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Fecal carriage of extended-spectrum beta-lactamase-producing *Escherichia coli* and *Klebsiella* spp. in a Turkish community

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Background/aim: The aim of this study was to determine the prevalence of fecal carriage of extended-spectrum beta-lactamase (ESBL)-producing bacteria, enzyme types, and risk factors affecting colonization.

Materials and methods: A total of 576 stool samples from outpatients were examined between October 2012 and May 2013. Screening was done with selective EMB plates. ESBL were detected by double-disk synergy and confirmed agar strip gradient methods. Enzyme types were determined by PCR.

Results: The prevalence of fecal carriage was found as 30% (173 of 576). Recent use of antibiotics, hospitalization and surgical operation, diabetes, crowded household populations, and old age were associated with higher carriage rates. Of the ESBL-producing bacteria, 87.5% were positive for $bla_{\text{CTX-M}}$ genes. Of the $bla_{\text{CTX-M}}$ gene-positive isolates, 95.2% were positive for $bla_{\text{CTX-M-1}}$ genes; among these, 82.2% were positive for $bla_{\text{CTX-M-3}}$ and 67.7% were positive for $bla_{\text{CTX-M-15}}$ genes while 62.5% isolates were positive for both $bla_{\text{CTX-M-3}}$ and $bla_{\text{CTX-M-15}}$ genes

Conclusions: A high rate (30%) of fecal carriage of ESBL bacteria was found in an adult population. The predominant beta-lactamase enzyme types were CTX-M-3 and CTX-M-15.

Key words: Extended-spectrum beta-lactamase, *Escherichia coli*, *Klebsiella* spp., fecal carriage

1. Introduction

Extended-spectrum beta-lactamases (ESBLs) are enzymes produced by bacteria that can degrade and confer resistance to some of the most commonly used antibiotics including penicillins, cephalosporins, and monobactams. Infections by ESBL-producing *Enterobacteriaceae* (ESBL-E) remain a great health concern in hospitals and long-term care facilities. Associated infectious syndromes include mainly urinary tract, bloodstream, and intraabdominal infections, and they may be serious enough to warrant hospitalization. Furthermore, infection or colonization of residents in long-term care facilities by ESBL-producing organisms may provide a means for the dissemination of ESBLs between hospitals and the community (1).

Colonization of the gastrointestinal tract plays a key role in the epidemiology and clinical significance of ESBL-producing bacteria. Intestinal colonization is demonstrated in most of the infections caused by these bacteria (2). Considering the probable increase in infections with ESBL-producing bacteria in patients who are already carriers of these bacteria, recent studies on the rates of carriage in the community and the relation between carriage and infections are attracting attention. While rates of 1%–8% carriage in healthy populations were reported in various European countries between 2003 and 2007, the rates were found to be increasing over time (2–4). On the other hand, up to 50%–60% rates of carriage were reported from countries in Asia and Africa such as Egypt (5) and China (6). When ESBL carriage was evaluated for travelers, rates were found to be low for those who had traveled to Europe (3%), while they were higher for people who had traveled to India, Egypt, and Thailand (36%) (7). The fecal carriage of ESBL-producing bacteria in Turkey has not been thoroughly investigated; among the few studies conducted (8–11), one reported a rate of 18.7% in İstanbul (12).

The purpose of this study was to investigate the prevalence and predisposing factors of fecal carriage of ESBL-E in the community in Zonguldak, Turkey, and to determine the enzyme types associated with carriage.

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2. Materials and methods

2.1. Patients and isolates

The study was performed from October 2012 to May 2013 at Bülent Ecevit University Hospital, Zonguldak, Turkey. Ethical approval for the study was obtained from the research ethics committee of the university. A total of 576 fecal samples were studied from individuals attending the routine check-up clinic. The demographic data of the patients, including age, sex, educational level, antibiotic usage in the past 3 months, urinary infection in the past 6 months, hospitalization, household population, keeping pets at home or feeding animals in the garden, and frequency of eating chicken, were collected through a questionnaire.

