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Risk Factors for Fecal Carriage of Extended-Spectrum Beta-Lactamase Producing *Escherichia coli* and *Klebsiella* spp. in the Community

Özlem KURT AZAP
Hande ARSLAN
Sedef Ö. KARAMAN
Turhan TOGAN

Department of Clinical Microbiology and Infectious Diseases, Faculty of Medicine, Başkent University, Ankara - TURKEY

Aim: Community-acquired infections caused by extended-spectrum beta-lactamase (ESBL)-producing bacteria are an emerging problem. Digestive tract colonization is a prerequisite for infections by ESBL-producing microorganisms. The aim of this study was to determine the prevalence of and risk factors for fecal carriage of ESBL-producing *Escherichia coli* (*E. coli*) or *Klebsiella* spp. in the community.

Materials and Methods: A total of 928 stool samples admitted to the laboratory during a four-month period were included in the study. Samples were diluted in saline and cultured in two EMB agar plates supplemented with either 1 µg/ml cefotaxime or 1 µg/ml ceftazidime. All isolates that grew were identified to the species level. *E. coli* and *Klebsiella* spp. strains were tested for ESBL production with ceftazidime and ceftazidime-clavulanate discs according to the Clinical and Laboratory Standards Institute (CLSI) Guideline.

Results: Of the 928 stool samples included, 133 (14%) were isolated from inpatients and 795 (86%) from outpatients. Sixty-three (47.3%) of 133 hospitalized and 121 (15.2%) of 795 outpatients harbored ESBL-producing *E. coli* and *Klebsiella* spp. (P = 0.000). Chronic hepatic failure (OR: 8.7, CI: 1.65-46.12; P = 0.011) and recent antibiotic use (OR: 4.4, CI: 1.76-11.16; P = 0.002) were found to be associated with ESBL positivity for the hospitalized patients. Recent antibiotic use (OR: 2.8, CI: 1.61-5.12; P = 0.000) was found to be the only independent variable associated with ESBL positivity for the outpatients.

Conclusions: The high prevalence (15.2%) of fecal carriage of ESBL-producing bacteria in the community warrants further study in this field including the consequences of this colonization in the hospital setting.

Key Words: ESBL positivity, fecal carriage, risk factors, *E. coli*, *Klebsiella* spp.

Toplumda Dışkıda Genişlemiş-Spektrumlu Beta Laktamaz Salgılayan *Escherichia coli* ve *Klebsiella* spp. Suşlarının Sıklığının Araştırılması

Amaç: Genişlemiş spektrumlu beta-laktamaz (GSBL) enzimi üreten bakterilerle gelişen toplum kökenli infeksiyonların sıklığı giderek artmaktadır. GSBL salgılayan bakterilerle oluşan infeksiyonlar için ilk aşama genellikle gastrointestinal sistem kolonizasyonudur. Bu çalışmanın amacı, toplumda dışkıda GSBL salgılayan *Escherichia coli* ve *Klebsiella* spp. suşlarının sıklığının araştırılmasıdır.

Yöntem ve Gereç: Çalışmaya 4 aylık çalışma dönemi boyunca Mikrobiyoloji Laboratuvarı'na kabul edilen 928 dışkı örneği dahil edilmiştir. Örnekler serum fizyolojik içinde sulandırıldıktan sonra 1 µg/mL sefotaksim ve 1 µg/mL seftazidim içeren EMB agar plaklarına ekilmiştir. Plaklarda üreyen tüm bakteriler tür düzeyine kadar isimlendirilmiştir. *E. coli* ve *Klebsiella* spp. suşları GSBL üretimi yönünden Clinical and Laboratory Standards Institute (CLSI) önerileri doğrultusunda seftazidim ve seftazidim-klavulonik asit diskleri ile test edilmiştir.

