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Predicting resistance to third generation cephalosporins in patients with Escherichia coli bacteraemia – a retrospective cohort study.

Degree Project in Medicine

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Abstract

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Introduction: Some Enterobacteriaceae can produce enzymes (extended spectrum beta-lactamases or ESBLs) which make them resistant to certain antibiotics. In Sweden, the number of ESBL-bacteremia are increasing and Escherichia coli (E. coli) is the dominant bacteria. There are difficulties to initially identify which septic patients are to be suspected of infection with multi-resistant Enterobacteriaceae, sometimes resulting in delayed effective treatment. There are several potential risk factors to consider when assessing whether a septic patient might be infected with ESBL-producing bacteria. Therefore, it would be valuable to have indicating factors to facilitate the identification of patients at risk in order to choose effective empiric treatment.

Methods: The patients were selected based on the occurrence of E. coli-positive blood cultures between 1 Jan - 31 Dec 2016. Analyzed factors were age, sex, previous hospitalization, recent surgery, use of urinary catheter, living in nursing home, previous antibiotic treatment, immunosuppression, hospital care abroad, hospital vs. community acquired infection, previous faeces culture with ESBL-E. coli and previous urine and/or blood culture with ESBL-E. coli. A multivariate analysis was performed for variables with a uni-variate p-value <0.2 and statistical significance was established at ≤ 0.05.

Results: A total of 470 patients were evaluated, with 485 cases of bacteremia. Three parameters gained statistical significance: hospital care abroad (OR=3.4, 95%CI=1.1-10.4, p=0.037), previously ESBL-positive in faeces (OR=8.1, 95%CI=1.0-65.4, p=0.050) and previously ESBL-positive in blood and/or urine (OR=54.5, 95%CI=11.2-256.5, p<0.001).

Conclusion: In this retrospectively conducted survey of patients with E. coli bacteremia, we found that previously detected ESBL-E. coli in blood, urine or faeces and previous hospital care abroad were predicting factor for bacteremia with ESBL-producing E. coli.

KEY WORDS: ESBL; E. coli; bacteremia; risk factors

Introduction

The development of antibiotics as a treatment of bacterial infections is to be regarded as one of the greatest successes in modern medicine (1). Thanks to antibiotics several simple as well as more difficult infections can be treated and thereby many patients can be cured. However, there is a growing threat to this way of treatment in terms of multi-resistant bacteria. Enterobacteriaceae producing enzymes, which make them resistant to third generation cephalosporins, also known as extended spectrum beta-lactamases (ESBLs), are a big problem worldwide. (2). In 1983 the first rapport regarding plasmid encoded β-lactamases with extended spectrum was published and since then several additional enzymes within this group have been identified (3). This is a growing issue in the treatment of bacterial infections also in the Swedish healthcare system. In 2014, 8 902 new cases of ESBL-producing bacteria were identified among Swedish patients of which Escherichia coli (E. coli) represented 89% (4). In 2016 respectively, 10 659 cases were identified, which shows of a significant increase in number of cases per year (5).

In Sweden, 609 cases of ESBL-bacteremia were reported in 2016 and E. coli is the dominant ESBL-producer (6). Other bacteria have also been identified with ESBL-producing abilities such as Klebsiella pneumoniae, Salmonella, Pseudomonas, Proteus and Enterobacter but in Sweden these are less common than E. coli. The second largest group after E. coli is K. pneumoniae which represented 7% of the ESBL-cases in Sweden 2014 (7).

Bacteria with the ability to produce ESBLs have found an effective way to evade several types of antibiotics. In many cases this leads to a delayed effective treatment which can result in severe consequences for the patient since the time from infection to correct treatment is of essence for these kind of systemic infections (8). ESBL-producers are in most cases susceptible to piperacillin/tazobactam and carbapenems which are subgroups of beta-lactams, but they are almost always resistant to cephalosporins (9). It is also common to see co-resistance to fluoroquinolones, aminoglycosides and trimethoprim-sulfamethoxazole which limits the choice of effective treatment even further.

Beta-lactams are considered to be the treatment of choice for several different types of infections such as urinary tract infections and septicemia. Patients arriving at the emergency department and who suffers from a systemic infection are often treated empirically while

awaiting analysis of the drug resistance pattern from the blood cultures. In majority of the cases the chosen antibiotic will not have any effect against ESBL-producing bacteria (10). The condition of a patient with a systemic infection with ESBL-producing bacteria can drastically deteriorate, and the risk of mortality increases, if effective treatment is not provided at an early stage (11).

Beta-lactams contain a structure called beta-lactam ring. This structure binds to penicillin-binding proteins (PBPs) in the bacterial cell wall by imitating its building blocks (12). The cell wall consists of alternating N-acetylmuramic acid (NAM) and N-acetylglucosamine (NAG) which creates a stable outer wall towards the surrounding environment. Every NAM-unit has an associated pentapeptide and the linkage between two NAM-pentapeptides is catalyzed by PBPs (13,14). When the beta-lactam ring binds to the PBPs the crosslinking between peptidoglycans is interrupted and the cell wall synthesis is thereby blocked resulting in lysis of the bacteria (15). Beta-lactamases work through hydrolyzing the beta-lactam ring of the antibiotic which make many penicillins and cephalosporins ineffective (16). Unlike regular beta-lactamases, ESBLs can inactivate beta-lactams with extended anti-bacterial spectrum such as third generation cephalosporins (17).