Fecal samples (approximately 0.1 g) were spread on two EMB agar plates (HiMedia, India), one supplemented with 2 μg/mL cefotaxime and the other supplemented with 2 μg/mL ceftazidime (13). Plates were incubated in ambient air at 35 °C for a minimum of 24 h before initial examination. Plates demonstrating no growth in primary examination were incubated for another 24 h. Samples yielding bacteria that grew on EMB agar plates were identified by conventional methods and by BBL Crystal Identification Systems E/NF (Becton Dickinson, USA) when necessary and they were initially identified as "suggestive of ESBL". Antimicrobial susceptibility was determined by the disk diffusion test in accordance with guidelines from the Clinical Laboratory Standards Institute (14). Double disk synergy test and agar strip gradient methods were performed to confirm ESBL production (Liofilchem, Italy). An eightfold difference between the minimal inhibitory concentrations of cefotaxime/ceftazidime and cefotaxime/ceftazidime + clavulanic acid was considered as ESBL positivity (14). *E. coli* strain ATCC 25922 and *K. pneumoniae* strain ATCC 700603 were used as negative and positive controls, respectively, for the quality control studies.

2.2. Molecular studies

DNA isolation was performed by a commercial DNA extraction kit (Thermo Scientific Gene JET Genomic). The PCR mixture was as follows: 25 μL of 2X mix solution, 1 μL of primer R, 1 μL of primer F, 0.5 μL of Taq polymerase, 0.5 μL of DNA, and 17.5 μL of distilled water. The primers used and the size of the expected DNA products for each enzyme group are shown in Table 1.

The amplification products were run on 2% agarose gel electrophoresis in 1X TBE buffer and visualized by the Gel Doc UV system (Bio-Rad, Italy).

2.3. Statistical analysis

Statistical analyses were performed with SPSS 18.0 (SPSS Inc., Chicago, IL, USA). Distribution of data was determined by Shapiro–Wilks test. Continuous variables were compared with the Mann–Whitney U test for two groups and categorical variables were compared using the Pearson chi-square test or Yates correction chi-square test. $P < 0.05$ was considered statistically significant for all tests.

3. Results

Bacterial isolates other than *E. coli* and *K. pneumoniae* that grew on the EMB agar were disregarded. Of the 576 stool samples tested, 173 (30%) yielded ESBL-producing organisms with a total of 192 ESBL-producing isolates on cultures. *E. coli* was the predominant ESBL-producing organism. Among the carriers, 51.2% were males and 48.8% were females. The mean age among the carriers was 44 ± 15.8 years, with a mean of 43.8 in males and 44.3 in females.

The susceptibility results for the isolates are summarized in Table 2. Carbapenems (imipenem and meropenem) and amikacin were the most active antibiotics against the ESBL-producing organisms.

Table 1. The primers and the size of the expected DNA products for each enzyme group.

Enzyme	Forward	Reverse	bp
CTX-M group	5'-TTTGCGATGTGCAGTACCAGTAA-3'	5'-CGATATCGTTGGTGGTGCCATA-3'	544 (18)
CTX-M-1 group	5'-AAAAATCACTGCGCCAGTTC-3'	5'-AGCTTATTCATCGCCACGTT-3'	415 (19)
CTX-M-2 group	5'-CGACGCTACCCCTGCTATT-3'	5'-CCAGCGTCAGATTTTTCAGG-3'	552 (19)
CTX-M-8 group	5'-TCGCGTTAAGCGGATGATGC-3'	5'-AACCCACGATGTGGGTAGC-3'	666 (19)
CTX-M-9 group	5'-CAAAGAGAGTGCAACGGATG-3'	5'-ATTGGAAAGCGTTCATCACC-3'	205 (19)
CTX-M-25 group	5'-GCACGATGACATTCGGG-3'	5'-AACCCACGATGTGGGTAGC-3'	327 (19)
CTX-M-3	5'-CGTCACGCTGTTGTTAGGAA-3'	5'-ACGGCTTTCTGCCTTAGGTT-3'	780 (20)
CTX-M-15	5'-CACACGTGGAATTTAGGGACT-3'	5'-GCCGTCTAAGGCGATAAACA-3'	996 (21)
TEM group	5'-ATGAGTATTCAACATTTCCG-3'	5'-CTGACAGTTACCATTGCTTA-3'	1075 (22)
SHV group	5'-GGGTTATTCTTATTTGTCGC-3'	5'-TTAGCGTTGCCAGTGCTC-3'	930 (22)