Bulgular: Çalışmaya alınan 928 örneğin 133'ü (%14) yatan hastalardan, 795'i (%86) ayaktan hastalardan elde edilmiştir. Yatan 133 hastadan 63'ünde (%47.3) ve ayaktan başvuran 795 hastanın 121'inde (%15.2) GSBL üreten *E. coli* veya *Klebsiella* spp. saptanmıştır (P = 0.000). Yatan hastalarda GSBL pozitifliği için bağımsız risk faktörleri; kronik karaciğer yetmezliği (OR: 8.7; CI: 1.65-46.12; P = 0.011) ve yakın zamanda antibiyotik kullanımı (OR: 4.4; CI: 1.76-11.16; P = 0.002) olarak belirlenmiştir. Ayaktan hastalarda ise GSBL pozitifliği yönünden belirlenen tek bağımsız risk faktörü yakın zamanda antibiotik kullanımıdır (OR: 2.8; CI: 1.61-5.12; P = 0.000).

Sonuç: Toplumda GSBL pozitifliği yönünden saptanan %15.2'lik yüksek oranın hastaneye yatış durumunda doğurabileceği sonuçları irdeleyen klinik çalışmalara gereksinim vardır.

Anahtar Sözcükler: GSBL pozitifliği, dışkı taşıyıcılığı, risk faktörleri, *E. coli*, *Klebsiella* spp.

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Correspondence
Özlem KURT AZAP
Department of Clinical Microbiology and Infectious Diseases, Faculty of Medicine, Başkent University,
Fevzi Çakmak Cad. 10. Sok. 45
06490 Bahçelievler
Ankara - TURKEY

okurtazap@baskent-ank.edu.tr

Introduction

Beta-lactamase production is the most common mechanism of bacterial resistance to beta-lactam antibiotics (1). Extended-spectrum beta-lactamases (ESBL) were first described in 1983 in Europe and were first reported from the United States in 1989 (2,3). Since then, infections due to ESBL-producing bacteria have increased sharply (4). Besides nosocomial infections, community-acquired infections caused by ESBL-producing bacteria are an emerging problem worldwide (5). Possible community acquisition of ESBL-producing bacteria was first reported in the urine of an elderly patient in 1998. The patient did not have a recent history of hospitalization but had received multiple courses of antibiotics (6). Risk factors for the development of infections caused by ESBL-producing bacteria in nonhospitalized patients were reported to be previous hospitalization in the past three months, antibiotic treatment in the past three months, age over 60 years, diabetes mellitus, male gender and *Klebsiella pneumoniae* infection (7). Digestive tract colonization is also a prerequisite for infections by ESBL-producing microorganisms (8). This issue was studied in the setting of an outbreak or in the patients admitted to an intensive care unit (9,10,11). Recently, Valverde and co-workers (12) reported an increase in prevalence of fecal carriage of ESBL-producing bacteria in the community. Millar and co-workers (13) screened healthy children for the carriage of antibiotic-resistant bacteria in mouth washings or stool samples. It was concluded that antibiotic-resistant bacteria are widely disseminated and may be acquired by children before exposure to specific selection pressure.

The aim of this study was to determine the prevalence of and risk factors for fecal carriage of ESBL-producing *Escherichia coli* (*E. coli*) or *Klebsiella* spp. in the community. To our knowledge, this is the first study reporting the prevalence of fecal carriage of ESBL-producing bacteria in Turkey and also the first study reporting the risk factors for fecal carriage of ESBL-producing bacteria in the community.

Materials and Methods

Patients and Data Collection

The stool samples admitted to the Microbiology Laboratory of Başkent University Hospital between the period of 1 June - 30 September 2005 were included in the study. One sample from each patient was included. A structured form was filled from the medical records of the patients, and collected data about demographic characteristics (age, sex), co-morbidities (chronic renal failure, chronic hepatic failure, diabetes mellitus, malignancy, transplantation), hospitalization within the last three months, and antibiotic usage in the last three months. One thousand and thirty-five stool samples from individual patients were admitted to the laboratory during the study period. Forms for 107 of these samples could not be completed because of insufficient data and were excluded.

This project was approved by the Başkent University Research Committee and was funded by Başkent University Research Foundation. Informed consent was not required because there was no patient intervention.

