Infection and Drug Resistance

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ORIGINAL RESEARCH

Clinical outcome of *Escherichia coli* bloodstream infection in cancer patients with/without biofilm formation: a single-center retrospective study

This article was published in the following Dove Medical Press journal:
Infection and Drug Resistance

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Background: Extended-spectrum β-lactamase-producing *Escherichia coli* (ESBL-EC) is one of the main antimicrobial-resistant pathogens. Little data are available on how biofilm formation (BF) contributes to EC-caused bloodstream infection (BSI) in cancer patients. This study investigated the impact of BF on clinical outcomes of cancer patients with EC-caused BSI.
Methods: Clinical outcome and microbiological characteristics including the presence of *bla* genes in ESBL-EC isolates were retrospectively collected from BSI cancer patients. Patients infected with ESBL-EC were compared with patients infected with third-generation cephalosporin-susceptible strains. Survival curves were generated by Kaplan–Meier analysis and the survival difference was assessed by the log-rank test. Risk factors for ESBL-EC infection, predictors of mortality, and outcome differences were determined by multivariate logistic regression and Cox regression analysis, respectively.
Results: A high prevalence of ESBL-EC with dominant $bla_{\text{CTX-M-15}}$, $bla_{\text{CTX-M-15}}$ plus $bla_{\text{TEM-52}}$ genotype was found in BSI cancer patients. Independent risk factors for infection with ESBL-EC were cephalosporins, chemotherapy, and BF. Metastasis, ICU admission, BF-positive ESBL-EC, organ failure, and the presence of septic shock were revealed as predictors for mortality. The ESBL characteristic was associated with the BF phenotype, and the overall mortality was significantly higher in cancer patients with BF-positive ESBL-EC-caused BSI.
Conclusion: $bla_{\text{CTX-M-15}}$ type ESBL-EC is highly endemic among cancer patients with BSI. BF is associated with multi-drug resistance by ESBL-EC and is also an independent risk factor of mortality for cancer patients with BSI. Our findings suggest that the combination of BF-positive ESBL-EC isolates with other appropriate laboratory indicators might benefit infection control and improve clinical outcomes.

Keywords: biofilm formation, extended-spectrum beta-lactamase, *Escherichia coli*, bloodstream infection

Introduction

Bloodstream infection (BSI) is one of the most severe forms of nosocomial infection, especially in immunocompromised cancer patients. *Escherichia coli* (EC) is a common cause of BSI, and production of extended-spectrum β-lactamase (ESBL) is the main mechanism conferring resistance to third-generation cephalosporins, which results in treatment problems, higher morbidity, mortality, and increased health care costs.[1] Previous studies showed that biofilm formation (BF) is associated with resistance of EC toward antimicrobial drugs, and BF markedly increases the incidence of health care-associated infections, especially in catheter-related BSI.[2–5] One study indicated that 60.2% of EC strains were multi-drug resistant (MDR, maximum resistance to

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http://dx.doi.org/10.2147/IDR.S192072

ampicillin), and 43% of MDR EC had a biofilm-positive phenotype.[2] BF results in serious clinical problems because of its resistance to host defense systems and to conventional antimicrobial therapy, which substantially hinders various treatments. Although many studies reported that BF is closely associated with EC-caused urinary tract infections,[2,6,7] more recent studies indicated that bacterial BF might act as a direct triggering factor contributing to cancer initiation and progression. For example, in colorectal cancer experimental models, biofilm microbial populations can significantly impair the intestinal epithelial barrier function, alter polyamine metabolism affecting cellular proliferation, enhance pro-inflammatory/pro-oncogenic responses, and exacerbate intestinal dysbiosis.[8] The invasive and co-aggregation capacity of microbiota may be essential for biofilm-promoted colon tumorigenesis. In addition, some studies attributed BF-related mortality to certain debatable factors, such as drugs with no activity against BF, biofilm heterogeneity, and the presence of comorbidities.[7,9] However, to our knowledge, there is little information about how BF contributes to EC-caused BSI, especially to extended-spectrum beta-lactamase-producing *Escherichia coli* (ESBL-EC)-caused BSI in cancer patients. Therefore, the aim of this study was to investigate the impact of BF-positive, EC-caused BSI on the clinical outcome of hospitalized cancer patients.

Methods

Setting and study design

A retrospective study was conducted at the Tianjin Medical University Cancer Institute and Hospital (http://www.tjmuch.com/, http://www.tjmuch.com/zlyjs/) between January 2013 and September 2017. All hospitalized cancer patients with the first episode of BSI were included in the study. Cancer patients with polymicrobial BSI, under the age of 18, or with non-EC-caused BSI were excluded from the study. Data retrospectively collected included age, sex, associated diseases, sources of BSI, invasive procedures, such as urinary catheterization or tracheostomy during the preceding 3 months, multiple shot antibiotics therapy during the preceding 3 months, the presence of severe sepsis or septic shock, and in-hospital mortality.

Depending on the different requirements, cancer patients included in this study were divided into the following groups: patients with BSI due to an isolate of ESBL-EC and those with non-ESBL-EC-caused BSI. The two groups were compared in order to identify independent risk factors for ESBL-EC infection. Patients who died were further compared with those who survived to determine predictors for mortality. Furthermore, the outcome differences between BF-positive and BF-negative EC-infected patients or ESBL-EC-infected patients were assessed.

Definition

All cases of cancer were confirmed by pathology. The BSI assessment of whether the isolated organisms represent true BSI, rather than contamination, was made based on the clinical or laboratory evidence of infection: fever, hypothermia, evidence of localized infection, inadequate organ perfusion, severe sepsis, and leukocytosis. The definitions of severe sepsis and septic shock were adapted from the American College of Chest Physicians/Society of Critical Care Medicine Consensus Conference Committee.[10] The source of BSI was determined according to the definition of nosocomial infections by the Center for Disease Control and Prevention, and the presence of clinical signs with EC isolation from the presumed source.[11] MDR was defined as acquired non-susceptibility to at least one agent in three or more antimicrobial categories. Once a species has intrinsic resistance to certain antimicrobial agents, related antimicrobial classes are not counted when calculating the number of classes to which the isolate is resistant.[11] In-hospital mortality was defined as death by any cause within the first 30 days after the onset of BSI during hospitalization.

Microbiological procedures

Blood cultures (8–10 mL blood from a patient) inoculated in BACTEC plus aerobic/F and anaerobic lytic/10 vials, were incubated using the automated blood culture system (BACTEC FX400; Becton Dickinson, NJ, USA) at 35°C for at least 5 days. Positive cultures were determined with gram staining, and then subcultured on both blood agar and MacConkey plates (JinZhangKeji, Tianjin, China) at 35°C for 18–24 hours. Pathogens identification and susceptibility tests were performed on the Vitek 2 Compact automated microbiology system (BioMerieux, Craponne, France) by using GN and GN67 cards. The Clinical and Laboratory Standards Institute criteria were used to define the susceptibility or the resistance to antimicrobial agents.[12]

ESBL screening, confirmatory test, and gene detection

Bacterial isolates identified as EC, were stored on glycerol tryptic soy broth in a –20°C freezer. When a fresh seed-stock vial was required, it was removed and used to inoculate a series of working cultures.

