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ORIGINAL RESEARCH

Mortality and molecular epidemiology associated with extended-spectrum β-lactamase production in *Escherichia coli* from bloodstream infection

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Background: The rate of infections due to extended-spectrum β-lactamase (ESBL)-producing *Escherichia coli* is growing worldwide. These infections are suspected to be related to increased mortality. We aimed to estimate the difference in mortality due to bloodstream infections (BSIs) with ESBL-positive and ESBL-negative *E. coli* isolates and to determine the molecular epidemiology of our ESBL-positive isolates.

Materials and methods: We performed a cohort study on consecutive patients with *E. coli* BSI between 2008 and 2010 at the Charité University Hospital. Collected data were ESBL production, basic demographic parameters, and underlying diseases by the Charlson comorbidity index (CCI). The presence of ESBL genes was analyzed by polymerase chain reaction (PCR) and sequencing. Phylogenetic groups of ESBL-positive *E. coli* were determined by PCR. Risk factors for mortality were analyzed by multivariable regression analysis.

Results: We identified 115 patients with BSI due to *E. coli* with ESBL phenotype and 983 due to ESBL-negative *E. coli*. Fifty-eight percent (n=67) of the ESBL-positive BSIs were hospital-acquired. Among the 99 isolates that were available for PCR screening and sequencing, we found mainly 87 CTX-M producers, with CTX-M-15 (n=55) and CTX-M-1 (n=21) as the most common types. Parameters significantly associated with mortality were age, CCI, and length of stay before and after onset of BSI.

Conclusion: The most common ESBL genotypes in clinical isolates from *E. coli* BSIs were CTX-M-15 (58%) and CTX-M-1 (22%). ESBL production in clinical *E. coli* BSI isolates was not related to increased mortality. However, the common occurrence of hospital-acquired BSI due to ESBL-positive *E. coli* indicates future challenges for hospitals.

Keywords: BSI, mortality, ESBL-genotype, sepsis

Background

Infections with extended-spectrum β-lactamase (ESBL)-producing Enterobacteriaceae are increasing worldwide.[1] In particular, bloodstream infections (BSIs) due to ESBL-producing Enterobacteriaceae have been reported to be related to increased mortality in the past 10 years.[2–4] However, recent studies showed no significant difference in mortality comparing ESBL-positive and ESBL-negative cases of BSI due to Enterobacteriaceae.[5,6] There is still a lack of current studies with large numbers of patients and adequate control cohorts consisting of infections with susceptible Enterobacteriaceae. Therefore, we present the results of a large study with more than 100 ESBL-positive patients and respective control patients with ESBL-negative *Escherichia coli* BSI.

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Materials and methods

Setting, study design, and definitions

The study was carried out at the Charité University Hospital in Berlin, a 3,213-bed tertiary care center. The study was performed in conformity with the ethical guidelines of the Declaration of Helsinki. It is based on secondary data, and ethical approval was not required.

We conducted a cohort study of all consecutive patients with BSI caused by *E. coli* between January 1, 2008 and December 31, 2010. Patients were retrospectively identified in the Charité microbiology database as patients with blood cultures positive for *E. coli*. For all patients enrolled in this study, the following demographic characteristics were collected from their electronic files: age, sex, in-hospital death, day of BSI onset, and underlying comorbidities by the Charlson comorbidity index (CCI) on the basis of the patients' International Classification of Diseases (ICD)-10-coded diagnoses.[7] If a patient had more than one episode of *E. coli* BSI within the analyzed period, the first episode was analyzed. Each patient was included in the analysis only once. Onset of BSI was defined as the date of the first blood culture positive for *E. coli*. BSI was considered hospital-acquired when the onset occurred at least 48 hours after admission.

Microbiological methods, resistance-gene screening, and bacterial typing

The VITEK 2 automated system was used for the identification of *E. coli* species and antimicrobial susceptibility testing. The results were interpreted according to the Clinical and Laboratory Standards Institute standards.[8] Confirmation of ESBL production was performed by a minimum inhibitory concentration dilution test on a multiwell microtiter plate. Three third-generation cephalosporins (cefoxitin, ceftazidime, cefpodoxime) were tested alone and in combination with ESBL inhibitor clavulanic acid (standard operating procedure according to the German Accreditation Council, registration DGA-ML-6243.03). All *E. coli* isolates with ESBL phenotype were screened for the presence of different ESBL genes ($bla_{\text{TEM-type}}$, $bla_{\text{SHV-type}}$, $bla_{\text{CTX-M-1/2/9 group}}$) by polymerase chain reaction (PCR) and subsequent sequencing.[9] If none of these ESBL genes could be identified, additional PCR tests for the presence of plasmid-mediated AmpC β-lactamases[10] and further ESBL genes ($bla_{\text{CTX-M-8-type}}$, $bla_{\text{CTX-M-26-type}}$, $bla_{\text{VEB-type}}$, $bla_{\text{PER-type}}$, $bla_{\text{GES-type}}$, $bla_{\text{OXA-1/2/10 group}}$) were performed. Furthermore, basic bacterial typing of all ESBL-positive *E. coli* isolates was performed by a PCR-based method for determination of the four major *E. coli* phylogenetic groups.[11]