Laboratory Methods

Samples were processed immediately after collection. A total of 0.5 g of each fecal sample was suspended in 5 ml of saline and aliquots of 200 mL were seeded into two EMB agar plates (Oxoid Ltd., Basingstoke, England) - one supplemented with 1 mg of cefotaxime per ml, the other supplemented with 1 mg of ceftazidime per ml and incubated at 37°C for 48 hours (12). These selective mediums with ceftazidime and cefotaxime were chosen because they inhibit the growth of susceptible gram-negative pathogens, yet still permit the isolation of ESBL-producing bacteria. All isolates that grew were identified to the species level. For this purpose, standard biochemical reactions were performed at the first step and BBL Crystal Enteric/NF 4.0 identification kits (Becton Dickinson®) were used when needed. *E. coli* and *Klebsiella* spp. strains were tested for ESBL production with ceftazidime and ceftazidime-clavulanate discs according to the Clinical and Laboratory Standards Institute (CLSI) Guideline (14). In this test, a ≥5 mm increase in zone diameter for ceftazidime/clavulanate disc versus ceftazidime disc alone demonstrates that the strain has the ESBL enzyme (14). When performing the ESBL confirmatory tests, *E. coli* ATCC 25922 (negative control) and *Klebsiella pneumoniae* ATCC 700603

(positive control) were tested as quality control strains (14). ESBL-producing isolates were tested against amikacin, amoxicillin-clavulanate, ampicillin-sulbactam, cefepime, ciprofloxacin, fosfomycin, gentamicin, imipenem, meropenem, nalidixic acid, nitrofurantoin, ofloxacin, piperacillin-tazobactam, sulfisoxazole, ticarcillin-clavulanate and trimethoprim-sulfamethoxazole. Antimicrobial susceptibility testing was performed by disc diffusion method according to the CLSI criteria (14). Quality was assured by testing *E. coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 in every batch. All zone determinations for these strains were within the ranges given by CLSI for the antibiotics tested in this study (14).

Statistical Methods

Data were analyzed using Stata Statistical Software, version 8.0 (STATA Corporation, Texas, USA). Proportion comparisons for categorical variables were done using chi-square tests, although Fisher's exact test was used when data were sparse. Significance was set at $P < 0.05$ using two-sided comparisons. A multivariate model was performed for ESBL positivity. In multivariate analysis, backward and forward selections revealed the same variables with the same odds ratios (OR) and confidence intervals (CI).

Results

Nine hundred and twenty-eight stool samples were included in the study. One sample was taken from each patient. Four hundred and forty-four (48%) of 928 patients were male. The mean age was 33.3 years with a range of 3 months-95 years. One hundred and thirty-three (14%) of 928 patients were hospitalized and 795 (86%) were ambulatory at the time of study. A total of 184 ESBL-producing bacteria were isolated, of which 170 (92%) were *E. coli* and 14 (8%) were *Klebsiella* spp. (9 *Klebsiella oxytoca*, 5 *Klebsiella pneumoniae*). Of the 170 *E. coli* strains, 55 (32%) were isolated from inpatients and 115 (68%) from outpatients.

Sixty-three (47.3%) of 133 inpatients and 121 (15.2%) of 795 outpatients harbored ESBL-producing bacteria ($P = 0.000$). In univariate analysis including both inpatients and outpatients, chronic renal failure ($P = 0.001$), diabetes mellitus ($P = 0.027$), malignancy ($P = 0.001$), chronic hepatic failure ($P < 0.001$), hospitalization at the time of study ($P < 0.001$), recent hospitalization ($P < 0.001$), and recent antibiotic use ($P < 0.001$) were found to be significantly associated with ESBL positivity (Table 1). In multivariate analysis, hospitalization at the time of study (OR: 3.3, CI: 2.17-5.11; $P = 0.000$), recent antibiotic use (OR: 3.3, CI: 2.17-5.11; $P = 0.000$), and chronic hepatic failure (OR:

Table 1. Univariate analysis of ESBL positivity among *E. coli* and *Klebsiella* spp. (n = 928).

