A multicentre cohort study on colonization and infection with ESBL-producing Enterobacteriaceae in high-risk patients with haematological malignancies

Maria J. G. T. Vehreschild1,2*, Axel Hamprecht1,3, Lisa Peterson1,4, Sören Schubert1,5, Maik Häntschel1,6, Silke Peter1,7, Philippe Schafhausen1,8, Holger Rohde1,9, Marie v. Lilienfeld-Toal1,10,11, Isabelle Bekeredjian-Ding1,12, Johannes Libam1,2, Martin Hellmich1,13, Jörg J. Vehreschild1,2, Oliver A. Cornely1,2,14,15 and Harald Seifert1,3

1German Centre for Infection Research (DZIF), Germany; 21st Department of Internal Medicine, University Hospital of Cologne, Cologne, Germany; 3Institute for Medical Microbiology, Immunology and Hygiene, University of Cologne, Cologne, Germany; 4Med. Klinik III, University of Munich—Campus Großhadern, Munich, Germany; 5Max von Pettenkofer-Institut, University of Munich—Campus Großhadern, Munich, Germany; 6Department of Oncology, Haematology, Immunology, Rheumatology and Pulmology, Internal Medicine II, University Hospital Tübingen, Tübingen, Germany; 7Institute of Medical Microbiology and Hygiene, University of Tübingen, Tübingen, Germany; 8Department of Oncology and Hematology, Hubertus Wald Tumorzentrum/University Cancer Center Hamburg, University Medical Center Hamburg-Eppendorf, Hamburg, Germany; 9Institute for Medical Microbiology, Virology and Hygiene, University Medical Center Hamburg-Eppendorf, Hamburg, Germany; 10Medizinische Klinik und Poliklinik I, Universitätsklinikum Bonn, Bonn, Germany; 11Klinik für Innere Medizin II—Abteilung für Hämatologie und Onkologie, Universitätsklinikum Jena, Jena, Germany; 12Institut für Medizinische Mikrobiologie, Immunologie und Parasitologie, Universitätsklinikum Bonn, Bonn, Germany; 13Department of Medical Statistics, Informatics and Epidemiology, University of Cologne, Cologne, Germany; 14Clinical Trials Center Cologne, ZKS Köln, BMBF 01KN1106, Medical Faculty, University of Cologne, Cologne, Germany; 15Center for Integrated Oncology CIO Köln/Bonn and Cologne Excellence Cluster on Cellular Stress Responses in Aging-Associated Diseases (CECAD), Medical Faculty, University of Cologne, Cologne, Germany

*Corresponding author. Klinik I für Innere Medizin, Kerpener Straße 62, 50937 Köln, Germany. Tel: +49-(0)221-478-6494; Fax: +49-(0)221-478-3611; E-mail: maria.vehreschild@ctuc.de

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Background: Bloodstream infections (BSIs) caused by enterobacteria remain a leading cause of mortality in patients with chemotherapy-induced neutropenia. The rate and type of colonization and infection with ESBL-producing Enterobacteriaceae (ESBL-E) and their mode of transmission in German cancer centres is largely unknown.

Methods: We performed a prospective, observational study at five German university-based haematology departments. Participating sites screened for intestinal ESBL-E colonization within 72 h of admission, every 10 ± 2 days thereafter and before discharge. Three of the five centres performed contact isolation for patients colonized or infected with ESBL-E. Molecular characterization of resistance mechanisms and epidemiological typing of isolates by repetitive extragenic palindromic PCR (rep-PCR) and PFGE was performed to assess strain transmission between patients.

Results: Between October 2011 and December 2012, 719 hospitalizations of 497 haematological high-risk patients comprising 20143 patient-days were analysed. Mean duration of in-hospital stay was 36.6 days (range: 2–159 days). ESBL-E were identified from screening samples (82.8% Escherichia coli and 14.6% Klebsiella pneumoniae) in 55/497 patients (11.1%; range by centre: 5.8%–23.1%). PFGE and rep-PCR revealed only a single case of potential cross-transmission among two patients colonized with K. pneumoniae. Six episodes of BSI with ESBL-E were observed. Multivariate analysis revealed previous colonization with ESBL-E as the most important risk factor for BSI with ESBL-E (OR 52.00; 95% CI 5.71–473.89).

Conclusions: Even though BSI with ESBL-E is still rare in this high-risk population, colonization rates are substantial and vary considerably between centres. In-hospital transmission of ESBL-E as assessed by molecular typing was the exception.