There are multiple ways for bacteria to develop resistance to beta-lactams besides producing beta-lactamases. One mechanism is the acquirement of changes in the active site of the PBPs, lowering the affinity of the beta-lactam ring to the PBPs (18). Another way to avoid the antibacterial effect of the beta-lactam is to decrease the number of outer membrane proteins (OMPs). The beta-lactam has to either diffuse through the membrane or be transported via protein channels in the outer membrane of gram-negative bacteria such as E. coli in order to reach the PBPs in the inner plasma membrane. By lowering the expression of OMPs, the permeability for beta-lactams is decreased and the antibacterial effect thereby impaired (19). The bacteria can also develop a resistance mechanism which consist of pumping the beta-lactam out of the bacteria via efflux pumps (20). In this way, antibiotics in the periplasm are pumped out into the surroundings and the antibiotic effect inside the bacteria is thereby absent. Consequently, there are several ways for bacteria to avoid antibiotics, but the production of beta-lactamase is the most common and important mechanism among gram-negative Enterobacteriaceae such as E. coli and thereby the main focus of this project (21).

The genes coding for the ESBL enzymes can be transferred to other bacteria through so-called plasmids (22). Plasmids are composed of double stranded DNA which can be replicated independently of the chromosomal genetic material in the bacteria. During the transmission of the ESBL-genes, specific mobile components known as insertion sequence common regions (ISCRs) have been identified on the plasmids. The ISCRs enable the transmission of genes to other plasmids as well as to chromosomal genome and through this kind of horizontal transmission bacteria can share enzymatic abilities among each other (23). Once the genes are transmitted, bacterial cloning enables the spreading within a bacterial strain. Bacterial cloning means that the same bacteria is multiplied through division and in that way ESBL-genes from one bacteria can be incorporated in the genome of an entire bacterial strain (24).

The most dominant ESBL-producers are the Enterobacteriaceae family such as E. coli and K. pneumoniae (25) and the bacterial flora inside the gut contains a massive number of bacteria and therefor constitutes an optimal environment for transmitting resistance genes. The plasmids holding the ESBL-genes can be transmitted between different strains of E. coli but also to other bacterial species (26). Besides the ESBLs, the plasmids transferred between the bacteria can contain other resistance genes which possibly could explain why Enterobacteriaceae often have co-resistance to various antibiotics limiting the methods of treatment (27). For several patients with severe infections, co-resistance can be detected towards well used treatment options in terms of trimethoprim-sulfamethoxazole, aminoglycosides and fluoroquinolones (28).

Furthermore, the ESBLs can be divided into different subgroups. According to the Swedish classification made by Giske et al three groups can be identified: $ESBL_A$, $ESBL_M$ and $ESBL_{CARBA}$ (29). Subgroup $ESBL_A$ contains the enzymes CTX-M, SHV and TEM and is internationally classified as ESBL. $ESBL_M$ refers to the acquirement of plasmid mediated AmpC (not chromosomal AmpC) and include enzymes such as AAC and CMY. Finally, $ESBL_{CARBA}$ contains enzymes with carbapenemase abilities, for example OXA, MBL and KPC (30). The term of $ESBL_{CARBA}$ is a subclassification primarily used in Sweden to describe this certain group of enzymes.

Bacteria producing $ESBL_A$ are commonly resistant to penicillin, cephalosporins with narrow and broad spectrum and the monobactam aztreonam but sensitive to carbapenems, cephamycins and ß-lactam/ß-lactamase inhibitor-combinations (31). Bacteria producing

$ESBL_M$ can inactivate penicillin, combinations of lactamase inhibitors and cephalosporins but are often susceptible to aztreonam (32). Due to their carbapenemase activity, bacteria producing $ESBL_{CARBA}$ are resistant to carbapenems which usually are the antibiotics of choice when treating infections with ESBL-producing bacteria (33).

The AmpC-enzymes of Enterobacteriaceae are commonly encoded in the chromosomal bacterial genome and expressed at low levels, usually not generating any beta-lactam resistance (34). However, an acquired mutation of the AmpC-promotor can induce overexpression of the gene, conferring resistance to extended spectrum cephalosporins (35). Besides the overexpression of chromosomal AmpC, the frequency of plasmid encoded AmpC is increasing (36). Plasmid-mediated AmpC-enzymes are not as common as the chromosomal variant but can confer a broader spectrum of resistance (37). There are also combinations of chromosomal and plasmid-encoded AmpC, further increasing the resistance to antibiotics. For the scope of this project, AmpC is included in the category of ESBLs due to the growing issue of E. coli producing AmpC-enzymes.

There has been much speculation regarding the potential risk factors for becoming colonized with ESBL-producing Enterobacteriaceae. One of the potential risk factors is travelling to countries with high prevalence of these multi-resistant bacterial strains. Studies made on healthy travelers who left stool samples after travelling outside northern Europe showed that 24% of the participants had become colonized with resistant Enterobacteriaceae (38). Based on studies conducted in Sweden, the public health authority estimates the prevalence of colonization within the Swedish population to be about 5% (39). Nevertheless, monitoring studies shows that 42% of colonized patients were still carriers after one year and 27% were again positive after one or more previous negative tests (40). Considering that it is possible to at first receive a negative carrier test and then a positive one, it becomes problematic to determine when a patient no longer is colonized solely based upon these screening tests.

Among patients colonized with ESBL-producing enterobacteriaceae who avoid developing an infection, the carriage will likely remain undetected and these patients will compose a risk of transmitting the multi-resistant bacteria to others. It is unfortunately difficult to predict which patients are at risk of becoming persistent carriers. For some patients the bacteria disappear within a year from infection, but studies show that colonization with ESBL-producing E. coli in the gut can lead to a persistent carriage for up to five years (41). The colonization entails an

increased risk of severe infection and previous studies have shown prolonged hospitals stays and increased mortality amongst patients who suffers from infection with ESBL-producing bacteria (42).