	Total n = 928	ESBL positivity n = 184 (%)	P value
Male	444	96 (22)	NS[1]
Co-morbidities			
Chronic renal failure	62	22 (35)	0.001
Diabetes mellitus	58	18 (31)	0.027
Malignancy	44	17 (39)	0.001
Chronic hepatic failure	38	16 (42)	< 0.001
Transplantation[2]	45	13 (29)	NS
Hospitalization[3]	133	63 (47)	<0.001
Recent hospitalization[4]	131	49 (37)	<0.001
Recent antibiotic use[5]	135	62 (46)	<0.001
Recent quinolone use	18	10 (56)	<0.001
Recent beta-lactam use	91	44 (47)	<0.001

[1] Non-significant
[2] Renal or liver transplantation
[3] Hospitalization at the time of study
[4] Hospitalization in the previous 3 months
[5] Antibiotic use in the previous 3 months

3.3, CI: 2.17-5.11; P = 0.000) were found to be associated with ESBL positivity (Table 2). Patients were then categorized into two groups according to hospitalization at the time of study and multivariate analysis was performed for each group. Chronic hepatic failure (OR: 8.7, CI: 1.65-46.12; P = 0.011) and recent antibiotic use (OR: 4.4, CI: 1.76-11.16; P = 0.002) were found to be associated with ESBL positivity for the hospitalized patients (Table 3). Although recent antibiotic use (P < 0.001) and beta-lactam use (P = 0.001) were found to be associated with ESBL positivity in the univariate analysis, recent antibiotic use (OR: 2.8, CI: 1.61-5.12; P = 0.000) was found to be the only independent variable associated with ESBL positivity for outpatients in the multivariate analysis (Tables 4, 5). Risk factors for ESBL positivity are summarized in the Figure.

There was no statistically significant difference between the susceptibility rates of ESBL-producing *E. coli* strains isolated from hospitalized patients and ambulatory patients (Table 6).

Table 2. Multivariate analysis of ESBL positivity among *E. coli* and *Klebsiella* spp. (n = 928).

	Odds ratio	Confidence interval	P value
Age	1.0	0.99-1.00	NS[1]
Male	0.8	0.61-1.23	NS
Chronic renal failure	1.1	0.59-2.17	NS
Diabetes mellitus	1.4	0.73-2.81	NS
Chronic hepatic failure	2.1	1.05-4.54	0.035
Hospitalization[2]	3.3	2.17-5.11	0.000
Recent hospitalization[3]	0.8	0.51-1.52	NS
Recent antibiotic use[4]	3.0	1.99-4.67	0.000

[1] Non-significant
[2] Hospitalization at the time of study
[3] Hospitalization in the previous 3 months
[4] Antibiotic use in the previous 3 months

Table 3. Multivariate analysis of ESBL positivity among *E. coli* and *Klebsiella* spp. isolated from hospitalized patients (n = 133).

	Odds ratio	Confidence interval	P value
Age	1.0	0.99-1.02	NS[1]
Gender	0.7	0.34-1.72	NS
Chronic renal failure	1.0	0.38-2.81	NS
Diabetes mellitus	2.4	0.67-8.55	NS
Chronic hepatic failure	8.7	1.65-46.12	0.011
Recent hospitalization[2]	1.3	0.55-3.36	NS
Recent antibiotic use[3]	4.4	1.76-11.16	0.002

[1] Non-significant
[2] Hospitalization in the previous 3 months
[3] Antibiotic use in the previous 3 months

Table 4. Univariate analysis of ESBL positivity among *E. coli* and *Klebsiella* spp. isolated from outpatients (n = 795).

	Total n = 795	ESBL positivity n = 121 (%)	P value
Male	372	59 (16)	NS[1]
Co-morbidities			
Chronic renal failure	33	7 (21)	NS
Diabetes mellitus	42	8 (19)	NS
Malignancy	24	5 (21)	NS
Chronic hepatic failure	25	5 (20)	NS
Transplantation[2]	26	4 (15)	NS
Recent hospitalization[3]	67	10 (15)	NS
Recent antibiotic use[4]	75	22 (29)	<0.001
Recent quinolone use	8	2 (25)	NS
Recent beta-lactam use	51	16 (31)	<0.001

[1] Non-significant

[2] Renal or liver transplantation

[3] Hospitalization in the previous 3 months

[4] Antibiotic use in the previous 3 months

Table 5. Multivariate analysis of ESBL positivity among *E. coli* and *Klebsiella* spp. isolated from outpatients (n = 795).