The VITEK 2 Compact was used to screen ESBL-EC. The ESBL confirmatory test was performed using cefotaxime (CTX, 30 μg), ceftazidime (30 μg) alone, or in combination with clavulanate (10 μg) on Mueller-Hinton agar.[12] Strains producing ESBL were confirmed by ≥5 mm increments in zone diameter. EC ATCC25922 (the negative control) and *Klebsiella pneumoniae* ATCC 700603 (the positive ESBL producer) (American Type Culture Collection, USA) were used for quality control.

Detection of ESBL genotype-related gene families (bla_{TEM}, bla_{SHV}, and bla_{CTX-M}) was performed using PCR with primers (Table S1) and conditions previously described.[13–15] PCR amplification for the individual *bla* gene was carried out in a Thermal Cycler (T100, Bio-Rad). A negative control (nuclease-free water) was included in each run. PCR products were electrophoresed on a 1% agarose gel containing ethidium bromide, and finally visualized in a gel documentation system (ChemiDoc XRS, Bio-Rad). Purified PCR products were sequenced (Sangon Biotech), and the sequencing results were forwarded for bioinformatics analysis using the Basic Local Alignment Search Tool online program (http://blast.ncbi.nlm.nih.gov/Blast.cgi).

BF detection

Crystal violet staining was performed in 96-well plates with all EC isolates sampled.[16] For each EC isolate, a single colony was incubated in test tubes containing 5 mL of Luria–Bertani (LB) medium and then shake-cultured at 37°C for 18 hours. Then, 1 mL of bacterial suspension was trans-inoculated into a new test tube containing 5 mL of sterile LB medium. Each well of the sterile 96-well flat-bottomed plastic tissue culture plate with a lid was filled with 200 μL of bacterial suspension. Negative controls (blank) were LB broth alone, which was dispensed into eight wells per tray. After aerobic incubation at 37°C for 24 hours, the content of the wells was carefully drawn off and each well was washed three times with 250 μL of sterile physiological saline. Biofilms were stained with 0.2 mL of crystal violet (2%) for 5 minutes at room temperature. Unspecific staining in the wells was rinsed out three times with PBS. The plates were inverted on a towel, allowed to air dry, and the dye-stained adherent cells in each well were dissolved with 200 μL 30% acetic acids. The OD of re-solubilized crystal violet in each well was measured at a wavelength of 590 nm absorbance. All tests were carried out three times, with three wells per culture, and the results were averaged (cutoff value is 0.218; Table S2).

Statistical analyses

The mean and SD were calculated for continuous variables. Continuous variables with normal distribution were compared with the Student's *t*-test, and variables that were not normally distributed were compared with the Mann–Whitney *U* test. The chi-squared test was used to compare categorical variables. All statistically significant variables with $P<0.05$ identified in the univariate analysis were further analyzed by multivariate logistic regression analysis. OR and 95% CI were calculated. Kaplan–Meier survival analysis was used to generate survival curves, and the difference between the survival curves was assessed by mean of the log-rank test. Cox regression analysis for the predictors of survival was performed. All significant variables related to mortality in the univariate analysis were entered into the multivariate model for further analysis. All analyses were performed using the SPSS version 23.0 software. Two-sided *P*-values <0.05 were considered to have statistical significance.

Ethics approval and consent to participate

The study was approved by the ethics committee of Tianjin Medical University Cancer Institute and Hospital. Waiving of informed consent was obtained due to the retrospective noninterventional study design. Data were collected anonymously.

Results

Risk factors of ESBL-EC-caused BSI in cancer patients

During the study period, 324 cases of EC-caused BSI in hospitalized cancer patients were identified, among which 160 episodes showed positive results and 164 episodes showed negative results in the ESBL confirmation test. In PCR detection of ESBL genotypes, 91.88% of the screened ESBL-positive EC isolates were found to possess one or more of the ESBL genes (bla_{TEM}, bla_{SHV}, and bla_{CTX-M}) tested in this study (Figure S1). The overall prevalence of ESBL genotypes in EC isolates is shown in Figure 1, and the main genotypes that predominated in these isolates were $bla_{CTX-M-15}$ (31.29%) and bla_{TEM-52} plus $bla_{CTX-M-15}$ (42.17%) (Table S3).

The clinical characteristics of cancer patients with EC-caused BSI are summarized in Table 1. EC-caused BSI occurred in 305 (94.14%) patients with solid tumors, among which most episodes were frequently acquired in patients with pancreatic cancer (61, 20.00%), hepatic carcinoma (49, 16.07%), followed by colorectal cancer (45, 14.75%), gastric carcinoma (43, 14.10%), and gynecological cancer (33,

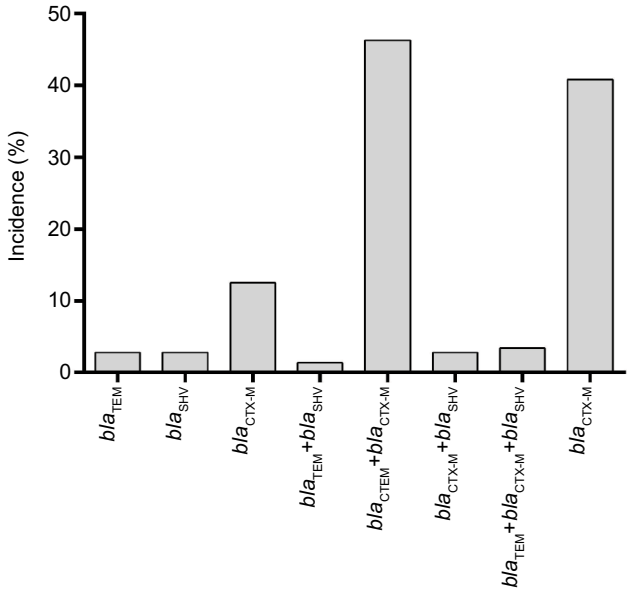

Figure 1 Distribution of ESBL genotypes in screened positive EC isolates.
Abbreviations: EC, *Escherichia coli*; ESBL, extended-spectrum β-lactamase.

10.82%). ESBL-EC-caused BSI was associated with recent exposure to antibiotics (cephalosporins and carbapenems), chemotherapy, and BF as determined by univariate analysis. Cancer patients with non-ESBL-EC-caused BSI had received more β-lactam/β-lactamase inhibitor combinations therapy compared to the ESBL-EC-caused BSI group. After applying a logistic regression model, independent risk factors for infection with ESBL-EC were therapy with third-generation cephalosporin (OR 0.30; 95% CI: 0.09–0.94; P=0.04), chemotherapy (OR 1.80; 95% CI: 1.12–2.89; P=0.02), and BF (OR: 2.79, 95% CI: 1.61–4.83; P<0.001). BF-producing EC-caused BSI was significantly observed in colorectal cancers and hematological malignancies (Table S4). To further determine the risk factors for BF, logistic regression analysis was again used in the regrouped data, and only the ESBL characteristic (OR 3.21; 95% CI: 1.86–5.53; P<0.001) was shown as an independent risk factor for the BF phenotype (Table 2).