Keywords: febrile neutropenia, bacteraemia, bloodstream infections, intestinal colonization, infection control, contact isolation
Introduction

Bloodstream infections (BSIs) remain the leading cause of mortality in patients with chemotherapy-induced neutropenia. A relevant number of these BSIs originate from bacterial penetration of the impaired mucosal barrier during neutropenia. This origin of BSIs might be gaining further importance, as the global spread of multidrug-resistant (MDR) bacteria is becoming a matter of increasing concern. Gut colonization with MDR bacteria may subsequently evolve into BSI after administration of chemotherapy. In the setting of febrile neutropenia, BSIs may benefit from faecal screening in combination with targeted empirical carbapenem treatment. However, this question has not been assessed in a structured fashion and the establishment of faecal screening as a standard of care is based on expert opinion only.

Concerning empirical treatment of febrile neutropenia, it has been proposed that patients treated at centres with high rates of colonization with ESBL-E may benefit from faecal screening in combination with targeted empirical carbapenem treatment. However, this question has not been assessed in a structured fashion and the establishment of faecal screening as a standard of care is based on expert opinion only.

Similarly, infection control measures for patients infected or colonized with ESBL-E are the subject of debate, particularly the need for contact isolation. The German commission KRINKO (Kommission für Krankenhaushygiene und Infektionsprävention) recommends contact isolation for patients infected or colonized with fluoroquinolone-resistant ESBL-E in high-risk settings, e.g. haematology/oncology wards and ICUs. In contrast to this statement, ESCMID recommends these precautions for carriers of ESBL-producing Klebsiella pneumoniae and for any ESBL-E outbreak setting only. An endemic setting, there is only a conditional recommendation for contact isolation of carriers of ESBL-producing Escherichia coli, even in high-risk settings.

While infection control measures have a significant impact on patient care, their development and implementation is often hampered by the lack of reliable clinical data.

This problem is further complicated by regional differences in the evolution of ESBL-E, both in the community and in the hospital setting. Large surveillance networks such as EARS-Net (European Antimicrobial Resistance Surveillance Network) or SMART (Study for Monitoring Antimicrobial Resistance Trends) provide highly valuable data on resistance trends at a national and international level. However, conclusions on local resistance rates in selected patient populations cannot be drawn from these sources. Detailed epidemiological information at the patient level isispensable to the design of any interventional study.

To improve epidemiological knowledge on ESBL-E in German haematological high-risk patients and thus facilitate the design of further interventional studies, a prospective multicentre study was performed to assess: (i) the rate of intestinal colonization with ESBL-E at hospital admission and during the course of hospitalization; (ii) the rate of patient-to-patient transmission of ESBL-E; (iii) the rate of BSIs with ESBL-E; and (iv) risk factors for the occurrence of BSIs with ESBL-E.

Study design

The present study was designed as a prospective, observational multicentre study, assessing the period from 1 October 2011 to 31 December 2012. All hospitalizations of high-risk patients admitted during this period were eligible for documentation in the study. Patients receiving one of the following cytotoxic treatments were considered high risk: (i) induction and consolidation chemotherapy for AML or ALL; (ii) high-dose chemotherapy with consecutive autologous stem cell transplantation (SCT); or (iii) conditioning chemotherapy with consecutive allogeneic SCT.

Intestinal colonization with ESBL-E was defined as detection of ESBL-E in at least one stool sample or rectal swab. BSI due to ESBL-E was defined as ESBL-E recovered from at least one blood culture. A patient was considered no longer colonized when two consecutive stool cultures or rectal swabs collected at a maximum interval of 3 weeks no longer yielded ESBL-E.

For determination of nosocomial colonization (NC) as opposed to community-acquired colonization (CAC), only patients whose first admission to a study site fell into the study period and from whom subsequent screening samples during the same hospitalization had been obtained were considered eligible for analysis. Since follow-up samples were not a standard of care at Centre E, patients from this centre were not included in this subanalysis. If consecutive screening samples from the same patient and hospital stay revealed a switch from a negative to a positive carrier status, this was considered NC. If a patient presented with a positive admission screening or if he changed his carrier status from negative to positive between two hospitalizations, this was considered CAC.

Anonymized data of eligible patients were entered into web-based electronic case report forms (eCRFs). A search of the literature was carried out to identify potential risk factors for ESBL-E BSIs. Documentation included age, gender, weight, height, underlying disease requiring chemotherapy, duration of hospital stay, information on allogeneic or autologous SCT and result and type of screening samples. If a screening sample or a blood culture positive for ESBL-E was entered into the eCRF, documentation was extended to include the following items: type of administered antimicrobial and chemotherapy agents; ICU treatment; total parenteral nutrition; haemodialysis; central venous catheter; neutropenia; and death. An intention-to-treat approach was used for inclusion of these variables in the analysis, considering a single dose/day relevant for the analysis. In case of a blood culture positive for ESBL-E, information on the type and duration of antimicrobial treatment, removal of central venous catheters and treatment outcome was documented as well. The documentation of each hospitalization ended with the day of discharge or death, whichever occurred first.