	Odds ratio	Confidence interval	P value
Age	1.0	0.99-1.01	NS[1]
Gender	0.8	0.59-1.31	NS
Chronic renal failure	1.4	0.54-3.61	NS
Diabetes mellitus	1.1	0.50-2.82	NS
Chronic hepatic failure	1.2	0.45-3.56	NS
Recent hospitalization[2]	0.5	0.25-1.27	NS
Recent antibiotic use[3]	2.8	1.61-5.12	0.000

[1] Non-significant

[2] Hospitalization in the previous 3 months

[3] Antibiotic use in the previous 3 months

Discussion

Colonization with multi-resistant isolates, including ESBL-producing isolates, is a prerequisite for infection. The importance of the detection of carriers harboring antimicrobial-resistant bacteria has been emphasized not only in patient populations but also in healthy people (15). Resistant bacteria may be transmitted from human-to-human or through the environment resulting in an increase in the proportion of carriers in the community (16). The admission of carriers harboring resistant bacteria to hospitals increases the risk of infection in other hospitalized patients (4,17). Antibiotic selective pressure in hospitals may amplify the number of carriers harboring resistant bacteria and enhance the opportunity for these bacteria to cause infections (17).

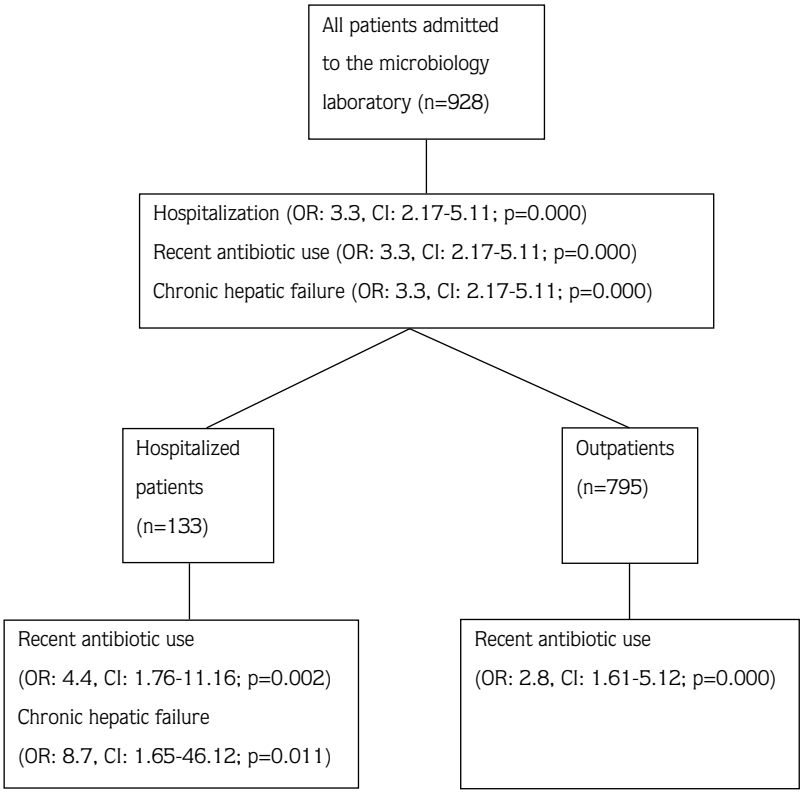

Figure. Summary of risk factors for ESBL positivity.

Table 6. The susceptibility rates of ESBL-producing *E. coli* strains against antimicrobial agents (n = 170).

	Total n = 55 (%)	ESBL positivity n = 115 (%)	P value
Amikacin	43 (78)	99 (86)	NS[1]
Amoxicillin-clavulanate	7 (13)	15 (13)	NS
Ampicillin-sulbactam	7 (13)	15 (13)	NS
Cefepime	12 (22)	31 (27)	NS
Ciprofloxacin	15 (27)	45 (39)	NS
Fosfomycin	54 (98)	113 (98)	NS
Gentamicin	23 (42)	49 (43)	NS
Imipenem	55 (100)	115 (100)	NS
Meropenem	55 (100)	115 (100)	NS
Nalidixic acid	15 (27)	37 (32)	NS
Nitrofurantoin	53 (96)	104 (90)	NS
Ofloxacin	15 (27)	45 (39)	NS
Piperacillin-tazobactam	37 (67)	77 (67)	NS
Sulfisoxazole	15 (27)	20 (17)	NS
Ticarcillin-clavulanate	20 (36)	48 (42)	NS
Trimethoprim-sulfamethoxazole	18 (33)	28 (24)	NS