Table 1 Clinical characteristic of cancer patients with ESBL/non-ESBL EC-caused BSI

Characteristics	ESBL (n=160), n (%)	Non-ESBL (n=164), n (%)	*P*-value	OR (95% CI)	*P*-value[a]
Demographics					
Males, n (%)	74 (46.25)	82 (50.00)	0.50		
Age (years), median (range)	61 (28–83)	62 (19–85)	0.26		
Comorbidities					
Hypertension	52 (32.50)	60 (36.59)	0.27		
Chronic heart disease	23 (14.38)	29 (17.68)	0.34		
Diabetes mellitus	23 (14.38)	26 (15.85)	0.71		
Underlying disease					
Solid tumor	149 (93.13)	156 (95.12)	0.44		
Hematological disease	11 (6.88)	8 (4.88)			
Source of BSI					
Abdominal infection	35 (21.88)	41 (25.00)	0.43		
Urinary tract infection	36 (22.50)	32 (19.51)	0.60		
Biliary infection	47 (29.38)	47 (28.66)	0.89		
Pulmonary infection	26 (16.25)	30 (18.29)	0.73		
Catheter-related	8 (5.00)	3 (1.83)	0.12		
Unknown origin	4 (2.50)	9 (5.49)	0.26		
Others[b]	4 (2.50)	2 (1.22)	0.39		
Previous exposure to antibiotics (within 1 month)					
Cephalosporin	34 (21.25)	20 (12.20)	0.03	0.30 (0.09–0.94)	0.04
β-lactam/β-lactamase inhibitor	17 (10.63)	36 (21.95)	0.01	0.98 (0.31–3.13)	0.98
Carbapenem	95 (59.38)	79 (48.17)	0.04	0.43 (0.15–1.21)	0.11
Combined	8 (5.00)	16 (9.76)	0.06		
Aminoglycosides	6 (3.75)	13 (7.93)	0.11		
LOS to the first positive culture	30.89±24.65	29.18±19.27	0.49		
Invasive procedure	81 (50.63)	79 (48.17)	0.66		
Chemotherapy	106 (66.25)	84 (51.22)	0.01	1.80 (1.12–2.89)	0.02
Surgery (within 1 month)	79 (49.38)	94 (57.32)	0.15		
BF	57 (35.63)	24 (14.63)	<0.001	2.79 (1.61–4.83)	<0.001

Notes: [a]Multivariate logistic regression analysis. [b]Intracranial infection; vagina infection; pelvic cavity infection.
Abbreviations: BF, biofilm formation; BSI, bloodstream infection; EC, *Escherichia coli*; ESBL, extended-spectrum β-lactamase; LOS, length of hospital stay (days).

Table 2 Clinical characteristic of cancer patients with BF/non-BF EC-caused BSI

Characteristics	BF-EC (n=81), n (%)	Non-BF-EC (n=243), n (%)	*P*-value	OR (95% CI)	*P*-value[a]
Demographics					
Males, n (%)	31 (38.27)	125 (51.44)	0.04	0.59 (0.35–1.01)	0.05
Age (years), median (range)	61 (34–84)	63 (19–85)	0.38		
Comorbidities					
Hypertension	28 (34.57)	84 (34.57)	1.00		
Chronic heart disease	13 (16.05)	39 (16.05)	1.00		
Diabetes mellitus	8 (9.88)	41 (16.87)	0.13		
Underlying disease					
Solid tumor	74 (91.36)	231 (95.06)	0.22		
Hematological disease	7 (8.64)	12 (4.94)	0.22		
Source of BSI			0.53		
Abdominal infection	17 (20.99)	59 (24.28)	0.50		
Urinary tract infection	18 (22.22)	50 (20.58)	0.81		
Biliary infection	23 (28.40)	71 (29.22)	0.89		
Pulmonary infection	19 (23.46)	37 (15.23)	0.09		
Catheter-related	1 (1.23)	10 (4.12)	0.22		
Unknown origin	3 (3.70)	10 (4.11)	0.50		
Others	1 (1.23)	5 (2.05)	0.64		
Previous exposure to antibiotics (within 1 month)			0.85		
Third-generation cephalosporins	16 (19.75)	38 (15.64)	0.39		
β-lactam/β-lactamase inhibitor	9 (11.11)	44 (18.11)	0.14		
Carbapenems	48 (59.26)	126 (51.85)	0.25		
Combined	5 (6.17)	19 (7.82)	0.62		
Aminoglycosides	3 (3.70)	16 (6.58)	0.34		
Invasive procedure	39 (48.15)	121 (49.79)	0.80		
Surgery (within 1 month)	44 (54.32)	129 (53.09)	0.90		
Chemotherapy	54 (66.67)	136 (55.97)	0.09		
ESBL	57 (70.37)	103 (42.39)	<0.001	3.21 (1.86–5.53)	<0.001

Note: [a]Multivariate logistic regression analysis.
Abbreviations: BF, biofilm formation; BSI, bloodstream infection; EC, *Escherichia coli*; ESBL, extended-spectrum β-lactamase.

Outcome difference among cancer patients with EC-caused BSI

The mortality rate was 21.91% (71/324) for all cancer patients with EC-caused BSI. The overall mortality was significantly higher in the BF-positive EC-caused BSI group compared to that in the BF-negative EC-caused BSI group (42.25% vs 20.16%; log-rank $P<0.001$) (Figure 2). Compared with the BF-negative ESBL-EC-caused BSI group, the overall mortality was significantly higher in the BF-positive ESBL-EC-caused BSI group ($P=0.001$) (Figure 3).

Risk factor analysis between the survival and non-survival cancer patients is summarized in Table 3. Univariate analysis results revealed that diabetes mellitus, LOS, ICU admission, metastasis, mechanical ventilation, BF, organ failure, and the presence of septic shock were significantly associated with death. After multivariate Cox regression analysis was performed, the variables that constituted independent predictors for mortality were metastasis (OR =2.71 95% CI: 1.59–4.63; $P=0.001$), ICU admission (OR =2.08, 95% CI: 1.13–3.84; $P=0.02$), BF (OR =2.20, 95% CI: 1.33–3.63; $P=0.002$), organ failure (OR =10.33, 95% CI: 5.92–18.03; $P<0.001$), and the presence of septic shock (OR =2.17, 95% CI: 1.24–3.78; $P=0.006$).