In addition, all participating centres received a questionnaire to assess current infection control standards concerning ESBL-E colonization and infection.

Methods

Setting

This study was conducted at five German university-based haematology/oncology departments. All departments had already implemented routine faecal screening for intestinal colonization with ESBL-E into their standard of care at the time of study initiation. Screening protocols included collection of a stool sample or rectal swab within 72 h of admission. At four of the five centres (A–D), additional samples were collected every 10 ± 2 days and within 72 h before discharge from hospital. At Centre E, follow-up samples were not part of the local standard of care. Readmitted patients were screened upon each new admission. At Centre A, patients colonized or infected with ESBL-E were placed into single-room contact isolation only if they had urinary or faecal incontinence or diarrhoea (more than three unformed stools within 24 h). At Centre B, only carriers of fluoroquinolone-resistant ESBL-E were placed into single-room contact isolation, irrespective of showing signs of incontinence. The remaining centres (C–E) followed a policy of single-room contact isolation for all patients colonized or infected with any kind of ESBL-E at all times. At all centres, cohorting of ESBL-E-colonized or -infected patients was only practised if no single rooms were available. As a standard of care at all participating sites, two blood culture sets were drawn in patients with neutropenic fever or suspicion of BSI.


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Statistical analysis
All statistical analyses were carried out using SPSS software (IBM SPSS Statistics, version 21; IBM, Armonk, NY, USA). We performed descriptive statistics as appropriate to evaluate the dataset. All risk factors identified during the literature search (see the Study design section) were first analysed using univariate statistics (Student’s t-test for continuous variables and Fisher’s exact test for dichotomous variables). To assess the independence of covariates, we performed binary logistic regression with occurrence of BSI as the dependent variable and potential risk factors as covariates. We then eliminated covariates from the analysis by a stepwise, backward approach, using Wald’s statistic with an exclusion margin of 0.1 to improve the model further. For multivariate analysis, we aggregated sequential hospitalizations of the same patient into a single dataset. For all analyses, a P value <0.05 was considered significant.

Microbiological analysis
Stool and blood specimens were processed at the local microbiology departments in accordance with ‘Microbiology Procedures Quality Standards (MIQ)’ issued by the German Society for Hygiene and Microbiology.17 Chromogenic culture media were used in addition to non-selective, non-chromogenic media to improve the detection of ESBL-E. Species identification and susceptibility testing including confirmatory tests for ESBL identification were performed using Vitek 2 (bioMérieux, Nürtingen, Germany) or a linear MALDI-TOF mass spectrometer (bioMérieux, Marcy l’Etoile, France or Bruker Daltonic, Bremen, Germany) according to CLSI or EUCAST standards.18,19 ESBL production was confirmed with the CLSI combination disc test where appropriate.

All E. coli and K. pneumoniae ESBL-E isolates, including those obtained from multiple screenings, were typed by repetitive extragenic palindromic PCR (rep-PCR) using the DiversiLab platform (bioMérieux) as described elsewhere.20 To maximize discrimination, E. coli and K. pneumoniae isolates with similar rep-PCR patterns (>95% similarity) as well as isolate pairs from stool and blood in a given patient were also typed by PFGE and interpreted as described elsewhere.11

Ethical considerations
Approval of the local Ethics Committee was obtained at all participating sites.

Results

Demographics
Between October 2011 and December 2012, 719 hospitalizations of 497 patients and 20143 patient-days were entered into the database (mean duration of hospitalization: 36.6 days; range: 2–159 days). There were no missing data. A total of 311 patients (62.6%) received intensive chemotherapy for acute leukaemia; 107 patients (21.5%) underwent autologous SCT and 223 (44.9%) allogeneic SCT. Three patients received two sequential autologous SCTs and nine patients two sequential allogeneic SCTs. Two patients received an autologous SCT followed by an allogeneic SCT. Two patients received an autologous SCT followed by an autologous SCTs and nine patients two sequential allogeneic SCTs. Three patients received two sequential allogeneic SCTs and nine patients two sequential allogeneic SCTs. Two patients received an autologous SCT followed by an allogeneic SCT. Table 1 summarizes the patient demographics.