Table 2. The susceptibility profiles of the ESBL-producing organisms.

Antimicrobial	Susceptible, n (%)
Amikacin	183 (95.3)
Ampicillin	2 (1.0)
Amoxicillin-clavulanic acid	88 (45.8)
Aztreonam	72 (37.5)
Ertapenem	188 (97.9)
Imipenem	191 (99.5)
Meropenem	191 (99.5)
Ciprofloxacin	120 (62.5)
Levofloxacin	133 (69.3)
Piperacillin	1 (0.5)
Piperacillin-tazobactam	116 (60.4)
Ceftazidime	105 (54.7)
Cefoxitin	164 (85.4)
Ceftriaxone	5 (2.6)
Cefotaxime	7 (3.6)
Cefepime	116 (60.4)
Cefuroxime	0
Trimethoprim-sulfamethoxazole	96 (50.0)
Gentamicin	139 (72.4)
Tobramycin	136 (70.8)

Statistical evaluation of the questionnaire and the carriage rates revealed that use of antibiotics in the past 3 months (P = 0.027), hospitalization (P = 0.022) and undergoing a surgical operation (P = 0.005) in the last 6 months, diabetes mellitus (P = 0.048), having a household population of more than three persons (P = 0.049), and age greater than 60 (P = 0.033) were associated with higher ESBL carriage rates while higher education (university graduate) was related to lower rates of carriage (P = 0.004). No statistically significant difference was found in carriage rates in different sexes (P = 0.820) and no significant association was demonstrated for urinary tract infection in the past 6 months, use of antacids and proton-pump inhibitors, chronic disease other than diabetes mellitus, keeping pets at home or in the garden, and frequency of eating chicken (Table 3).

Of the 192 ESBL-producing bacteria, 168 (87.5%) yielded CTX-M group enzymes while 144 (75%) and 11 (5.7%) had TEM and SHV group enzymes, respectively (Figure 1). Of the 168 CTX-M group enzymes, 160 (95.2%) belonged to the CTX-M-1 group; among these 160 CTX-M-1-positive isolates, 158 yielded CTX-M-3 and 130 yielded CTX-M-15 enzymes while 120 isolates had both CTX-M-3 and CTX-M-15 (Figure 2). Eight isolates were negative for CTX-M-1, of which four had CTX-M-9, two had CTX-M-2, and one had both CTX-M-9 and CTX-M-2 (Figure 3). None of the five enzyme groups was found for one isolate with a positive CTX-M group PCR result. The distribution of the enzyme types with respect to bacterial species is shown in Table 4.

4. Discussion

Colonization of the gastrointestinal tract with ESBL-E plays a key role in the epidemiology and clinical significance of infections with these bacteria. Intestinal colonization is demonstrated in most people infected by these bacteria. The rate of fecal carriage of ESBL-producing bacteria was found as 30% in the present study. There is a limited number of studies evaluating fecal carriage of ESBL in Turkey, which has land in both Asia and Europe. Of the few studies, two reported carriage rates of 3% (8) and 15% (9) in two cities in Central Anatolia, while studies conducted in İstanbul yielded rates of 14%–21.3% (10–12). Considering the geographic status of the country being like a bridge between Asia and Europe, the rates reported until recently are lower than the ones from Asia and Africa while they are higher than those from Europe.

