Incidence of fecal *Enterobacteriaceae* producing broad-spectrum beta-lactamases in patients with hematological malignancies

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Aim. Given the steadily increasing numbers of resistant bacteria, the frequency and severity of infections are on the rise. In patients with hematological malignancies, the treatment itself increases the risk of complicating bacterial infections. One important mechanisms of resistance is production of broad-spectrum beta-lactamases, increasingly detected not only in bacterial pathogens but also in bacteria contained in the normal microflora of the human body. The objectives of this study were determination and analysis of the prevalence of multiresistant ESBL- and AmpC-positive *Enterobacteriaceae* in the gastrointestinal tract (GIT) of patients with hematological malignancies.

Methods. For 3 months, rectal swabs were taken from patients with hematological malignancies and analyzed using chromogenic screening plates to isolate ESBL- and AmpC-producing *Enterobacteriaceae*. Beta-lactamase production was determined by phenotype tests and confirmed by detecting genes encoding ESBL and AmpC types. At the same time, ESBL- and AmpC-positive *Enterobacteriaceae* were isolated from clinical samples collected from patients with bacterial infection.

Results. Over the study period, fifteen patients (21%) of all patients treated at the Department of Hemato-Oncology were shown to have ESBL- or AmpC-positive *Enterobacteriaceae* in their GIT. Most frequently identified were ESBL-positive strains of *Klebsiella pneumoniae* and AmpC-positive strains of *Citrobacter freundii*. The ESBL enzymes were mainly of the CTX-M type. Isolates producing AmpC were found to contain genes for enzymes mainly from the CIT and DHA groups.

Conclusion. The study identified patients diagnosed with urinary tract and bloodstream infections caused by ESBL-positive strain of *Klebsiella pneumoniae* and AmpC-positive strain of *Enterobacter cloacae* contained in the GIT microflora.

Key words: *Enterobacteriaceae*, ESBL, AmpC, fecal carriage

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INTRODUCTION

The immune system of patients with hematological malignancies is seriously weakened due to the myelosuppressive and immunosuppressive effects of cytostatic agents. As a result, their prognosis is negatively affected by potential bacterial infections that have been – and are very likely to continue to be – of major importance. One of the main reasons is the fact that a large proportion of these infections are of endogenous origin, causative agent being a part of the human microflora. Another factor, is the changing distribution of individual bacterial species. Until recently, Gram-positive bacteria were most prevalent. Today, Gram-negative bacteria are becoming increasingly serious, in particular *Enterobacteriaceae* and strains of *Pseudomonas aeruginosa*[1-3].

Serious bacterial pathogens seen in patients with hematological malignancies include *Enterobacteriaceae*. In their study of patients with hematological malignancies, Cattaneo et al. reported strains of *Escherichia coli* to be the most frequent isolates (almost 25%) in bacteremia[4]. A multicenter study of bacteremia in patients with hematological malignancies treated in Czech and Slovak centers demonstrated that *Enterobacteriaceae* accounted for more than 50% of all isolates[5]. At the present time, the clinical significance of *Enterobacteriaceae* is significantly intensified by their increasing resistance to antimicrobial agents. In the case of beta-lactam antibiotics, the most important mechanism of resistance is production of ESBL and AmpC types of broad-spectrum beta-lactamases[6,7].

Bacterial complications in patients with hematological malignancies pose a serious threat, particularly in the case of neutropenia. Effective initial antibiotic therapy for febrile neutropenia decreases mortality to 2-10% (ref.[8]). At present, however, there is a significantly increasing risk of mortality rising due to growing bacterial resistance and the associated failure of antibiotic therapy. For instance, patients with hematological malignancies have 25% higher mortality due to bacteremia caused by ESBL-positive *Enterobacteriaceae* and 69% higher death rates in the case of positive carbapenemase production[9,10].

It should be stressed that as an effect of the hospital

environment, the bacterial microflora of patients with hematological malignancies is changed from natural primary to secondary, with a higher proportion of multiresistant bacteria posing a risk because of a role they may play in the etiology of complicating bacterial infections. Arnan et al. documented that 29% of neutropenic patients carried ESBL-positive *Escherichia coli* strains, compared with only 14% on admission[11].

The aims of this study were determining the prevalence of carrier multiresistant ESBL- and AmpC-positive *Enterobacteriaceae* in patients with hematological malignancies, their molecular genetic analysis and assessment of their clinical significance. The study was the first of its kind in the Czech Republic.

MATERIAL AND METHODS

Over 3 months (1 November 2012 – 31 January 2013), rectal swabs were taken from all patients treated at the Department of Hemato-Oncology, University Hospital Olomouc, Czech Republic. From the samples, *Enterobacteriaceae* were isolated using chromogenic screening plates for the detection of ESBL- and AmpC-producing strains (Brilliance™ ESBL Agar, Oxoid). At the same time (plus an additional month), clinical samples (blood, urine, other body fluids and exudates, lower airway samples and intravascular catheters) collected from patients with febrile neutropenia were investigated for ESBL- and AmpC-positive *Enterobacteriaceae* causing complicating bacterial infections.