Statistical methods

Parameters in the descriptive analysis of patients with ESBL-positive and ESBL-negative *E. coli* BSIs were tested using the Wilcoxon rank-sum test for continuous variables and Fisher's exact test for categorical variables. A multivariable analysis was performed to estimate the effects of multiple factors associated with mortality using a stepwise forward regression. Included variables were ESBL production, age, sex, CCI, hospital acquisition, and length of stay before and after BSI onset. Variables with P-values<0.05 were included, and variables with $P \geq 0.05$ were excluded. Odds ratios and their 95% confidence intervals were calculated. All tests of significance were two-tailed, with a P-value<0.05 considered to be significant. Data were analyzed using PASW Statistics 18 (IBM, Armonk, NY, USA).

Results

We identified 1,098 consecutive patients with *E. coli* BSI. One hundred and fifteen patients (10.5%) with ESBL-positive due to *E. coli*, and 983 patients with BSI due to ESBL-negative *E. coli* (89.5%). The baseline characteristics of patients with ESBL-positive and ESBL-negative *E. coli* BSI are shown in Table 1. Overall and regardless of ESBL production, 449 (40%) *E. coli* BSIs were hospital-acquired and associated with an in-house mortality of 24% (n=107), compared to 16% (n=104, P=0.001) among community-acquired cases. Fifty-eight percent (n=67) of the ESBL-positive BSIs and 38% (n=382) of ESBL-negative *E. coli* BSIs ($P<0.001$) were hospital-acquired.

The blood culture specimens of 99 (86%) BSI patients were available for molecular analysis. ESBL genes were identified in 95 *E. coli* isolates. One isolate was positive for AmpC β-lactamase CMY-2, and three isolates were only positive for β-lactamase type TEM-1 (n=2) and TEM-181 (n=1), respectively. All four of these patients were removed from the analysis, leaving 95 analyzed patients. Among the remaining 95 ESBL-producing isolates, CTX-M enzymes (n=87, 92%) were predominant, with CTX-M-15 (n=55, 58%), CTX-M-1 (n=21, 22%), and CTX-M-14 (n=4, 4%) the most common ESBL-types (Figure 1A). Furthermore, 43 (45%) of all ESBL-producing *E. coli* additionally harbored TEM-type β-lactamases. Among the 54 hospital-acquired ESBL-positive *E. coli* isolates that were available for molecular analysis, the most common genotypes were CTX-M-15 (n=33, 61%), CTX-M-1 (n=10, 19%) and CTX-M-14 (n=3, 6%).

Bacterial typing (Figure 1B) revealed that the 95 ESBL-producing *E. coli* isolates belonged to phylogenetic groups B2 (n=31, 33%), D (n=28, 29%), A (n=26, 27%), and B1 (n=10,

Table 1 Baseline characteristics of 1,098 patients with *Escherichia coli* BSI stratified by ESBL production

Parameter/ category	ESBL-positive (n=115)	ESBL-negative (n=983)	*P*-value
Hospital-acquired BSI*	67 (58%)	382 (39%)	<0.001
Age, years	59 (44–71)	67 (54–75)	<0.001
Male*	74 (64%)	521 (53%)	0.018
In-house mortality*	30 (26%)	181 (18%)	0.076
Length of hospital stay, total days	27 (12–53)	15 (8–32)	<0.001
Length of stay before BSI onset, days	7 (0–20)	1 (0–10)	<0.001
Charlson comorbidity index	5 (3–8)	5 (3–8)	0.227
Myocardial infarct*	5 (4%)	28 (3%)	0.381
Congestive heart failure*	19 (17%)	148 (15%)	0.681
Peripheral vascular disease*	19 (17%)	83 (8%)	0.007
Cerebrovascular disease*	8 (7%)	56 (6%)	0.673
Dementia*	2 (2%)	21 (2%)	1.000
Chronic lung disease*	11 (10%)	109 (11%)	0.643
Rheumatic disease*	0	32 (3%)	0.050
Peptic ulcer*	6 (6%)	36 (4%)	0.436
Mild liver disease*	12 (10%)	121 (12%)	0.652
Diabetes without complication*	14 (12%)	192 (20%)	0.058
Diabetes with complication*	8 (7%)	62 (6%)	0.839
Renal disease*	55 (48%)	425 (43%)	0.372
Malign tumor*	25 (22%)	237 (24%)	0.644
Liver disease*	9 (8%)	70 (7%)	0.848
Metastasis*	14 (12%)	139 (14%)	0.577
HIV*	2 (2%)	9 (1%)	0.617
Hemiplegia*	5 (4%)	37 (4%)	0.795
Leukemia*	15 (13%)	85 (9%)	0.124
Lymphoma*	5 (4%)	66 (7%)	0.424