The over-prescription of antibiotics is known to be a prominent factor in the emergence of multi-resistant strains among bacteria (43). According to WHO 2016, cephalosporins and quinolones compose two of the most critically important subclasses of antibiotics in human medicine when treating serious bacterial infections (44). Necessary and unnecessary prescription of primarily extended spectrum cephalosporins and the uncontrolled use of antibiotics abroad promote development of resistance in general and the development of multi-resistant Enterobacteriaceae in particular (45). The use of antibiotics to animals contributes to the problem because of the evolvement of resistant bacterial strains in this area as well. These strains can then be transmitted further to humans through direct contact or through the food chain (46).

Hospitalization is another potential risk factor of being colonized and infected with multi-resistant Enterobacteriaceae (47). Bacteria can circulate due to insufficient hygiene among patients or health care professionals, through overcrowded hospital rooms or to inadequately disinfected medical instruments. During 2016, a hospital in Stockholm county suffered from an outbreak of ESBL-producing Enterobacteriaceae. In total, 55 patients were affected from autumn 2015 to June 2016 (48). This resulted in several measurements regarding hygiene routines to limit further transmission. Studies have also shown a connection between colonization with primarily ESBL-producing E. coli and living in nursing homes (49). However, it is difficult to establish the origin of the bacteria since patients living in nursing homes often need hospital care and might have brought the bacteria to the hospitals and the other way around (50, 51).

Surgical procedures are considered to be another risk factor for colonization and infection with resistant bacteria (52). Whether the increased risk of infection after surgical procedures is due to prior colonization of the patient, multi-resistant bacteria in the surrounding hospital environment or contamination during or after the procedure is difficult to determinate. For patients undergoing complicated surgery it is common with urinary catheterization during and/or after the procedure. This alone composes a risk of infection with ESBL-producing

Enterobacteriaceae (53). Consequently, the risk of infection among patients hospitalized due to surgical procedures with remaining catheters is most likely significantly increased.

Our immune system is an important part in the defense against bacteria. Due to this known fact it is near at hand to draw the conclusion that different forms of immunosuppression are likely to compose another risk for infection with above mentioned bacteria. Studies made from a urological section in a Spanish hospital show a connection between immunocompromised patients and a higher incidence of ESBL-producing bacteria (54).

In conclusion, there are many potential risk factors to consider when attempting to assess whether a patient with bacteremia is infected with ESBL-producing Enterobacteriaceae or not. Therefore, it would be of value to have a few indicating factors to facilitate the identification of these patients at risk in order to choose adequate treatment. Besides potentially contributing to an improved patient health care it would also lower unnecessary prescription of extended spectrum antibiotics by further indicating which patients are especially at risk. By investigating whether above mentioned risk factors are current in the Gothenburg health care system, the hope is to ease the clinical assessment and to further indicate optimal treatment for patients with bacteremia.

Aim

The aim of this study was to find predicting factors to identify patients with ESBL-E. coli bacteremia within the Gothenburg health care system in order to provide adequate treatment.

Method

Setting and study design

The study was retrospectively conducted at the Sahlgrenska University Hospital. Sahlgrenska University Hospital is a large tertiary hospital at five different locations in Gothenburg and Mölndal, with approximately 1 950 hospital beds distributed on 50 departments.

Data collection

The electronic patient record system Melior was used to examine the medical history of the patients. Information on patient laboratory data was obtained from the laboratory data system.

Ethics

According to current regulation on student degree projects, ethical review board clearance was not needed for this project. Patients were not asked for consent to participate in this survey. However, to preserve the patients' integrity the data was unidentified after the data collection was completed.

Inclusion and exclusion criteria

The patients were selected based on the occurrence of a positive blood culture with E. coli. The blood samples were collected between 1 January and 31 December 2016. A list with all positive blood culture results was obtained from the laboratory. The list was further adjusted to solely include one E. coli-sample per patient per month. This limitation was made to demarcate the number of bacteremic episodes for each patient. Several positive results within one month were therefor considered to be one infection with E. coli.

Variables analyzed

The factors chosen for analysis were age, sex, hospitalization within a year before admission, surgical procedure within a month, urinary catheter within a month, living in a nursing home, treatment with fluoroquinolones or cephalosporins within three months, immunsuppression, hospital care abroad, if the current bacteremia was hospital or community acquired, positive faeces culture with ESBL-producing E. coli within two years and positive urine and/or blood culture with ESBL-producing E. coli within two years.

The potential risk factors were analyzed based on the occurrence of ESBL-bacteremia or not. Among the patients classified as ESBL-positive, both $ESBL_A$ and $ESBL_M$ were included. Consequently, E. coli non-susceptible to one or more 2nd-3rd generation cephalosporins and/or with AmpC-acquirement were included.

The definition of hospitalization was set to at least one overnight hospital stay within the last year in addition to the episode when the positive culture was obtained. This category only comprises hospital care registered in the Sahlgrenska University Hospital electronic patient records system. It does not include private hospital care or if a patient received hospital care outside Gothenburg, if not specifically stated in the patient medical records. In a handful of cases, information concerning previous hospitalization or transfer from another hospital were obtainable and these patients were consequently classified with a positive result.