Discussion

In this study, we retrospectively collected the clinical microbiological characteristic of cancer patients with EC-caused BSI. We systematically evaluated the risk factors, survival difference, and mortality predictors for cancer patients with bloodstream-infected by EC with/without BF. The results showed that prior exposure to cephalosporins, chemotherapy, and BF were three independent risk factors for ESBL-EC-caused BSI. Cancer patients infected by BF-positive ESBL-EC had a lower survival rate. Metastasis, ICU admission,

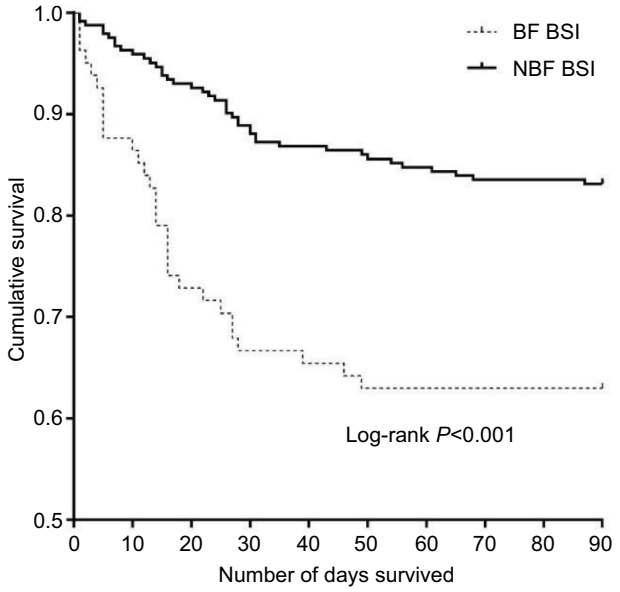

Figure 2 Survival analysis of EC-caused BSI in cancer patients with/without BF.
Notes: The Kaplan–Meier curve shows the survival curves until day 90 for the two infection groups. The mortality risk was higher among the cancer patients with BF-positive EC-caused BSIs compared with those with BF-negative EC-caused BSIs ($P<0.001$).
Abbreviations: BF, biofilm formation; BSI, bloodstream infection; EC, *Escherichia coli*; NBF BSI, BF-negative EC-caused bloodstream infections.

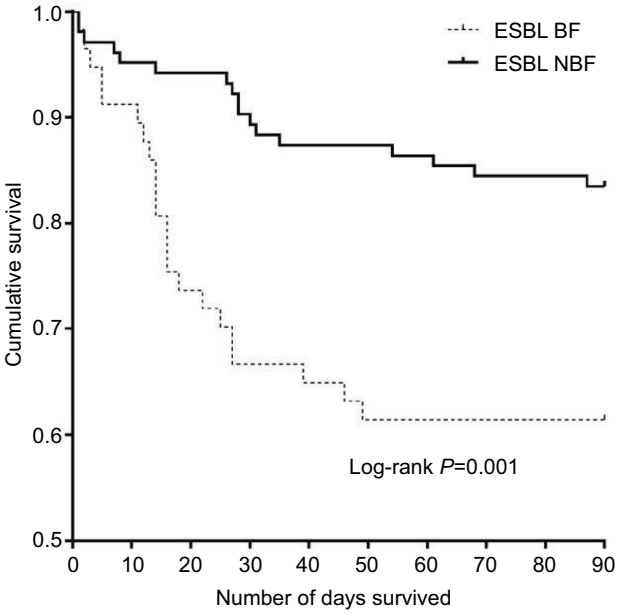

Figure 3 Survival analysis of ESBL-EC-caused BSI in cancer patients with/without BF.
Notes: The Kaplan–Meier curve shows the survival curves until day 90 for the two infection groups. The mortality risk was higher among the cancer patients with BF-positive ESBL-EC-caused BSIs compared with those with BF-negative EC-caused BSIs ($P=0.001$).
Abbreviations: BF, biofilm formation; EC, *Escherichia coli*; ESBL, extended-spectrum β-lactamase.

organ failure, septic shock, and BF-positive ESBL-EC infection were five independent risk factors for mortality. These data highlight that BF is closely associated with ESBL-EC-caused BSI and prominently affects the survival of BSI cancer patients.

EC is one of the most frequent causes of BSI in many tertiary care hospitals. Our previous studies revealed that cancer patients often suffer from treatment problems due to EC-caused BSI.[17,18] In the present study, we further showed the high prevalence of MDR EC (49.38%) in cancer patients with BSI. PCR amplification based on gene-specific primers followed by nucleotide sequencing provides the easiest and most reliable method to detect the ESBL genotype.[15,19,20] Previous studies reported a high prevalence of ESBL-EC in the Asia-Pacific area.[15,19,21] Although the CTX-M type of ESBL is believed to be the dominant type in Europe and Asia,[15,19,22,23] it was remarkable that in the present study, only 40.82% of ESBL-EC carried the $bla_{\text{CTX-M}}$ genotype whereas 46.26% of ESBL-EC carried the bla_{TEM} plus $bla_{\text{CTX-M}}$ genotype (Figure 1; Table S3). This result was similar to several clinical observation conducted in other patients,[24–27] showing that TEMs genotype are frequently encountered in clinical isolates expressing the CTX-Ms genotype. The reason for co-occurrence of TEM and CTX-M genotypes in ESBL-EC is not clear. It may reflect the redistribution of resistant genes in immunocompromised cancer patients, or provide complementary contributions to the overall resistance of the ESBL producers.[28] In addition, it was found that among the isolated ESBL-ECs, 49.38% carried two or three *bla* genes, which might also account for the high-level β-lactam-resistant phenotype.

BF is a unique mechanism exhibited by several microbes in order to survive in unfavorable conditions. It is a structured community of bacterial cells enclosed in a polymeric matrix and adherent to a surface.[9] Biofilm-producing bacteria are highly resistant to antibiotic treatment. The ability of bacterial BF to adhere to medical devices and the use of invasive medical devices are widely recognized.[29] It was reported that the prevalence of MDR in BF isolates are more common in comparison to non-BF isolates.[3] We found that 81 (25.00%) EC isolates from 324 BSI cancer patients were biofilm producers. To our knowledge, this is the first report of BF in cancer patients with EC-caused BSI. Interestingly, it was found that BF was significantly higher in ESBL-positive EC isolates than in that of ESBL-negative isolates (Table 1). This finding is similar to the investigations on other infectious types. The studies by Subramanian et al and Neupane et al

Table 3 Risk factors associated with the mortality of cancer patients with EC-caused BSI

Characteristics	Survivors (n=253)	Non-survivors (n=71)	*P*-value	OR (95% CI)	*P*-value[a]
Demographics					
Age (years), median (range)	63 (19–85)	61 (34–80)	0.235		
Males (n %)	128 (50.59)	28 (39.44)	0.112		
Comorbidities					
Hypertension	92 (36.36)	20 (28.17)	0.192		
Chronic heart disease	42 (16.60)	10 (14.08)	0.558		
Diabetes mellitus	32 (12.65)	17 (23.94)	0.020	1.21 (0.67–2.17)	0.53
Underlying disease					
Solid tumor	240 (94.86)	65 (91.55)	0.231		
Hematological disease	13 (5.14)	6 (8.45)			
LOS	29.05±21.69	34.70±23.45	0.048	1.00 (0.99–1.01)	0.96
ICU admission	19 (7.51)	15 (21.13)	0.002	2.08 (1.13–3.84)	0.02
Invasive procedure	122 (48.22)	38 (53.52)	0.494		
Mechanical ventilation	56 (22.13)	42 (59.16)	<0.001	1.27 (0.44–3.63)	0.66
Metastasis	100 (39.53)	46 (64.79)	<0.001	2.71 (1.59–4.63)	0.001
Chemotherapy	147 (58.10)	43 (60.56)	0.712		
Surgery	134 (52.96)	39 (54.93)	0.833		
Previous blood transfusion	98 (38.74)	31 (43.66)	0.520		
ESBL	121 (47.83)	39 (54.93)	0.310		
BF	51 (20.16)	30 (42.25)	<0.001	2.20 (1.33–3.63)	0.002
Organ failure	12 (4.74)	47 (66.20)	<0.001	10.33 (5.92–18.03)	<0.001
Sepsis shock	17 (6.72)	29 (40.85)	<0.001	2.17 (1.24–3.78)	0.006