Colonization and transmission
Overall, 1931 screening samples were obtained. The distribution of patients, hospitalizations and samples by centre is shown in Table 2. Overall, 163 screening samples positive for ESBL-E were obtained (i.e. E. coli, 135 isolates (82.8%); K. pneumoniae, 24 isolates (14.6%); Klebsiella oxytoca, 2 isolates (1.2%); and Enterobacter cloacae, 2 isolates (1.2%)). These isolates were detected in samples from 55/497 patients (11.1%; range by centre: 5.8%–23.1%). The distribution of colonization with different ESBL-E species was E. coli in 49 patients (89.1%), K. pneumoniae in 2 patients (3.6%), K. oxytoca in 2 patients (3.6%) and E. cloacae in 1 patient (1.8%). Two patients experienced simultaneous colonization with ESBL-producing E. coli and K. oxytoca (3.6%) and one patient with ESBL-producing E. coli and K. pneumoniae (1.8%). Table 1 shows the demographic details of patients with intestinal colonization with ESBL-E.

Overall, 26/55 (47.2%) patients colonized with ESBL-E were eligible for analysis of their origin of colonization. Based on this analysis, CAC and NC occurred in 15/26 (57.7%; E. coli only) and 11/26 (42.3%; E. coli = 9, E. cloacae = 1 and K. oxytoca = 1) patients, respectively. The rate of NC with ESBL-E was low at all centres included in the analysis (A, 1.1 cases per 1000 patient-days; B, 1.4 cases per 1000 patient-days; C, 1.3 cases per 1000 patient-days; and D, 0.54 cases per 1000 patient-days). To further assess...
potential nosocomial patient-to-patient transmission, molecular epidemiologic analyses of ESBL-E isolates of all colonized and infected patients (E. coli, 49 patients; and Klebsiella spp., 8 patients) was carried out and revealed only a single case of potential cross-transmission between two patients colonized with K. pneumoniae; however, these patients were not hospitalized simultaneously. Two isolates of patients colonized with E. coli were missing and could not be included in this analysis.

Twenty-two of the 55 patients (40.0%) colonized with ESBL-E converted to a negative ESBL-E carrier status during the study period. Among these 22 patients, 18 (81.8%) maintained this status until their last documented observation; in 3 patients (13.6%), ESBL-E were again isolated from subsequent screening samples. To assess potential factors responsible for successful decolonization, previously administered antimicrobials with activity against ESBL-E were analysed. Before conversion to a negative carrier status, 13 patients (59.1%) had received treatment with a carbapenem, 11 with a fluoroquinolone (50.0%) and 2 (9.1%) with an aminoglycoside. There was no significant change in the colonization rate over the study period.

### BSIs

Six episodes of BSI with ESBL-E (E. coli, 4; K. pneumoniae, 1; and E. cloacae, 1) were detected in 5/497 patients (1.0%) accounting for 6/719 hospitalizations (0.8%). Four of the five patients with BSIs belonged to the group of 55 patients that had been previously found to be colonized with ESBL-E, while the other patient belonged to the group of 442 patients not colonized with ESBL-E (P=0.0006, Fisher’s exact test). In all four cases, the bacteria isolated from blood cultures were identical to the colonizing isolate as assessed by PFGE. Multivariate analysis revealed previous colonization with ESBL-E as the most important risk factor for BSI with ESBL-E, with an associated OR of 52.00 (95% CI 5.71–473.89). Detailed results of the univariate and multivariate analyses are shown in Table 3.

Patients with BSI due to ESBL-E were treated with carbapenems (n=5 episodes) and a fluoroquinolone (n=1 episode), respectively. In the patient in whom a fluoroquinolone was used, the isolate tested susceptible. The mean time to treatment initiation was 1.3 days (range: 0–4 days). During five of the six corresponding hospitalizations, a central venous catheter had been placed and was removed after detection of ESBL-E from blood cultures in four patients. After onset of BSI due to ESBL-E, one patient required transfer to the ICU, but there were no fatalities. No other invasive infections due to ESBL-E were observed during the study period.

### Discussion

Our study demonstrates that BSI with ESBL-E was a rare event in a high-risk population with haematological malignancies in Germany. However, the rate of intestinal colonization with ESBL-E varied considerably between centres and was as high as 23%

Epidemiological analysis yielded 11 cases of NC. In the interpretation of this figure, it should be considered that a case of NC as defined here is not necessarily the result of in-hospital transmission. Low-level colonization on admission may have gone undetected using our culture-based protocol. It may, however, have become apparent once bacterial density increased under the selective pressure exerted by antimicrobial treatment for febrile neutropenia.

Furthermore, it is noteworthy that the estimated NC rate was not only low (range: 0.54–1.4 cases per 1000 patient-days), but also independent of enforcement of contact isolation. This finding
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Discusses the issue of ESBL-E colonization and infection, emphasizing the need for contact isolation. Highlights the importance of community acquisition of ESBL-E in high-risk settings and its implications for patient management.

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