1 Non-significant

Several studies have focused on beta-lactam resistance in Enterobacteriaceae isolated from stools in healthy people, but they did not specifically investigate ESBLs (18,19). Recently, Mirelis (20) reported that 2.1% of outpatients were fecal carriers of ESBL–producing bacteria in 2001 and this percentage increased to 3.8% one year later. Valverde (12) also reported that rates of fecal carriage of ESBL-producing isolates increased significantly ($P < 0.001$) in both hospitalized patients and outpatients, from 0.3% and 0.7%, respectively, in 1991 to 11.8% and 5.5%, respectively, in 2003. The rate of ESBL-producing isolates among healthy volunteers was reported to be 3.7%. In the present study, 63 (47.3%) of 133 inpatients and 121 (15.2%) of 795 outpatients harbored ESBL-producing bacteria ($P = 0.000$). Inpatients were not further categorized into intensive care unit patients or ward patients. Because this study was not planned to determine the prevalence of ESBL positivity in hospitalized patients, stool samples were not obtained from all patients in the hospital. So this high rate (47.3%) among inpatients is probably far from the actual rate. The prevalence of ESBL harboring *E. coli* or *Klebsiella* spp. is strikingly high (15.2%) among outpatients. The definition of "outpatients" in the present study obviously does not correspond to "healthy volunteers". Stool samples of these patients were admitted to the microbiology laboratory usually because the patient had diarrhea, but because they were not hospitalized at the time of study, they were considered as presenting the community profile.

Until recently, most infections caused by ESBL-producing *E. coli* or *Klebsiella* spp. have been described as nosocomially acquired or nursing home-related (1,21). However, some recent data suggest that infections due to ESBL-producing microorganisms might be an emerging problem in the outpatient setting (5,6,7,22). Previous antimicrobial treatment, older age, diabetes mellitus, and previous hospitalization were identified as risk factors for community-acquired infections caused by ESBL-producing bacteria in two articles (7,22). To our knowledge, risk factors for fecal carriage of ESBL-producing *E. coli* and *Klebsiella* spp. in the community have not been studied. In the present study, recent antibiotic use was determined as the only independent variable in outpatients. Surprisingly, hospitalization in the previous three months was not found to be associated with ESBL positivity. Although older age and diabetes mellitus were found to be risk factors for community-acquired infections caused by ESBL-producing bacteria, no relation between age, underlying disease and fecal carriage of bacteria harboring ESBL could be demonstrated (7,22). Among the recent antibiotics used, only beta- lactams and not quinolones were found to be significantly associated with ESBL positivity among outpatients. This finding is also distinct from the other two studies which demonstrated that previous quinolone use was one of the risk factors for community-acquired infections caused by ESBL-producing bacteria (7,22).

Uropathogens are assumed to originate primarily from the bowel flora, i.e. resistance in urinary isolates is closely associated with the resistance in the fecal bacteria (23). This is one of the reasons for giving careful attention to this issue. ESBL-producing isolates are known to be frequently resistant to antimicrobial classes including aminoglycosides, quinolones and trimethoprim-sulfamethoxazole (24). Multi-drug resistance causes difficulties in the management of urinary tract infections. Table 6 shows the susceptibility rates of the antibiotics including fosfomycin, nitrofurantoin and sulfisoxazole against 170 *E. coli* strains isolated from inpatients and outpatients. These three antibiotics were tested because *E. coli* is the most common cause of urinary tract infections. Recently, ESBL production was found to be significantly associated with ciprofloxacin resistance in urinary *E. coli* isolates (25). The susceptibility rates were also found to be low in the present study for the ESBL-producing *E. coli* strains. There was no statistically significant difference between the strains isolated from inpatients and outpatients (Table 6).

In conclusion, community-acquired infections caused by ESBL-producing bacteria are an emerging problem. Digestive tract colonization of resistant bacteria is a prerequisite for infection. When urinary system infections are taken into consideration, a high prevalence (15.2%) of fecal carriage of ESBL-producing bacteria in the community will probably cause difficulties in the treatment of outpatients. These findings warrant further study in this field including the consequences of colonization with ESBL-producing bacteria both in the community and in the hospital setting.

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