Each isolate was identified by standard microbiological techniques with the Phoenix™ automated system (BD Diagnostics) and MALDI Biotyper system (Bruker Daltonics) (ref.[12]). The strains were selected in such a manner that from each patient, a single strain of each species isolated as the first one was included.

Phenotypic detection of ESBL and AmpC beta-lactamases was carried out using a modified double-disk synergy test and a modified AmpC test[13]. In all positive isolates, polymerase chain reaction (PCR) detected the presence of genes encoding relevant beta-lactamases[14-18].

All isolates were analyzed by comparing whole-genome DNA with pulsed-field gel electrophoresis (PFGE) (ref.[19]).

RESULTS

Over the study period, a total of 71 patients were enrolled. The most frequent diagnoses were acute myeloid leukemia, acute lymphoblastic leukemia, chronic lymphocytic leukemia, chronic myelomonocytic leukemia, multiple myeloma, Hodgkin lymphoma, diffuse large B-cell lymphoma and other non-Hodgkin lymphoma. There were 43 males and 28 females with ages ranging from 21 to 76 years. The mean age was 54 years; the median age was 58 years. Over the study period, febrile neutropenia was diagnosed in 46 patients (64.8%).

A total of 15 patients (21.1%) were demonstrated to have *Enterobacteriaceae* producing broad-spectrum beta-lactamases in their gastrointestinal tract (GIT). ESBL- and AmpC-positive *Enterobacteriaceae* were found in 8 (11.3%) and 7 (9.8%) patients, respectively.

Of the 15 patients carrying ESBL- and AmpC-positive *Enterobacteriaceae* in their GIT, nine (60.0%) had febrile neutropenia. In the group of the 56 patients (no ESBL- and AmpC-positive strains detected in the GIT), febrile neutropenia was noted in 66.1% of subjects. Thus, carriage of *Enterobacteriaceae* producing broad-spectrum beta-lactamases in the GIT was not shown to be a risk factor for the development of febrile neutropenia.

Among the 15 patients with the GIT colonized by producers of broad-spectrum beta-lactamases, only one was hospitalized for the first time. The mean length of hospital stay was 25 days (range, 3-70 days). Thirteen patients (86.7%) had been hospitalized at the Department of Hemato-Oncology in the preceding six months (previous hospital stays ranging from 3 to 58 days; a mean of 21 days).

The 8 cases of ESBL-positive *Enterobacteriaceae* comprised strains of *Klebsiella pneumoniae* (75.0%), *Escherichia coli* (12.5%) and *Enterobacter aerogenes* (12.5%). The AmpC-positive bacteria were *Citrobacter freundii* (42.8%), *Enterobacter cloacae* (28.6%), *Escherichia coli* (14.3%) and *Klebsiella pneumoniae* (14.3%) strains.

PCR revealed the presence of a gene for beta-lactamase from the CTX-M-1-like group in 7 ESBL-positive isolates (87.5%), the bla_{SHV} gene was detected in 6 cases (75.0%) and the bla_{TEM} gene was noted in all ESBL-positive isolates. Isolates producing AmpC were found to contain genes for enzymes from the CIT, DHA and EBC groups. The species composition of ESBL- and AmpC-positive *Enterobacteriaceae* and genes encoding

Table 1. ESBL-positive *Enterobacteriaceae* isolated from GIT of 71 patients and detected genes for beta-lactamases.

Species	No. of isolates	Beta-lactamase type
Enterobacter aerogenes	1	CTX-M-1-like, TEM
Escherichia coli	1	CTX-M-1-like, TEM
Klebsiella pneumoniae	1	SHV, TEM
Klebsiella pneumoniae	5	CTX-M-1-like, SHV, TEM

Table 2. AmpC-positive *Enterobacteriaceae* isolated from GIT of 71 patients and detected genes for beta-lactamases.

Species	No. of isolates	Beta-lactamase type
Citrobacter freundii	2	CIT
Citrobacter freundii	1	CIT, DHA
Enterobacter cloacae	1	EBC
Enterobacter cloacae	1	EBC, DHA
Escherichia coli	1	CIT
Klebsiella pneumoniae	1	DHA

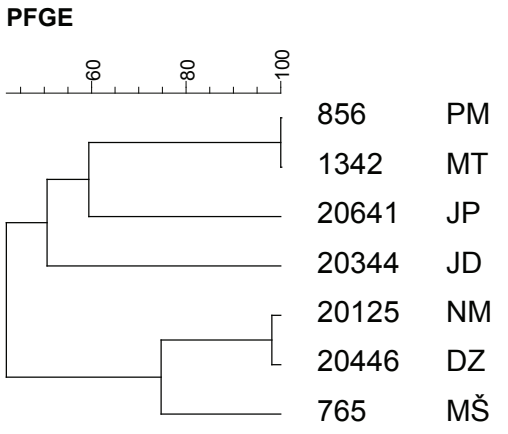

Fig. 1. Dendrogram of *Klebsiella pneumoniae* isolates.

beta-lactamases detected in individual isolates are shown in Tables 1 and 2.