Note: [a]Multivariate logistic regression analysis.
Abbreviations: BF, biofilm formation; BSI, bloodstream infection; EC, *Escherichia coli*; ESBL, extended-spectrum β-lactamase; ICU, intensive care unit; LOS, length of hospital stay (days).

reported similar observations in patients with urinary tract infections that ESBL-EC strains more frequently formed biofilm in comparison to that of non-ESBL-EC isolates.[30,31] The reason of the higher ability of ESBL-EC strains to form biofilm is not clear. Activation of several stress response genes and expression of certain virulence genes during bacterial chromosomal gene rearrangements to acquire the ESBL plasmids may be the underlying mechanism.[30–33] Among these studies, enhanced biofilm formation capacity in ESBL-plasmid-carrying ST131 and ST648 strains that synthesize virulent- and survival-associated extracellular matrix components (eg, curli fimbriae and/or cellulose) was particularly notable. A deeper understanding of the contribution of transcription factor csgD to biofilm formation and motility capacity in pandemic ESBL-producing EC lineages may be the solution.[33]

Although BF has been repeatedly described to increase the antibiotic resistance of microorganisms, one study showed it was not associated with any clinical characteristic in patients with EC bacteremia.[34] Our study found that BF was not only significantly associated with ESBL-EC-caused BSI but was also an independent risk factor for mortality in BSI cancer patients (Table 3). This finding highlights the equal importance of the BF contribution to mortality as the traditional factors, such as ICU admission, metastasis, organ failure, and shock. Our finding that BF was an independent predictor for mortality further confirms this point. These results are in line with a previous study on enterobacteriaceae-caused infections in non-cancer patients.[33] The higher ability of the ESBL-EC to form biofilm increases the mortality and infection severity of cancer patients, making current treatments even more difficult. Therefore, adoption of alternative antibiotics (eg, rifampicin, gentamicin, azithromycin, clarithromycin, tigecycline, colistin, and daptomycin) that can disrupt or inhibit BF may be a good choice.[32] Clinicians should take into account the use and doses of BF-response antibiotics to be on the safe side, as exposure to sub-inhibitory concentrations of these antibiotics can enhance BF. In addition, innovative approaches aimed at overcoming biofilm resistance to conventional antimicrobial agents should be tested in small-scale clinical trials. These include the use of weak organic acids (acetic acid used in hepatology/oncology and citric acid used in hematology/oncology/renal), photo irradiation (blue light therapy), and the application of bacteriophages (phage endolysin with anti-Gram-negative activity).[24] Perhaps,

combining aspects of these approaches can greatly benefit cancer patients with BF-positive ESBL-EC infection.

Limitations

This study was performed based on a heterogeneous cancer patients group in a single institution, which may present different clinical characteristics than other types of patients and may not reflect the epidemiology of other cancer centres in China. In addition, it is a pity that detailed typing of the ESBL genes was not done.

Conclusion

This study showed that BF is not only significantly associated with the ESBL production by EC but is also an independent risk factor for mortality in hospitalized cancer patients with BSI. Our findings suggest that the combination of BF-positive ESBL-EC isolates with other appropriate laboratory indicators might benefit infection control and improve clinical outcomes, which may facilitate current antibiotic treatment and prognosis to BSI cancer patients.

Acknowledgments

The authors wish to thank their colleagues who assisted in data collection. Thanks to Dr Edward C Mignot, Shandong University, for linguistic advice. This work was supported by the National Natural Science Foundation of China (No. 30700829, No. 81373318), the Natural Science Foundation of Tianjin (No. 16JCYBJC23900), and the Tianjin Municipal Science and Technology Commission (No. 2016KYZQ03).

Disclosure

The authors report no conflicts of interest in this work.