Notes: *Categorical variables were tested using Fisher's exact test, and are presented as number (percentage). Continuous variables were tested using the Wilcoxon rank-sum test, and are presented as medians (interquartile range).
Abbreviations: BSI, bloodstream infection; ESBL, extended-spectrum β-lactamase; HIV, human immunodeficiency virus.

10%). The distribution of the phylogenetic groups among the 54 hospital-acquired *E. coli* isolates was nearly identical. However, considering only the isolates with CTX-M-15 (n=55) and CTX-M-1 (n=21), phylogenetic group B2 was mainly associated with CTX-M-15 (n=24, 44%) in contrast to isolates of phylogenetic group A producing mainly CTX-M-1 (n=10, 48%) (Figure 1C and D).

The results of the regression analysis are shown in Table 2. Age, Charlson comorbidity index, and length of stay before and after onset of BSI were significantly associated with hospital mortality. ESBL production was not a significant risk factor in the multivariable analysis.

Discussion

In the present single-center cohort study of patients with *E. coli* BSI, we analyzed 115 patients with ESBL-positive and 983 patients with ESBL-negative *E. coli.* BSI due to ESBL-positive *E. coli* was significantly more often hospital-acquired than ESBL-negative *E. coli* BSI. However, in this study, ESBL production was not associated with increased mortality risk. In our study, ESBL *E. coli* BSI was related to an in-house mortality of 26%. This result is comparable to several recent studies that reported ESBL BSI mortality (30-day or in-hospital) between 20% and 25%.[6,12–14] Nevertheless, few published studies have assessed the mortality of ESBL or third-generation cephalosporin-resistant *E. coli* BSI in comparison to patients with BSI due to susceptible *E. coli*.[3–6] Our data are comparable with the results of a study by Nasa et al that analyzed cases of Enterobacteriaceae BSI on intensive care units.[5] Another study used a multistate approach to analyze the burden of ESBL-positive BSI.[6] Even though they found increased costs and length of stay, the mortality of their ESBL-positive cases was not significantly increased.

In contrast, de Kraker et al analyzed data from 13 European tertiary care centers.[4] They found high mortality rates of BSI cases due to third-generation cephalosporin-resistant *E. coli* that were significantly increased compared to their susceptible controls (36% versus 17%). However, many of their data derived from countries with high prevalence of carbapenem nonsusceptible Enterobacteriaceae. Since the study included no information on additional carbapenem resistance of the isolates, the possibility that carbapenemase production influenced the calculated mortality rate cannot be excluded.[15] A further study conducted in 2010 found a significantly increased mortality of patients with ESBL *E. coli* BSI compared to patients with non-ESBL *E. coli* BSI (30% versus 6%).[3] Here, the majority of ESBL-positive patients received inadequate initial antimicrobial chemotherapy. Moreover, in a meta-analysis on BSI cases due to ESBL-producing Enterobacteriaceae between 1996 and 2003, significantly increased mortality (pooled crude mortality 34% versus 20%) and a significant delay in administration of appropriate antimicrobial therapy was associated with ESBL production.[2] A meta-analysis by Rottier et al furthermore found that increased mortality in cases of BSI due to ESBL-positive Enterobacteriaceae is strongly influenced by the administration of empirically adequate antimicrobial therapy.[16] In the present study, data on antimicrobial therapy were not available. Hence we cannot evaluate the definite influence of appropriate antimicrobial therapy in the present cohort. However, internal antibiotic policy was