The global dissemination of the CTX-M beta-lactamases since the mid-1990s is a challenging issue. Unlike the TEM and SHV variants, CTX-M-producing isolates, particularly urinary isolates of *Escherichia coli*, are increasingly recovered from patients with community-onset infections and from populations with minimal healthcare risks (15). A multicenter study performed between 1999 and 2006 indicated CTX-M-15 as the predominant enzyme worldwide, including most of the European countries, Middle Eastern countries, North America, Canada, and Turkey (16). However, CTX-M-9 and CTX-M-14 were reported to be exceptionally endemic in Spain (13,16). CTX-M-2 was found to be the most common ESBL enzyme type in France in 2006 (3), while the most common types were the CTX-M-1 group and TEM-52 in the Netherlands (17), the CTX-M-1 group in the Czech Republic (4), and CTX-M-14 in China (18). The limited number of reports from Turkey found the CTX-M group as the most predominant enzyme group (10,12), in concordance with the present results, which revealed the CTX-M group of enzymes in 168 of the total 192 isolates (87.5%), CTX-M-3 (a member of the CTX-M-1 group) being the most frequent among these isolates (82.3%). Another member of the CTX-M-1 group, CTX-M-15, which was previously reported to be the most common type from our country, was found to be the second most predominant enzyme type in the present study (67.7%, 130/192). The coexistence of CTX-M-3 and CTX-M-15 was observed for a total of 120 isolates (62.5%).

Table 3. Risk factors for ESBL carriage.

Risk factors	ESBL carriers	ESBL noncarriers	P-value
	n = 173	n = 403	
Antibiotic intake	44.6	34.8	0.027
Urinary infection	13.1	9.8	0.247
Hospitalization	22	14.2	0.022
Operation	14.3	6.9	0.005
Chronic diseases	42.3	40.4	0,682
Diabetes mellitus	12.5	37.4	0.048
Ulcerative bowel disease	3.6	6.9	0.184
PPI usage	48.0	40.1	0.212
Feeding animal	32.7	29.7	0.277
Eating chicken	89	92.3	0.277

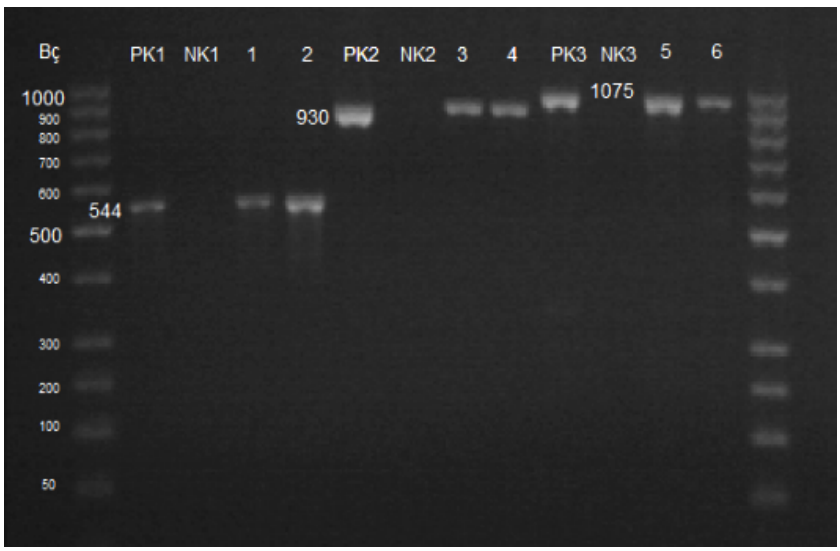

Figure 1. CTX-M, TEM, and SHV group enzymes in agarose gel electrophoresis. PK1: CTX-M group positive control, NK1: CTX-M group negative control, 1–2: CTX-M group enzymes in our isolates (544 bp), PK2: SHV group positive control, NK2: SHV group negative control, 3–4: SHV group enzymes in our isolates (930 bp), PK3: TEM group positive control, NK3: TEM group negative control, 5–6: TEM group enzymes in our isolates (1075 bp), Bç: marker, 50–1000 bp DNA ladder.