Comparison of whole-genome DNA with PFGE in *Klebsiella pneumoniae* revealed only one identical pair of isolates from two different patients and one pair of isolates with very similar restriction profiles, very likely corresponding to a single genetic change. This result is clearly shown as a dendrogram in Fig. 1. The similarity of the other isolates within individual species, in accordance with defined parameters – Dice (Tol 1.0%-1.0%) – was lower than 60% (dendrograms not shown).

Over the study period, two patients with a confirmed presence of ESBL- or AmpC-positive *Enterobacteriaceae* in the GIT were diagnosed with bacterial infections, one patient with urinary tract infection and the other with bloodstream infection. The etiological agents were ESBL-positive *Klebsiella pneumoniae* isolated from urine and AmpC-positive *Enterobacter cloacae* from blood. In both pathogenic strains, a restriction profile identical to that in GIT isolates was found.

DISCUSSION

Comprehensive treatment of patients with hematological malignancies increases the risk of complicating bacterial infections. Given the characteristics of these patients, antibiotic therapy must be initiated when the first signs of bacterial infection appear. There is no time to wait for the results of microbiological tests, including identification of the bacterial agent and determination of its susceptibility to antimicrobials.

Important pathogenic bacteria found in patients with hematological malignancies are *Enterobacteriaceae* producing both ESBL and AmpC broad-spectrum beta-lactamases. These multiresistant bacteria were found in the GIT microflora in patients with hematological malignancies[15, 20]. Liss et al. documented that 17.5% of patients with hematological and oncological malignancies were colonized with ESBL-positive *Enterobacteriaceae*[21].

This study demonstrated a 21% prevalence of ESBL- and AmpC-positive *Enterobacteriaceae* carriage in the GIT of patients with hematological malignancies; at the same time, it confirmed the effect of previous hospital stays on positive carriage of these bacteria in the GIT. A total of 93% of patients with ESBL- and AmpC-positive *Enterobacteriaceae* in their GIT had been hospitalized in the preceding 6 months. Most frequently, ESBL-positive strains of *Klebsiella pneumoniae* and AmpC-positive strains of *Citrobacter freundii* were identified. The ESBL enzymes were mainly of the CTX-M type. This is consistent with data from other studies confirming changes in the prevalence of broad-spectrum beta-lactamases and prevailing strains producing CTX-M as compared with bacteria with TEM and SHV enzymes[22,23]. A high proportion (81%) of strains producing CTX-M types of beta-lactamases among ESBL-positive bacteria isolated from rectal swabs taken from patients with hematological malignancies was also reported by Arnan et al.[11]. A group of isolates in this Spanish study comprised strains with CTX-M-9 and CTX-M-1 enzymes, as opposed to our study that detected only bacteria producing beta-lactamases from the CTX-M-1 group[11]. Calatayud et al. analyzed rectal swabs from cancer patients and identified ESBL-positive bacteria with genes for CTX-M-9 and CTX-M-1 beta-lactamases[20].

In our study, the AmpC enzymes were most frequently of types CIT and DHA. In contrast to ESBL, intestinal colonization due to AmpC-producing *Enterobacteriaceae* in patients with hematological malignancies has not been studied yet. However, Korona-Glowniak et al. obtained 3 AmpC-positive isolates of *Enterobacteriaceae* from patients with chronic lymphocytic leukemia testing the upper respiratory tract colonization of these hematological patients[24]. Gudiol et al. documented that Gram-negative bloodstream infections in neutropenic cancer patients were associated with high rates of multiresistant *Enterobacteriaceae*, including AmpC-positive *Enterobacter cloacae*[25].

An important task of today's clinical microbiology is to determine potentially identical selected multiresistant bacteria in order to define their likely horizontal clonal spread. It must be noted that this study identified identical strains in two pairs of patients, clearly suggesting interpersonal transmission. However, most isolates had unique restriction profiles. Thus, no clinically significant horizontal spread of identical strains was demonstrated. A small percentage of identical strains and a large diversity of ESBL-positive strains isolated from cancer patients' rectal swabs were also reported by Calatayud et al.[20].

The danger of ESBL- and AmpC-positive *Enterobacteriaceae* in the normal microflora rests on two aspects. First, they are a source of resistance genes for other bacteria. Second, they are potential etiological agents whose high resistance to antibiotic therapy may lead to a failure of initial antibiotic therapy and thus to higher morbidity and mortality rates. Arnan et al. found no clinical significance of ESBL-positive strains of *Escherichia coli* colonizing the GIT of patients with hematological

malignancies and do not consider monitoring of these strains in the GIT as useful[11]. However, our study identified two patients diagnosed with urinary tract and bloodstream infections caused by ESBL- or AmpC-positive *Enterobacteriaceae* contained in the GIT microflora. This is evidence that these strains may act as agents causing bacterial complications.

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