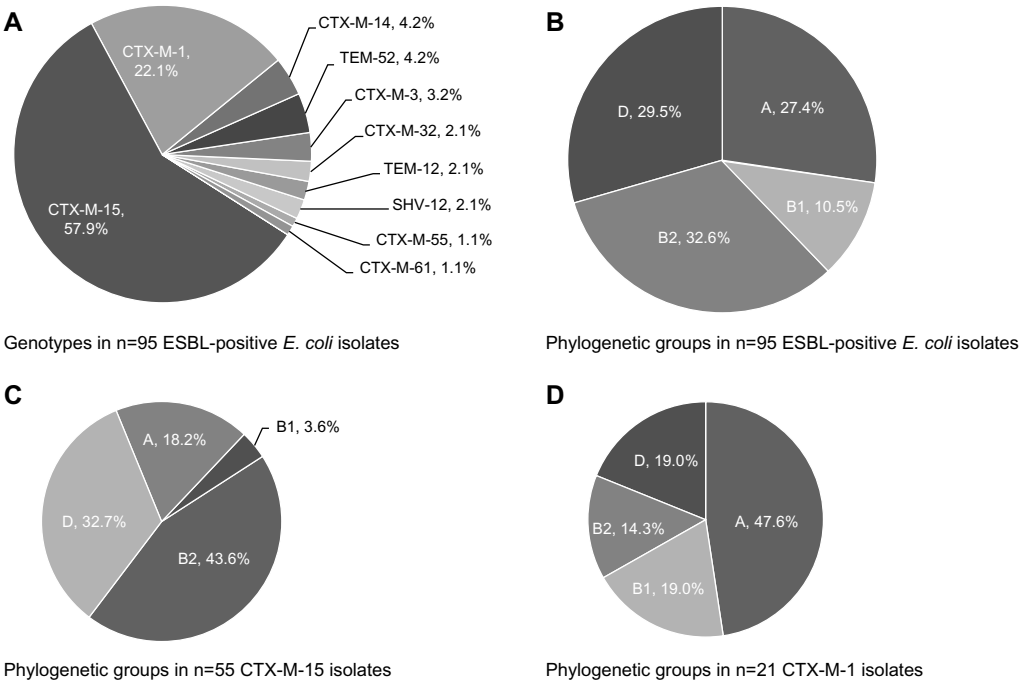

Figure 1 (**A**–**D**) Distribution of genotypes and phylogenetic groups of 95 extended-spectrum β-lactamase (ESBL)-producing *Escherichia coli* isolates from patients with bloodstream infection. (**A**) ESBL genes in all 95 ESBL *E. coli* isolates (n=95); (**B**) phylogenetic groups of all 95 ESBL *E. coli* isolates; (**C**) phylogenetic groups of CTX-M-15 *E. coli* isolates (n=55); (**D**) phylogenetic groups of CTX-M-1 *E. coli* isolates (n=21).
Abbreviation: ESBL, extended-spectrum β-lactamase.

communicated to us as the following: for patients with severe sepsis or septic shock, empirical treatment was initiated with carbapenems and less severe infections with third-generation cephalosporins or piperacillin/tazobactam. Furthermore, the mortality rate in our cohort is comparable with many other recent studies, and documents the current outcome associated with ESBL *E. coli* BSI.[6,12–14]

The most common ESBL genotypes identified in the present study (CTX-M-15, 58%; CTX-M-1, 22%) were also found in other studies from Europe. However, differences in distribution of these three CTX-M-types and other ESBLs in European countries have been reported, eg, the dominant ESBL type in Spain is CTX-M-14.[12,17] A possible explanation could be delivered by the ongoing discussion on ESBL distribution through the food chain. While recent studies from Germany have shown that diet could play an important role in the distribution of CTX-M-1,[18,19] a study from Spain showed in contrast that CTX-M-14 is the most common ESBL enzyme in *E. coli* from Spanish retail meat.[20]

Table 2 Results of the multivariable regression analysis of risk factors for mortality in cases of *Escherichia coli* bloodstream infection (BSI)

Parameter	Odds ratio	95% confidence interval	*P*-value
Age	0.985	0.975–0.995	0.004
Charlson comorbidity index	1.303	1.238–1.372	<0.001
Length of stay before BSI onset	1.017	1.009–1.024	<0.001
Length of hospital stay after BSI	0.988	0.980–0.996	0.002

The finding that four *E. coli* isolates with ESBL phenotype in our study harbored no ESBL type is most likely due to overexpression of TEM β-lactamases in three isolates and one false-positive ESBL confirmation test for one CMY-producing isolate. Typing of our ESBL *E. coli* isolates revealed a high proportion of CTX-M-15-positive isolates belonging to phylogenetic group B2. This may partly be due to the spread of strains belonging to the internationally disseminated clonal lineage *E. coli* O25b:H4-ST131.[21] In contrast, the majority of CTX-M-1-positive isolates (48%) belonged to phylogenetic group A, which is generally associated with less virulence than group B2 or D isolates. CTX-M-1-producing *E. coli* are more often described in animals, food, or community-acquired isolates than CTX-M-15, indicating an association of distinct CTX-M-types with different settings due to various ways of transmission.[3,18,21–25]

In conclusion, although there was no difference in mortality of BSIs with ESBL-positive or ESBL-negative

E. coli in our study, the significantly higher number of hospital-acquired BSIs due to *E. coli* with ESBLs indicates the importance of hygiene precautions. Measures like high compliance in hand hygiene, preemptive isolation of high-risk patients, and prudent use of antibiotic agents are needed to prevent further dissemination and selection of these drug-resistant bacteria.

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Disclosure

The authors report no conflicts of interest in this work.

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