Regarding surgical procedures, the assessment was made based on the performed procedure. Surgery involving larger incisions in the skin to remediate underlying structures was classified as positive result because of the generally increased risk of infection. According to this division, e.g. abdominal and thoracic surgery, orthopedic procedures as well as skin transplants were included in the surgical category. Also, other invasive procedures such as transuretral resection of the prostate (TURP), transuretral elimination of vesical tumors and prostate biopsies were equate with a positive result. Procedures considered less relevant for the current question and therefore classified with a negative result, were for instance smaller skin excisions, suturing of traumatic wounds, transluminal procedures such as percutaneous coronary intervention (PCI) and diagnostically endoscopic examinations e.g. gastroscopy, colonoscopy and cystoscopy. The evaluation and classification were made individually for each patient because of the great variation of surgical procedures performed. The timeframe for the procedures was set to a month preceding the positive E. coli blood culture.

Previous use of urinary catheter was the next factor which was assessed. Patients who had a temporary or indwelling urinary catheter within a month until the day before obtaining a positive blood culture, were classified with a positive result in this category. Also, patients with suprapubic catheters were included. This category contains solely patients who received catheters while hospitalized or if information about previous or current catheter use outside the hospital was given from the medical records.

Furthermore, patients living in a nursing home before the E. coli-bacteremia were noted with a positive score. The definition of nursing home in this paper includes all varieties of residency except the patient's own home, for example short-term accommodations, elderly care facilities or variations of temporary group residences. Patients who received health care through advanced hospital care at home were categorized with a negative result considering that the treatment was performed in the patient's own habitation.

The use of antibiotics was another risk factor which was evaluated. In this category, treatment within the last three months with fluoroquinolones or 2nd-3rd generation cephalosporins were examined. This group solely include treatment prescribed and documented during hospital care or if information about such treatment in e.g. the primary care was included in the medical records.

In question whether a patient was considered to be immunosuppressed or not, an evaluation was made individually for each patient. Several varieties of immunsuppression occurred such as chemotherapy, combinations of steroid treatments, rheumatic pharmaceuticals etc. Concerning chemotherapy, a timeframe was constructed to within the last year. If ongoing steroid treatment, doses equal to a daily dosage of 20 mg Prednisolone for at least 30 days were classified as positive.

Hospital care abroad is considered another potential risk factor and therefore also examined in the medical records. The information was obtained from emergency charts which are filled in when the patients arrive to the emergency room. These charts contain information regarding previous hospital care abroad within the last year and in some cases even further back in time. However, the information given on the emergency charts does not generally include in which country the patient received hospital care or the acquired therapy. Consequently, all patients who received hospital care abroad, regardless of the country or treatment, were categorized with a positive score. In several cases the information in question were absent, and those patients were classified with an unknown/negative result in this category.

To determine whether the infection was to be considered hospital or community acquired, a timeframe was set. Patients with onset of symptoms of infection within 2 days from arriving to hospital were classified as community acquired and attained a negative result in this group.

Consequently, patients who obtained symptoms more than 2 days after admission to hospital were classified as hospital acquired and received a positive result.

Finally, every positive culture consisting of ESBL-producing E. coli in the last two years from the current septic episode were noted. Patients with one or more positive ESBL-culture within the last two years, were classified as positive. The assessed material was separated into two groups, previous ESBL-producing E. coli in blood and/or urine, and previous ESBL-producing E. coli in faeces.

When all the data was collected from the medical records, remaining variables such as age at the time for the blood samples and gender were compiled.

Statistical analyses

Statistical analysis was performed using the statistical software package SPSS version 25.0.

The categorical variables were studied separately to observe frequency and distribution within each parameter. Proportions were compared using univariate Pearson's Chi-square test to determine significant factors influencing the acquisition of ESBL/AmpC-positive strains. Continuous variables were compared by Mann–Whitney *U*-test.

After the univariate analysis, a multivariate analysis was performed using a backward logistic regression model for all variables with a calculated p-value <0.2 in the Chi-square analysis. Odds ratios (OR) and 95% confidence interval (CI) were calculated to evaluate the strength of any association that emerged and statistical significance was established at ≤0.05.

Results

A total of 470 patients were evaluated in this study, with 485 cases of bacteremia caused by E. coli. There were 218 male patients with a total of 228 blood cultures and 252 female patients with a total of 257 blood cultures. The median age in the ESBL-positive group was 72 years and 74 years in the ESBL-negative group. Strains without production of ESBLs, were located in 438 blood cultures (90.3%) while ESBL-producing isolates were identified in 47 blood cultures (9.7%).

Among the patients included in this study, a total of 12 patients were classified with more than one septic episode. Nine of these patients experienced two separate infectious episodes and three patients had three separate infectious episodes.

A Chi-Square test with a cut-off p value of <0.2 was performed (Table 1), which resulted in the following factors for further statistical examination: sex ($p = 0.015$), treatment with fluoroquinolones or 2nd-3rd generation cephalosporins within the last three months ($p = 0.095$), hospital care abroad ($p = 0.006$), ESBL-positive in faeces within the last two years ($p = 0.001$) and ESBL-positive in blood and/or urine within the last two years ($p < 0.001$). These factors were further evaluated in a multivariate backward logistic regression model to confirm any potential significant value. The residual parameters, described below, were not associated with significant outcomes and consequently not further investigated.

Results from the univariate analysis

Previous hospital admission within the preceding year was not associated with ESBL-bacteremia ($p = 0.378$). In total, 236 cases had no previous hospital admission (216 ESBL-negative blood cultures and 20 ESBL-positive blood cultures) and 249 patients had spent at least one night in hospital during the last year (222 ESBL-negative blood cultures and 27 ESBL-positive blood cultures).

Surgical procedure within one month preceding the blood culture did not result in a significantly increased risk for bacteremia with ESBL-E. coli ($p = 0.927$). In 421 cases, no history of recent surgery was discovered (380 ESBL-negative and 41 ESBL-positive blood cultures). The number of cases with a positive result in this category was 64 (58 ESBL-negative blood cultures and six ESBL-positive blood cultures).