References

1. Schwaber MJ, Carmeli Y. Mortality and delay in effective therapy associated with extended-spectrum beta-lactamase production in Enterobacteriaceae bacteraemia: a systematic review and meta-analysis. *J Antimicrob Chemother*. 2007;60(5):913–920.
2. Tajbakhsh E, Ahmadi P, Abedpour-Dehkordi E, Arbab-Soleimani N, Khamesipour F. Biofilm formation, antimicrobial susceptibility, serogroups and virulence genes of uropathogenic *E. coli* isolated from clinical samples in Iran. *Antimicrob Resist Infect Control*. 2016;5:11.
3. Costerton JW, Stewart PS, Greenberg EP. Bacterial biofilms: a common cause of persistent infections. *Science*. 1999;284(5418):1318–1322.
4. Costerton B. Microbial ecology comes of age and joins the general ecology community. *Proc Natl Acad Sci U S A*. 2004;101(49): 16983–16984.
5. Fux CA, Costerton JW, Stewart PS, Stoodley P. Survival strategies of infectious biofilms. *Trends Microbiol*. 2005;13(1):34–40.
6. Mittal S, Sharma M, Chaudhary U. Biofilm and multidrug resistance in uropathogenic *Escherichia coli*. *Pathog Glob Health*. 2015;109(1):26–29.
7. Tascini C, Sozio E, Corte L, et al. The role of biofilm forming on mortality in patients with candidemia: a study derived from real world data. *Infect Dis (Lond)*. 2018;50(3):214–219.
8. Li S, Konstantinov SR, Smits R, Peppelenbosch MP. Bacterial biofilms in colorectal cancer initiation and progression. *Trends Mol Med*. 2017;23(1):18–30.
9. Rajendran R, Sherry L, Nile CJ, et al. Biofilm formation is a risk factor for mortality in patients with *Candida albicans* bloodstream infection – Scotland, 2012-2013. *Clin Microbiol Infect*. 2016;22(1):87–93.
10. Bone RC, Balk RA, Cerra FB, et al. Definitions for sepsis and organ failure and guidelines for the use of innovative therapies in sepsis. The ACCP/SCCM Consensus Conference Committee. American College of Chest Physicians/Society of Critical Care Medicine. *Chest*. 1992;101(6):1644–1655.
11. Bereket W, Hemalatha K, Getenet B, et al. Update on bacterial nosocomial infections. *Eur Rev Med Pharmacol Sci*. 2012;16(8):1039–1044.
12. Institute CaLS. *Performance Standards for Antimicrobial Susceptibility Testing Supplement. Twenty-Second Informational Supplement*. Vol. M100-S22. Wayne, PA, USA: CLSI; 2012.
13. Sharma J, Sharma M, Ray P. Detection of TEM & SHV genes in *Escherichia coli* & *Klebsiella pneumoniae* isolates in a tertiary care hospital from India. *Indian J Med Res*. 2010;132:332–336.
14. Lee SH, Kim JY, Lee SK, Jin W, Kang SG, Lee KJ. Discriminatory detection of extended-spectrum beta-lactamases by restriction fragment length dimorphism-polymerase chain reaction. *Lett Appl Microbiol*. 2000;31(4):307–312.
15. Edelstein M, Pimkin M, Palagin I, Edelstein I, Stratchounski L. Prevalence and molecular epidemiology of CTX-M extended-spectrum beta-lactamase-producing *Escherichia coli* and *Klebsiella pneumoniae* in Russian hospitals. *Antimicrob Agents Chemother*. 2003;47(12):3724–3732.
16. Stepanovic S, Vukovic D, Dakic I, Savic B, Svabic-Vlahovic M. A modified microtiter-plate test for quantification of staphylococcal biofilm formation. *J Microbiol Methods*. 2000;40(2):175–179.
17. Zhang Q, Zhang W, Li Z, et al. Bacteraemia due to AmpC β-lactamase-producing *Escherichia coli* in hospitalized cancer patients: risk factors, antibiotic therapy, and outcomes. *Diagn Microbiol Infect Dis*. 2017;88(3):247–251.
18. Zhang Q, Li D, Bai C, et al. Clinical prognostic factors for time to positivity in cancer patients with bloodstream infections. *Infection*. 2016;44(5):583–588.
19. Kiratisin P, Apisarnthanarak A, Laesripa C, Saifon P. Molecular characterization and epidemiology of extended-spectrum-beta-lactamase-producing *Escherichia coli* and *Klebsiella pneumoniae* isolates causing health care-associated infection in Thailand, where the CTX-M family is endemic. *Antimicrob Agents Chemother*. 2008;52(8):2818–2824.
20. Ehlers MM, Veldsman C, Makgotlho EP, Dove MG, Hoosen AA, Kock MM. Detection of blaSHV, blaTEM and blaCTX-M antibiotic resistance genes in randomly selected bacterial pathogens from the Steve Biko academic hospital. *FEMS Immunol Med Microbiol*. 2009;56(3):191–196.
21. Hirakata Y, Matsuda J, Miyazaki Y, et al. Regional variation in the prevalence of extended-spectrum beta-lactamase-producing clinical isolates in the Asia-Pacific region (SENTRY 1998-2002). *Diagn Microbiol Infect Dis*. 2005;52(4):323–329.
22. Livermore DM, Canton R, Gniadkowski M, et al. CTX-M: changing the face of ESBLs in Europe. *J Antimicrob Chemother*. 2007;59(2):165–174.
23. Feizabadi MM, Delfani S, Raji N, et al. Distribution of bla(TEM), bla(SHV), bla(CTX-M) genes among clinical isolates of *Klebsiella pneumoniae* at Labbafinejad Hospital, Tehran, Iran. *Microb Drug Resist*. 2010;16(1):49–53.
24. Fang H, Ataker F, Hedin G, Dornbusch K. Molecular epidemiology of extended-spectrum beta-lactamases among *Escherichia coli* isolates collected in a Swedish hospital and its associated health care facilities from 2001 to 2006. *J Clin Microbiol*. 2008;46(2):707–712.
25. Arpin C, Quentin C, Grobost F, et al. Nationwide survey of extended-spectrum {beta}-lactamase-producing Enterobacteriaceae in the French community setting. *J Antimicrob Chemother*. 2009;63(6):1205–1214.
26. Mulvey MR, Bryce E, Boyd D, et al. Ambler class A extended-spectrum beta-lactamase-producing *Escherichia coli* and *Klebsiella* spp. in Canadian hospitals. *Antimicrob Agents Chemother*. 2004;48(4):1204–1214.

27. Romero ED, Padilla TP, Hernández AH, et al. Prevalence of clinical isolates of *Escherichia coli* and *Klebsiella* spp. producing multiple extended-spectrum beta-lactamases. *Diagn Microbiol Infect Dis*. 2007;59(4):433–437.
28. Salverda ML, De Visser JA, Barlow M. Natural evolution of TEM-1 β-lactamase: experimental reconstruction and clinical relevance. *FEMS Microbiol Rev*. 2010;34(6):1015–1036.
29. Donelli G, Vuotto C. Biofilm-based infections in long-term care facilities. *Future Microbiol*. 2014;9(2):175–188.
30. Neupane S, Pant ND, Khatiwada S, Chaudhary R, Banjara MR. Correlation between biofilm formation and resistance toward different commonly used antibiotics along with extended spectrum beta lactamase production in uropathogenic *Escherichia coli* isolated from the patients suspected of urinary tract infections visiting Shree Birendra Hospital, Chhauni, Kathmandu, Nepal. *Antimicrob Resist Infect Control*. 2016;5:5.
31. Subramanian P, Umadevi S, Kumar S, Stephen S. Determination of correlation between biofilm and extended spectrum β lactamases producers of Enterobacteriaceae. *Scho Res J*. 2012;2(1):2.
32. Jolivet-Gougeon A, Bonnaure-Mallet M. Biofilms as a mechanism of bacterial resistance. *Drug Discov Today Technol*. 2014;11:49–56.
33. Schaufler K, Semmler T, Pickard DJ, et al. Carriage of extended-spectrum beta-lactamase-plasmids does not reduce fitness but enhances virulence in some strains of pandemic *E. coli* lineages. *Front Microbiol*. 2016;7:336.
34. Martínez JA, Soto S, Fabrega A, et al. Relationship of phylogenetic background, biofilm production, and time to detection of growth in blood culture vials with clinical variables and prognosis associated with *Escherichia coli* bacteremia. *J Clin Microbiol*. 2006;44(4):1468–1474.