Coexistence of more than two enzymes in the same isolate was reported before but coexistence of more than one CTX-M enzyme type was not reported in the literature. This coexistence was also observed in another study that evaluated ESBL fecal carriage of children aged between 0 and 15 years (unpublished data). Lee et al. reported *K. pneumoniae* isolates that harbored two different SHV-type beta-lactamases genes bounded by an insertion sequence in a single transferable plasmid (19). We planned an investigation of our isolates to evaluate whether a similar situation existed or not.

Community-acquired ESBL-E-associated infections typically affect patients with various complicating factors. A case-control study demonstrated risk factors for community-acquired infection by ESBL-producing *E. coli*, including female sex, increased age, diabetes mellitus, recurrent urinary tract infections, previous instrumentation of the urinary tract, follow-up in

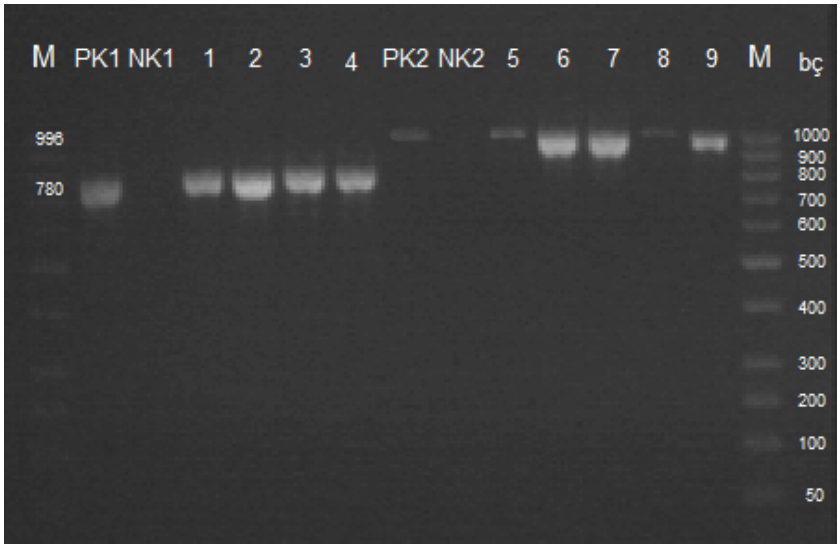

Figure 2. CTX-M-15 and CTX-M-3 group enzymes in agarose gel electrophoresis. PK1: CTX-M-3 group positive control, NK1: CTX-M-3 group negative control, 1–4: CTX-M-3 group enzymes in our isolates (780 bp), PK2: CTX-M-15 group positive control, NK2: CTX-M-15 group negative control, 5–9: CTX-M-15 group enzymes in our isolates (996 bp), M: marker, 50–1000 bp DNA ladder.