The analysis did not demonstrate any increased risk for patients living in nursing homes (p = 0.590). In total, there were 410 cases where the patients were living in their own homes (369 ESBL-negative blood cultures and 41 ESBL-positive blood cultures) and 75 cases where the patients lived in some sort of nursing home (69 ESBL-negative blood cultures and six ESBL-positive blood cultures).

Concerning the use of urinary catheter within a month preceding the positive blood culture, no elevated risk of infection was detected (p = 0.246). Among these cases, 401 patients had no record of using urinary catheter within the last month (365 ESBL-negative blood cultures and 36 ESBL-positive blood cultures) in contrast to 84 patients who previously had received a urinary catheter (73 ESBL-negative blood cultures and 11 ESBL-positive blood cultures).

Furthermore, no association was found between immunosuppression and ESBL-bacteremia (p = 0.918). Overall, 405 cases concerned patients without immunomodulating treatment (366 ESBL-negative blood cultures and 39 ESBL-positive blood cultures) and 80 cultures were connected to immunodepressed patients (72 ESBL-negative blood cultures and eight ESBL-positive blood cultures).

Whether the infection was acquired at hospital or in the community did not significantly affect the occurrence of ESBL-bacteremia (p = 0.815). In total, there were 407 community acquired infections (367 ESBL-negative blood cultures and 40 ESBL-positive blood cultures) and 78 cases were classified as hospital acquired (71 ESBL-negative blood cultures and seven ESBL-positive blood cultures).

Age was analyzed with the Mann-Whitney U-test and not proven to be a significant factor concerning the risk of developing an ESBL-E. coli bacteremia (p = 0.595). Within this category, the mean age in the ESBL-negative group was 69 years and the median 74 years. Similarly, in the ESBL-positive group the mean age was 68 years and the median 72 years. When separated according to gender, in the entire cohort, the mean age for men was 71 years and 67 years for women respectively.

Results from the logistic regression model

Treatment with fluoroquinolones or 2nd-3rd generation cephalosporins within the preceding three months did not result in a statistically increased risk to acquire a systemic infection with ESBL-E. coli ($p = 0.461$). In total, 420 cases had no history of treatment with the above-mentioned antibiotics within this period of time (383 ESBL-negative blood cultures and 37 ESBL-positive blood cultures) and 65 cases had received such treatment (55 ESBL-positive blood cultures and ten ESBL-negative blood cultures).

Concerning sex, there was a trend towards a lower risk for women in terms of ESBL-bacteremia. However, significant results were not obtained in this category in the multivariate analysis (p=0.093). In total, 228 blood cultures from male patients were obtained (198 ESBL-negative blood cultures and 30 ESBL-positive blood cultures) and 257 blood cultures from women (240 ESBL-negative blood cultures and 17 ESBL-positive blood cultures).

Hospital care abroad was significantly associated with ESBL-bacteremia ($p = 0.037$). In 462 cases, no history of hospital care abroad was discovered (421 ESBL-negative blood cultures and 41 ESBL-positive blood cultures) in contrast to 23 patients who previously received health care internationally (17 ESBL-negative blood cultures and six ESBL-positive blood cultures).

A statistical association was found with previously detected rectal colonization with ESBL-E. coli within two years preceding the current blood culture (p=0.050). Within this group, 479 cases had no history of previously known rectal colonization (436 ESBL-negative blood cultures and 43 ESBL-positive blood cultures) in contrast to six cases which were associated with a former positive faeces test (two ESBL-negative blood cultures and four ESBL-positive blood cultures).

A previously positive ESBL-culture from blood and/or urine within the last two years was significantly associated with ESBL-bacteremia ($p < 0.001$). In total, 472 cases had no previous record of ESBL-E. coli in blood and/or urine (436 ESBL-negative blood cultures and 36 ESBL-positive blood cultures), while 13 cases had a positive preceding ESBL-culture (two ESBL-negative and 11 ESBL-positive blood cultures).

In summary, three parameters gained statistical significance in the multivariate logistic regression analysis: hospital care abroad, previously positive ESBL-cultures from faeces within the last two years and previously positive ESBL-cultures from blood and/or urine within the last two years (Table 2, Figure 1). Hospital care abroad, as a single predictive clinical risk factor, had a sensitivity of 12.8% and a specificity of 96.1%. PPV was calculated to 26.0% and NPV to 91.1%. Previously positive ESBL-cultures from faeces gained a sensitivity of 8.5% and a specificity of 99.5%. The PPV was calculated to 66.7% and NPV 91.0%. Previously positive ESBL-cultures from blood and/or urine received a sensitivity of 23.4% and a specificity of 99.5% when analyzed as an indicator. The PPV for this factor was 84.6% and NPV 92.4%.

To further assess the results, we created a model to perceive the occurrence of a positive result in either one of the parameters which had gained a p-value < 0.05, e.g. hospital care abroad and previously positive ESBL-cultures from blood and/or urine. Accordingly, the sensitivity was calculated to 34.0% and the specificity to 95.7%. This generated a PPV-value of 45.7% and an NPV-value of 93.1%.

TABLE 1. Univariate analysis with Chi-Square and Mann-Whitney *U*-test to evaluate risk factors indicating bacteremia with ESBL-producing E. coli.