Supplementary materials

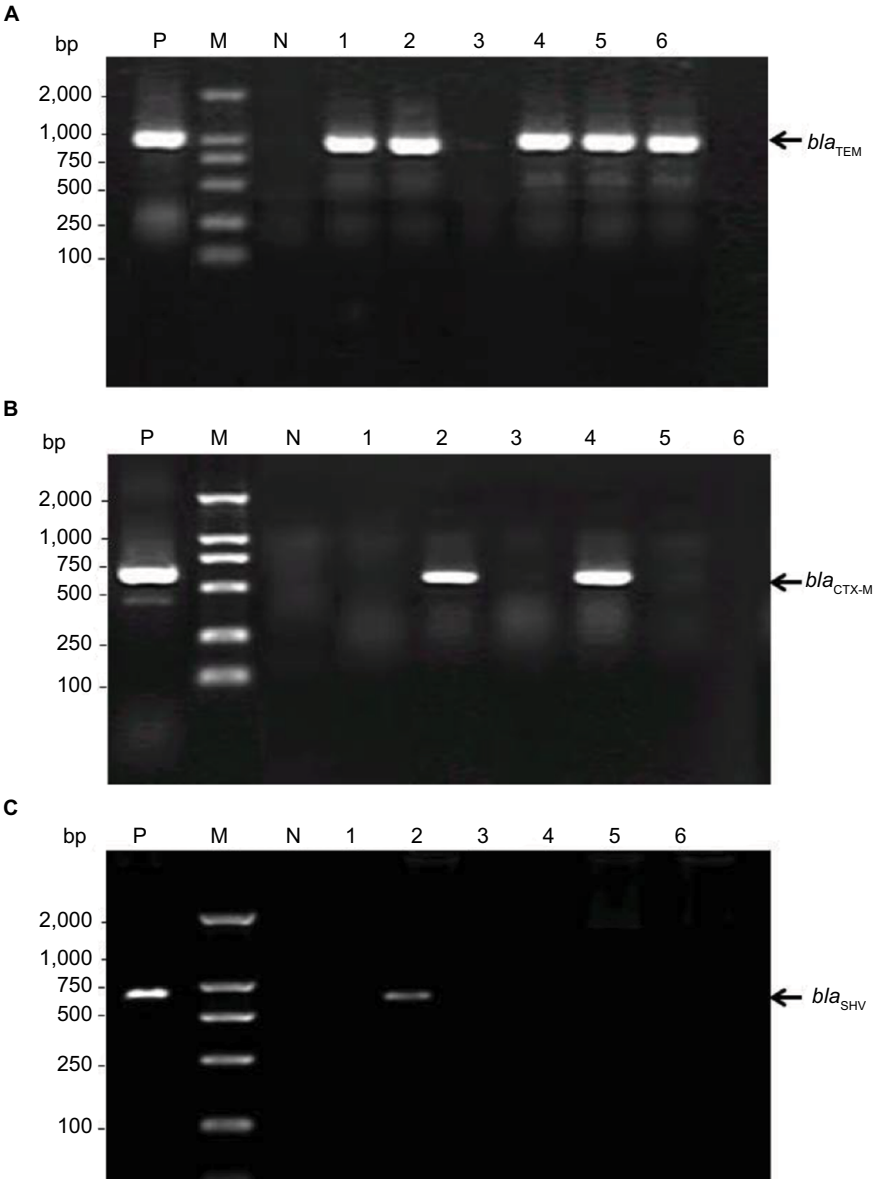

Figure S1 Multiplex PCR analysis of ESBL-EC isolates.
Notes: PCR products from partial samples were separated in a 1% agarose gel. M, DNA ladder. P, positive control. Previously confirmed EC isolates containing bla_{TEM}, bla_{SHV}, and bla_{CTX-M} genes. N, negative control (ddH_2O). Lanes 1–6 in (**A**–**C**), clinical EC isolates. The amplified product from each PCR is indicated on the right, and the size of the marker in base pairs is shown on the left.
Abbreviation: ESBL-EC, extended-spectrum β-lactamase-producing *Escherichia coli*.

Table S1 PCR primers used for amplification of ESBL-related genes

Target gene	Primer	Sequence (5′–3′)	Amplicon size (bp)
bla_{TEM}	Forward Reverse	AAAATTCTTGAAGACG TTACCAATGCTTAATCA	1,080
bla_{SHV}	Forward Reverse	GGGTTATTCTTATTTGTCGCT TAGCGTTGCCAGTGCTCG	929
bla_{CTX-M}	Forward Reverse	TTTGCGATGTGCAGTACCAGTAA CGATATCGTTGGTGGTGCCATA	544

Abbreviation: ESBL, extended-spectrum β-lactamase.

Table S2 BF detection values by crystal violet staining

Strains	OD_{590} value	Strains	OD_{590} value	Strains	OD_{590} value	Strains	OD_{590} value
1	0.352±0.025	82	0.258±0.107	163	0.175±0.028	244	0.613±0.059
2	0.140±0.039	83	0.162±0.044	164	0.200±0.015	245	0.213±0.026
3	0.412±0.092	84	0.099±0.014	165	0.142±0.057	246	0.140±0.052
4	0.153±0.056	85	0.118±0.033	166	0.154±0.043	247	0.276±0.057
5	0.135±0.066	86	0.220±0.088	167	0.124±0.028	248	0.172±0.038
6	0.149±0.08	87	0.159±0.030	168	0.178±0.025	249	0.271±0.047
7	0.211±0.096	88	0.100±0.019	169	0.155±0.048	250	0.175±0.026
8	0.156±0.054	89	0.432±0.065	170	0.170±0.033	251	0.170±0.039
9	0.401±0.067	90	0.137±0.078	171	0.139±0.052	252	0.179±0.025
10	0.150±0.052	91	0.172±0.037	172	0.165±0.026	253	0.376±0.052
11	0.275±0.05	92	0.129±0.052	173	0.188±0.029	254	0.120±0.033
12	0.271±0.102	93	0.130±0.079	174	0.195±0.009	255	0.136±0.057
13	0.234±0.049	94	0.168±0.039	175	0.275±0.053	256	0.166±0.049
14	0.364±0.059	95	0.128±0.089	176	0.184±0.007	257	0.325±0.057
15	0.148±0.064	96	0.172±0.038	177	0.166±0.031	258	0.127±0.051
16	0.155±0.06	97	0.150±0.039	178	0.156±0.056	259	0.152±0.046
17	0.146±0.039	98	0.137±0.056	179	0.154±0.024	260	0.184±0.031
18	0.155±0.062	99	0.123±0.054	180	0.168±0.022	261	0.149±0.034
19	0.128±0.039	100	0.156±0.061	181	0.586±0.038	262	0.354±0.047
20	0.146±0.057	101	0.110±0.038	182	0.185±0.014	263	0.371±0.053
21	0.154±0.058	102	0.144±0.054	183	0.141±0.021	264	0.137±0.051
22	0.481±0.054	103	0.167±0.045	184	0.169±0.022	265	0.145±0.037
23	0.565±0.073	104	0.322±0.051	185	0.197±0.014	266	0.164±0.028
24	0.538±0.091	105	0.173±0.044	186	0.126±0.078	267	0.486±0.044
25	0.152±0.037	106	0.286±0.091	187	0.179±0.038	268	0.581±0.062
26	0.125±0.043	107	0.140±0.071	188	0.419±0.042	269	0.206±0.011
27	0.145±0.042	108	0.128±0.059	189	0.152±0.049	270	0.185±0.012
28	0.150±0.057	109	0.118±0.049	190	0.173±0.023	271	0.168±0.034
29	0.340±0.048	110	0.169±0.046	191	0.154±0.057	272	0.118±0.033
30	0.135±0.058	111	0.159±0.058	192	0.140±0.067	273	0.179±0.038
31	0.158±0.045	112	0.262±0.099	193	0.195±0.013	274	0.191±0.015
32	0.241±0.058	113	0.161±0.034	194	0.181±0.027	275	0.138±0.068
33	0.270±0.068	114	0.149±0.067	195	0.169±0.044	276	0.294±0.042
34	0.271±0.048	115	0.169±0.039	196	0.496±0.070	277	0.271±0.060
35	0.142±0.064	116	0.152±0.057	197	0.252±0.114	278	0.179±0.037
36	0.290±0.069	117	0.155±0.030	198	0.246±0.066	279	0.183±0.029
37	0.152±0.05	118	0.331±0.043	199	0.137±0.03	280	0.189±0.014
38	0.481±0.054	119	0.167±0.044	200	0.178±0.038	281	0.123±0.039
39	0.158±0.037	120	0.139±0.051	201	0.341±0.055	282	0.330±0.060
40	0.149±0.049	121	0.150±0.055	202	0.160±0.046	283	0.399±0.058
41	0.231±0.040	122	0.162±0.051	203	0.139±0.059	284	0.111±0.017
42	0.295±0.074	123	0.139±0.047	204	0.188±0.028	285	0.417±0.086
43	0.335±0.074	124	0.490±0.050	205	0.171±0.029	286	0.275±0.077
44	0.212±0.058	125	0.165±0.042	206	0.151±0.055	287	0.194±0.016
45	0.133±0.064	126	0.138±0.070	207	0.184±0.027	288	0.163±0.054
46	0.149±0.051	127	0.196±0.011	208	0.150±0.037	289	0.253±0.053
47	0.422±0.107	128	0.628±0.064	209	0.140±0.074	290	0.129±0.047
48	0.147±0.043	129	0.116±0.063	210	0.529±0.045	291	0.191±0.025
49	0.305±0.079	130	0.173±0.044	211	0.169±0.038	292	0.156±0.052
50	0.111±0.018	131	0.162±0.032	212	0.149±0.062	293	0.198±0.014
51	0.157±0.047	132	0.581±0.075	213	0.144±0.055	294	0.816±0.043
52	0.214±0.052	133	0.153±0.044	214	0.143±0.052	295	0.266±0.042
53	0.364±0.060	134	0.189±0.019	215	0.132±0.082	296	0.090±0.013