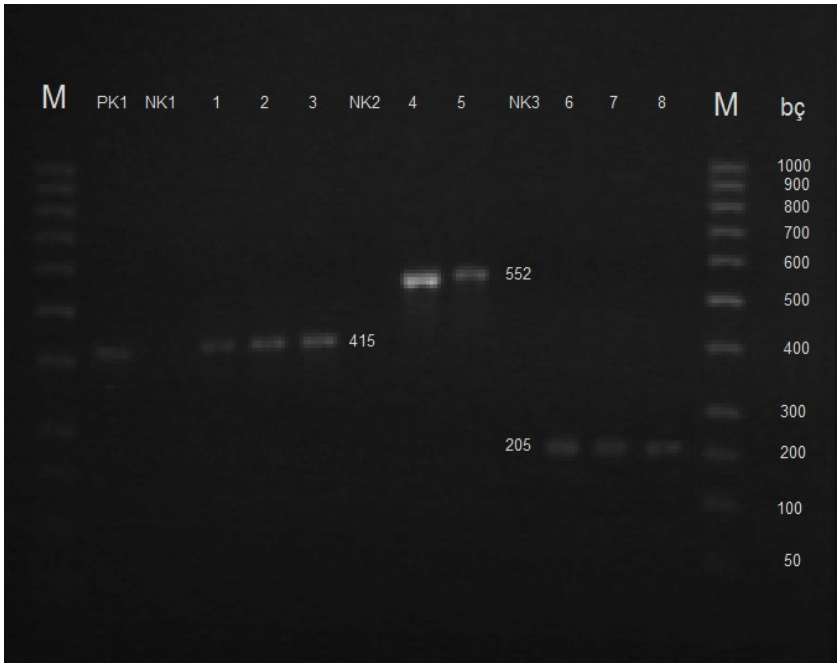

Figure 3. CTX-M-1, CTX-M-2, and CTX-M-9 group enzymes in agarose gel electrophoresis. PK1: CTX-M-1 group positive control, NK1: CTX-M-1 group negative control, 1–3: CTX-M-1 group enzymes in our isolates (415 bp), NK2: CTX-M-2 group negative control, 4–5: CTX-M-2 group enzymes in our isolates (552 bp), NK3: CTX-M-9 group negative control, 6–8: CTX-M-9 group enzymes in our isolates (205 bp), M: marker, 50–1000 bp DNA ladder.

outpatient clinics, and previous usage of various antibiotics. In agreement with that study, use of antibiotics in the past 3 months was indicated as a risk factor for ESBL carriage in the present study. Age greater than 65 was represented as one of the risk factors for ESBL carriage in previous studies (16,20,21). Likewise, among the population included in our

Table 4. Distribution of enzyme types with respect to bacterial species.

Bacterial species	n (%)	CTX-M group	CTX-M-1 group	CTX-M-15	CTX-M-3	TEM	SHV
E. coli	174 (90.6)	154	147	118	145	131	5
K. pneumoniae	17 (8.9)	14	13	11	13	13	6
K. oxytoca	1 (0.5)	0	0	0	0	0	0
Total	192	168	160	130	158	144	11

study, which had an average age of 44 ± 15.8, age older than 60 was significantly associated with higher rates of carriage. There are variable results in different studies about the association of sex and the rates of carriage; some reported higher rates in females while others reported it in males (16,20). There was no statistically significant difference in rates of different sexes in the present study (P = 0.820).

A study by Friedmann et al. showed that the rate of rectal carriage of ESBL-E bacteria at hospital admission was 8%; the rate of nosocomial acquisition of these bacteria increased with extended duration of hospitalization, doubling to 17% by day 4 or 5 after admission and gradually increasing up to 33% after 10 or more days of hospitalization (21). Another study demonstrated that the patients in intensive care units were colonized after an average of 7 days of stay in the ICU (2–14 days) (22). Colonization during hospital stay leads to the transfer of the ESBL-E to the community after discharge from the hospital. Research on the ongoing colonization time after hospital discharge is limited and an average of 4–7.5 months is reported (23,24); however, up to 59 months has been reported (25). In our study, carriage rates were found to be significantly higher in those who were hospitalized in the past 6 months. Apart from hospitalization, undergoing a surgical operation was reported to increase ESBL colonization (26). Similarly, in the present study, undergoing a surgical operation in the past 6 months was significantly related to increased ESBL carriage rates.