Parameter	*p* value	ESBL+	ESBL-
Age, median	0.595	72	74
Sex	0.015		
Men		30	198
Women		17	240
Hospitalization[a]	0.378	27	222
Surgery[b]	0.927	6	58
Nursing home[c]	0.590	6	69
Prev. catheter use[d]	0.246	11	73
Prev. antibiotic treatment[e]	0.095	10	55
Immunosuppressions	0.918	8	72
Health care abroad	0.006	6	17
Hospital acquired infection[f]	0.815	7	71
Prev. ESBL-positive in faeces[g]	0.001	4	2
Prev. ESBL-positive in blood and/or urine[h]	<0.001	11	2

The quantities given for ESBL+ and ESBL- represent the number of blood cultures and consequently not the number of patients. The quantities given for age represent median age.
ESBL = Extended spectrum beta-lactamase
E. coli = Escherichia coli
[a] Spent at least one night at hospital within the last year in addition to the episode when the E. coli-positive blood culture was obtained.
[b] Surgery within the last month preceding the E. coli-positive blood culture.
[c] Catheter use within one month preceding the E. coli-positive blood culture.
[d] All varieties of residency except the patient's own home.
[e] Treatment with fluoroquinolones or 2nd-3rd generation cephalosporins within the last three months preceding the E. coli-positive blood culture.
[f] Patients who obtained symptoms of infection more than two days after admission to hospital.
[g] Previously ESBL-positive in faeces within the last two years preceding the E. coli-positive blood culture.
[h] Previously ESBL-positive in blood and/or urine within the last two years preceding the E. coli-positive blood culture.

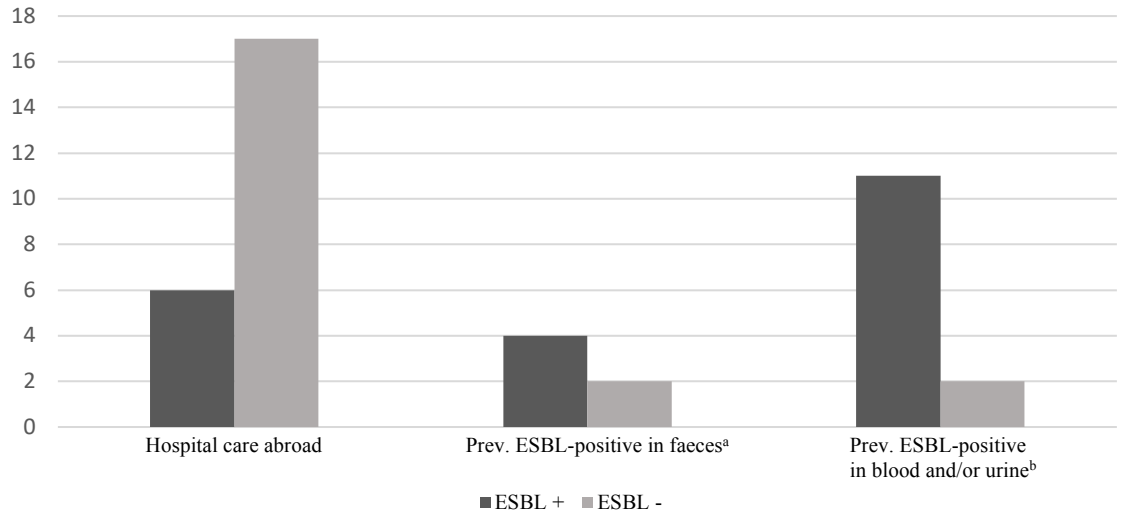
TABLE 2. Multivariate logistic regression analysis of risk factors for ESBL-producing E. coli isolation.

Parameter	OR 95% CI	*p* value
Hospital care abroad	3.4 (1.1-10.4)	0.037
Prev. ESBL-positive in faeces[a]	8.1 (1.0-65.4)	0.050
Prev. ESBL-positive in blood and/or urine[b]	54.5 (11.2-265.5)	<0.001

ESBL = Extended spectrum beta-lactamase
E. coli = Escherichia coli

[a] Previously ESBL-positive in faeces within the last two years preceding the E. coli-positive blood culture.
[b] Previously ESBL-positive in blood and/or urine within the last two years preceding the E. coli-positive blood culture.

FIGURE 1. Diagram displaying the quantities within each parameter with significant results in the multivariate logistic regression analysis. The logistic regression analysis evaluate risk factors indicating bacteremia with ESBL-producing E. coli



The quantities given for ESBL+ and ESBL- represent the number of blood cultures and consequently not the number of patients.
ESBL = Extended spectrum beta-lactamase
E. coli = Escherichia coli

[a] Previously ESBL-positive in faeces within the last two years preceding the E. coli-positive blood culture.
[b] Previously ESBL-positive in blood and/or urine within the last two years preceding the E. coli-positive blood culture.

Discussion

In this retrospectively conducted survey of patients with E. coli bacteremia, we found that previously detected ESBL-producing bacteria in blood, urine or faeces and previous hospital care abroad were predicting factors for bacteremia with ESBL-producing E. coli.

Infections with ESBL-producing Enterobacteriaceae have evolved greatly within the last few years (55). The treatment of severe infections caused by these bacteria, such as septicemia with ESBL-producing E. coli, becomes problematic due to the risk of an inefficient empirical treatment (56). Previous studies have revealed that infections with multi-resistant Enterobacteriaceae have a great impact on the clinical outcome (57, 58). Furthermore, studies show that early administration of effective antimicrobial treatment is the most important parameter regarding survival from septicemia and the mortality increases every hour of delayed treatment (59, 60). In the light of this knowledge, multiple studies worldwide have been published that have aimed at finding parameters predicting which patients are at risk of having bacteremia with ESBL-producing Enterobacteriaceae (61, 62, 63). Different factors have been found significant depending on the geographic area and the study population where the material is collected, and it is therefore problematic to identify generalizable factors.

