Cip, Tob, SxT (2) SxT (2) Gen, Tob, SxT (1) Cip, Gen, Tob (1) S (2)	Cip (4) Cip, SxT (4) SxT (1)	Tob (1) S (1)

416 NOTE: ESBL-EC: extended-spectrum β-lactamase *E. coli*

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418 Table 4. Outcomes of the study population according to the extended-spectrum β-
419 lactamase faecal carriage.

Characteristics	**ESBL-EC carriage N=63 (%)**	**ESBL-EC non-carriage N=154 (%)**	***p***
One or more febrile episodes	61 (97)	145 (94)	0.3
Infections per episode (mean ± SD)	1.57±0.99	1.41±0.72	0.3
Episodes of infection	67	123	-
Clinically documented	6 (9)	7 (6)	0.4
Microbiologically documented	61 (91)	116(94)	0.4
Non-ESBL-EC infections	15 (22)	15 (12)	0.07
Bactaraemia	8 (13)	13 (11)	0.7
Urinary tract infection	7 (10.4)	3 (2.4)	0.018
ESBL-EC infections	1 (1.5)	2 (1.6)	0.9
Bactaraemia	1 (1.5)	1 (1)	0.7
Urinary tract infection	0	1 (1)	0.5
Days of hospitalization (mean ± SD)	30.15± 13.70	29.05± 12.11	0.6
Days of antibiotic therapy (mean ± SD)	19.03± 12.34	19.39±13.55	0.9
Early mortality during hospitalization (7 days)	1 (1.6)	0	0.1
Overall mortality during hospitalization	6 (10)	17 (11)	0.74

420 NOTE: ESBL-EC: extended-spectrum β-lactamase *E. coli*

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