E. coli is the causative agent of more than 80% of community-acquired urinary tract infections, which usually result from contamination of the urethra by gastrointestinal bacteria. Fecal colonization of ESBL bacteria in the community has been linked to the increase in infections caused by these bacteria (27). On the other hand, urinary tract infection has been defined as an independent risk factor for ESBL fecal carriage (28). However, there are also reports indicating no link between urinary tract infection and urinary catheterization and fecal ESBL carriage (13). In the present study, we could not demonstrate a significant association between urinary tract infection and carriage.

Chronic diseases, particularly diabetes mellitus, were accused of being risk factors for increased ESBL carriage (12,13,29). Urinary tract infections are also more frequent in diabetic patients; female diabetic patients are reported to have fivefold and threefold more pyelonephritis and asymptomatic bacteriuria, respectively. It was also determined that 26% of the diabetic patients with asymptomatic bacteriuria had urethral colonization with the same bacteria for at least 6 months (30). Some researchers noted that diabetic patients had more likelihood to have infections with more resistant uropathogens (31). In concordance with most of these reports, we found higher ESBL carriage rates in patients with diabetes mellitus.

High rates of ESBL-producing *E. coli* carriage are reported in various animals. A study conducted in Hong Kong in 2011 revealed rates of 58.5% in chickens, 32.9% in cattle, 63.6% in swine, 13.3% in cats, and 14.7% in dogs (32). Keeping pets at home was accused of causing up to sevenfold increase in the carriage rates (33). There are also reports, in contrast, reporting no significant increase in carriage in those who have contact with animals or who keep pets (3,13). We could not demonstrate a significant association between keeping pets and ESBL carriage rates (P = 0.277). Determination of the same ESBL genes and plasmids in patients, in chickens, and in other fowls led to the idea that ESBL could be transferred from fowls to people via the food chain. In their study conducted in 2011 in the Netherlands, Hall et al. reported ESBL carriage rates of 35% in the population and 49% in chickens and noted that 19% of the isolates obtained from both humans and chickens were ESBL-producing *E. coli* with common genetic properties (17). On the other hand, a study including vegetarians revealed similar rates of colonization in those who were not vegetarians (34). In addition, carriage was found high in children with malnutrition in India and Africa, suggesting that carriage was not only related to food intake but also to water and various environmental conditions (35,36). We could not demonstrate a significant relation between the habit of eating chicken and ESBL carriage in the present study.

It has been stated that the rate of carriage is higher when the family has a low socioeconomic profile and the head of the family is unemployed. Poverty was identified as the main factor affecting this rate and higher carriage

rates have been observed in people with low or moderate incomes. Poor health and sanitation conditions were set forth to increase the spread of ESBL by fecal-oral route and compliance with the rules of hygiene was suggested to be worth investigation (37). In a study in India, the ESBL fecal carriage rate was high in people with high levels of education and this situation was thought to be associated with the fact that it was easier for these people to obtain antibiotics (38). In our study, the carriage rates in university graduates were determined to be significantly lower. With the increase in community-acquired infections with ESBL-producing bacteria, intrafamilial spread between in-house contacts has been suggested (13,29,39). Furthermore, there are reports indicating significant differences in rates of carriage in children living with parents who are CTX-M carriers and those who are not CTX-M carriers (40). In our study, having a household population of more than three persons was found to be significantly associated with higher fecal carriage.

In conclusion, a 30% fecal carriage rate of ESBL bacteria in the adult population was found in the present study, the predominant beta-lactamase enzyme types being CTX-M-3 and CTX-M-15. Use of antibiotics in the past 3 months, hospitalization and undergoing a surgical operation in the past 6 months, diabetes mellitus, having a household population of more than three persons, and age greater than 60 were associated with higher ESBL carriage rates. The carriage of resistant bacteria is a significant challenge for public health and clinicians, clinical microbiologists, and general practitioners must keep in mind that ESBL-producing bacteria have reached high levels not only in hospital settings but also in the community. Awareness should be raised in the community about the means of distribution of and possible results of infection with resistant bacteria.

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