The available material regarding such indicative factors for patients in Sweden is limited. The results available from international studies are difficult to generalize to patients in Sweden due to epidemiological differences and because of differences in health care systems and antibiotic prescription tradition. The prevalence of multi-resistant bacteria in Sweden is relatively low and it is therefore problematic to generalize results obtained in countries with a higher prevalence (64). Risk factors which have been found significant in international studies should therefore also be evaluated in this population and hopefully later on be implemented in the clinical assessment of these patients.

Our study found three significant parameters indicative for when patients are to be suspected to have bacteremia caused by ESBL-producing E. coli. The first factor was previous hospital care abroad, without consideration to country or treatment (OR 3.4, 95% CI 1.1-10.4, *p* value = 0.037). This parameter resulted in a positive statistical association and can consequently be regarded as a stated risk factor. When analyzed as a predictive parameter, the calculated sensitivity was 12.8% and specificity was 96.1% with a PPV and NPV of 26.0% and 91.1%

respectively. Consequently, the analysis resulted in a relatively high PPV why it may have some value to consider this component in the clinical assessment. A previous Swedish study showed an association between the carriage of ESBL-producing E. coli and traveling abroad to primarily Asia and Africa where the prevalence of multi-resistant bacteria is high (65). Consequently, the risk of colonization and infection with multi-resistant Enterobacteriaceae seems to be increased when receiving hospital care abroad or when travelling to areas with a high prevalence. The information we received from the emergency charts did not specify the country where the hospital care was given. However, one might speculate that receiving hospital care in countries with high prevalence of multi-resistant bacteria would compose an even higher risk.

The second indicative parameter was previously documented ESBL-producing E. coli in faeces (OR 8.1, CI 1.0-65.4, *p* value = 0.050). When viewed as a single predictor, sensitivity was 8.5% and specificity 99.5%. PPV and NPV were 66.7% and 91.0% respectively. Since statistical significance was established at ≤0.05, this factor barely reached significance. Consequently, the importance of previous rectal colonization in this population can be regarded as a bit uncertain. However, a previous study from the Netherlands indicate that isolation of multi-resistant Enterobacteriaceae from any site ought to be regarded as a risk factor for ESBL-bacteremia (66). The results from our survey could be explained by the limited number of cases. If the same analysis would be made on a larger population, the results might be more definite.

The third parameter that significantly predicted ESBL-bacteremia, was previously documented blood and/or urine cultures with ESBL-producing E. coli (OR 54.5, 95% CI 11.2-265.5, *p* value < 0.001). When analyzing this as an individual risk factor, sensitivity and specificity were 23.4% and 99.5% respectively. PPV and NPV were calculated to 84.6% and 92.4% respectively. The results indicate that this variable ought to be regarded as an important predictor in septic patients. A study performed in Baltimore, USA, display a similar association (67). The results from that study revealed that previous colonization or infection with ESBL-producing Enterobacteriaceae within the last 6 months was one of the strongest associated variables regarding the risk of acquiring ESBL-bacteremia.

To further assess the results, we created a model to analyze the occurrence of a positive outcome in either one of the two parameters which had gained a p-value <0.05, e.g. hospital care abroad and previously positive ESBL-cultures from blood and/or urine. Accordingly, the

sensitivity was calculated to 34.0% and specificity 95.7%. The PPV and NPV were 45.7% and 93.1% respectively. According to our constructed model, the sensitivity score did not demonstrate a notably high value. Approximately 34% of patients with ESBL-bacteremia will be identified based on the occurrence of the analyzed factors. However, the high PPV illustrates that these parameters still might have a high indicative value. If E. coli is suspected to be the causal agent and the patient have a positive score in either one of these two variables, approximately 46% will be infected with an ESBL-producing E. coli-strain and antibiotics effective against ESBL-producing bacteria should be administrated.

If either of the variables are negative on the other hand, the probability of bacteremia caused by ESBL-producing E. coli is lower and empiric antibiotic coverage for ESBL-producing bacteria is not necessary unless the patient is severely ill. Nonetheless, approximately 7% of these patients will have an ESBL-bacteremia despite negative scoring on either of these parameters and might consequently not respond to treatment with third generation cephalosporins.

All of these parameters, are easily accessible in the acute assessment of a patient with suspected sepsis. Information whether a patient has received hospital care abroad is documented on the emergency chart, filled in when the patient arrives to the emergency room. These charts are commonly close at hand in the emergency rooms and the physician in charge can easily survey the current category on the chart and be further directed in etiology and treatment. Likewise, information regarding previously obtained blood, urine or faeces cultures is also easily accessible. All analyzed cultures are registered in the laboratory data system in a relatively approachable way and therefore also a parameter which is clinically valuable. To obtain information concerning these parameters do not require significant loss of critical time. Instead, the acquired information can possibly result in important evidences to determine the origin.

The remaining factors analyzed in this survey did not result in significant values. However, several of these parameters have attained significant results in studies performed in other countries. For example, we did not find a connection between previous treatment with fluoroquinolones or 2nd-3rd generation cephalosporins within the last three months, and an increased risk of infection with ESBL-E. coli. Although, this association was found in a study

performed in Italy (68). The same study also revealed previous hospitalization and urine catheterization to be significant risk factors in the examined population.