(*Continued*)

Table S2 (*Continued*)

Strains	OD_{590} value	Strains	OD_{590} value	Strains	OD_{590} value	Strains	OD_{590} value
54	0.197±0.072	135	0.157±0.05	216	0.161±0.046	297	0.142±0.040
55	0.145±0.064	136	0.187±0.015	217	0.189±0.025	298	0.130±0.029
56	0.174±0.034	137	0.185±0.028	218	0.292±0.052	299	0.151±0.043
57	0.237±0.088	138	0.182±0.013	219	0.369±0.045	300	0.146±0.040
58	0.171±0.037	139	0.173±0.038	220	0.193±0.016	301	0.128±0.059
59	0.282±0.087	140	0.206±0.007	221	0.139±0.055	302	0.103±0.023
60	0.533±0.108	141	0.159±0.042	222	0.178±0.037	303	0.167±0.024
61	0.129±0.012	142	0.196±0.017	223	0.183±0.027	304	0.198±0.017
62	0.161±0.055	143	0.129±0.034	224	0.154±0.042	305	0.125±0.015
63	0.260±0.080	144	0.167±0.041	225	0.171±0.037	306	0.179±0.037
64	0.146±0.059	145	0.150±0.058	226	0.138±0.047	307	0.259±0.030
65	0.475±0.059	146	0.396±0.027	227	0.117±0.042	308	0.183±0.032
66	0.150±0.056	147	0.181±0.015	228	0.174±0.042	309	0.162±0.029
67	0.371±0.099	148	0.174±0.022	229	0.167±0.026	310	0.325±0.049
68	0.171±0.027	149	0.205±0.009	230	0.248±0.029	311	0.173±0.019
69	0.134±0.072	150	0.115±0.052	231	0.156±0.037	312	0.138±0.064
70	0.144±0.063	151	0.142±0.041	232	0.142±0.038	313	0.203±0.013
71	0.150±0.048	152	0.150±0.039	233	0.184±0.027	314	0.134±0.048
72	0.150±0.054	153	0.104±0.041	234	0.189±0.026	315	0.175±0.041
73	0.127±0.034	154	0.073±0.022	235	0.123±0.061	316	0.169±0.044
74	0.129±0.058	155	0.290±0.054	236	0.333±0.064	317	0.128±0.063
75	0.156±0.054	156	0.225±0.065	237	0.110±0.024	318	0.151±0.048
76	0.556±0.070	157	0.159±0.038	238	0.197±0.041	319	0.595±0.088
77	0.157±0.052	158	0.173±0.039	239	0.166±0.038	320	0.170±0.035
78	0.133±0.071	159	0.168±0.037	240	0.174±0.042	321	0.162±0.043
79	0.155±0.046	160	0.147±0.045	241	0.140±0.060	322	0.200±0.017
80	0.142±0.064	161	0.169±0.033	242	0.164±0.043	323	0.146±0.040
81	0.124±0.063	162	0.162±0.036	243	0.180±0.004	324	0.132±0.033

Abbreviation: BF, biofilm formation.

Table S3 Molecular characterization of *bla* genes in ESBL-EC isolates

Genotype of the *bla* gene	No. of isolates
TEM-52	4
SHV-11	2
SHV-12	2
CTX-M-1	1
CTX-M-14	8
CTX-M-15	46
CTX-M-28	2
CTX-M-19	2
CTX-M-27	1
TEM-3, SHV-12	2
TEM-52, CTX-M-14	6
TEM-52, CTX-M-15	62
SHV-12, CTX-M-15	4
SHV-12, TEM-52, CTX-M-15	5

Abbreviation: ESBL-EC, extended-spectrum β-lactamase-producing *Escherichia coli*.

Table S4 Distribution of BF/non-BF EC-caused BSI in cancer patients

Tumor type	BF-EC (81)	non-BF-EC (243)	*P*-value
Hematological malignancies	9 (11.11)	10 (4.12)	0.020
Lung cancer	5 (6.17)	21 (8.64)	0.479
Mammary cancer	9 (11.1)	16 (6.58)	0.187
Gynecological cancer[a]	6 (7.41)	27 (11.11)	0.341
Colorectal cancer	17 (20.99)	28 (11.52)	0.033
Pancreatic cancer	10 (12.35)	51(20.99)	0.085
Hepatic carcinoma	14 (17.28)	35 (14.40)	0.532
Gastric carcinoma	8 (9.88)	35 (14.40)	0.299
Bladder cancer	3 (3.70)	7 (2.88)	0.711
Prostate cancer	0	6 (2.47)	0.154
Renal carcinoma	0	2 (2.1)	0.413
Others[b]	0	5 (2.06)	0.194

Notes: [a]Includes cervical cancer, endometrial carcinoma, ovarian cancer. [b]Includes melanoma, buccal cancer, glioma, meningioma.
Abbreviations: BF, biofilm formation; BSI, bloodstream infection; EC, *Escherichia coli*.