Another study, performed in Taiwan, identified a connection between patients living in nursing homes and an increased risk of bacteremia with Enterobacteriaceae (69). We did not find such association in our survey, possibly due to good hygiene routines in many Swedish nursing homes. The Taiwanese study also showed recent invasive procedures to be a risk factor for bacteremia with above mentioned bacteria, a parameter which was not found statistically significant in our population. Accordingly, the results vary depending on localization and study population. If this is due to epidemiological differences or as a result of differences in health care systems etc., is difficult to determine.

Sex was a parameter which displayed a trend towards a significant value but did not attain a significant result in the multivariate analysis. One can speculate that male patients might have a higher prevalence of urinary tract pathology due to conditions such as benign prostate hyperplasia, prostate cancer etc. These conditions are often treated surgically and are also associated with a high prevalence of recurrent infections in need of antibiotic treatment. It is known from previous studies that frequent antibiotic treatment benefits the selection of multi-resistant bacteria such as ESBL-producing E. coli (70, 71). Because of this, speculations can be made that male patients might have an increased risk of ESBL-bacteremia. However, such speculations were not validated in our study.

Limitations to this study should be noted. There are a few potential sources of error which should be taken into consideration. The study material is collected from patient medical records and is dependable upon the factual documentation of the requested parameters in the medical history. If the parameters were not documented in the records, the collected data will be misleading. Also, the accuracy of the collected data is dependent upon whether the documentation was made consistently in all records. For example, information regarding the use of urinary catheter was discovered to be documented within various posts in the medical records. The number of positive outcome in this category might therefore be underestimated due to difficulties to thoroughly find the correct information. Consequently, the use of urinary catheter might have a larger impact as a risk factor than revealed in this survey. Likewise, the collected information regarding previously received medical treatment outside the

geographical area comprehended in this study, or the prescription of antibiotics from the primary care, might also be underestimated due to the absence of documentation.

One should also mention that the number of cases included in this survey is limited and a larger study population would have been desirable to further statistically confirm the results. Moreover, one can discuss the individual evaluations made regarding both surgical procedures as well as the assessment of what should be considered as immunocompromising. These divisions were made primarily based on acquired results from international studies, but also on clinical experience attained from medical physicians.

Finally, this survey was performed on patients treated at the Sahlgrenska University Hospital and the results can therefore be problematic to generalize to areas with other epidemiological conditions. Similar national studies are needed to further confirm these results. The outcome should also preferably be evaluated in additional scoring models.

In summary, three specific factors were found associated with increased risk of bacteremia with ESBL-producing E. coli. These parameters are easily obtainable without significant loss of time in the acute assessment of septic patients. By taking these predicting factors into account, we hope to have contributed with an easy to use tool in the evaluation of whether antibiotics effective against ESBL-producing bacteria should be administrated to patients with suspected gram-negative sepsis.

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Populärvetenskaplig sammanfattning

Förutsägande faktorer för att insjukna i blodförgiftning med multiresistenta tarmbakterier

Under 2016 drabbades drygt 600 patienter i Sverige av blodförgiftning till följd av multiresistenta tarmbakterier. Karaktäristiskt för dessa bakterier är att de producerar ett protein som gör dem motståndskraftiga mot vanligt förekommande antibiotika som ofta används vid akut behandling av patienter med blodförgiftning. I de flesta fall kan dock dessa bakterier behandlas med en annan antibiotikatyp som därmed bör ges när det finns misstanke om att en patient kan vara infekterad med multiresistenta tarmbakterier.

I dagsläget är det svårt att identifiera vilka patienter som bör misstänkas vara infekterade med multiresistenta tarmbakterier och i många fall leder detta till att den botande behandlingen blir fördröjd, något som kan innebära svåra konsekvenser för patienten. Det vore därför värdefullt om det kunde finnas hjälpmedel att tillgå som underlättar identifieringen av dessa riskpatienter så att rätt behandling kan sättas in i ett tidigt skede.

Studien gjordes på patienter som vårdats på Sahlgrenska Universitetssjukhuset under 1 jan-31 dec 2016 där sjukvården erhållit en positiv bakterieodling avseende den utvalda tarmbakterien. Dessa patienters journaler granskades sedan utefter ett flertal förvalda faktorer.

De faktorer där man kunde se en koppling till en ökad risk att drabbas av blodförgiftning med multiresistenta tarmbakterier var erhållen sjukhusvård utomlands samt att man tidigare funnit multiresistenta tarmbakterier i blod, urin eller avföring. Resultaten visade att för en patient som kommer till sjukhus med tecken på blodförgiftning och antingen har vårdats på sjukhus utomlands eller har tidigare odlingar med multiresistenta tarmbakterier så är risken ca 46% att orsaken till patientens blodförgiftning är multiresistenta tarmbakterier. Om patienten saknar dessa faktorer så är risken istället ca 7%. Genom att beakta dessa parametrar beräknas ca 34% av de patienter som drabbats av denna variant av blodförgiftning kunna urskiljas.

I vår studie har vi kunnat identifiera tre specifika faktorer för att insjukna i blodförgiftning med multiresistenta tarmbakterier för patienter inom Göteborgs sjukhusvård. Information kring dessa parametrar kan enkelt fås fram utan att det innebär en allt för stor tidsförlust vid den akuta behandlingen av patienterna. Upplysningar avseende sjukhusvård utomlands finns

på de akutblad som fylls i då en patient anländer till Akutmottagningen. Information om tidigare bakterieodlingar där man funnit multiresistenta tarmbakterier är även den lättillgänglig i sjukhusens datorsystem. Genom att beakta dessa parametrarna hoppas vi därmed kunna bidra med ett enkelt hjälpmedel vid bedömningen av vilken typ av behandling en patient bör få om det föreligger misstanke om multiresistenta tarmbakterier som orsak till blodförgiftningen.

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