Abstract

Fecal carriage is one of the most important reasons for extended spectrum beta-lactamase-producing Enterobacteriaceae (ESBL-PE) causing infections. We aimed to demonstrate epidemiological features for a subtype of ESBL-PE-encoding TEM, SHV, and CTX-M as well as for qnrA, qnrB, qnrC, qnrS, aac-(6)-lb genes through polymerase chain reaction (PCR) in patients undergoing transrectal needle prostate biopsy (TRNBP). Between October 2008 and February 2010, we collected 400 fecal swabs from patients prior to TRNBP in four separate centers. After detecting ESBL-PE isolates in the material, we further analyzed TEM, SHV, and CTX-M enzymes, as well as three types of qnr genes of qnrA, qnrB, qnrC and aac-(6)-lb by PCR. We detected 80 ESBL-PE isolates in 400 fecal samples. Of the 80 isolates; blaSHV, blaTEM, and blaCTX-M were observed in 12, 46 and 79 isolates, respectively. All three genes were present in eight isolates. Resistance to quinolone was identified in 67 (83.7%) isolates, resistance to aminoglycoside in 52 (65%) isolates, and resistance to both antibiotics in 46 (57.5%) isolates. Subsequently, we determined qnrB, qnrS and aac-(6)-lb genes in 7 (8.8%), 11 (14%) and 60 (76%) isolates, respectively. qnrA and qnrC were not detected in any of the isolates. CTX-M-producing ESBL-PE is the most common pathogen responsible for fecal carriage in the community. Plasmid-mediated quinolone resistance genes (qnr, aac-(6)-lb) are the reason behind the dissemination of ESBLs.

Introduction

Extended spectrum beta-lactamase producing Enterobacteriaceae (ESBL-PE) carriage is rising in the community with intestinal colonization accounting as the most common factor for the escalation (1,2). A Herindrainy et al. study in Madagascar revealed the carriage rate as 10.1%, one of the highest in the literature (3). Figures for Brazil and Spain were 1.5% and 3.7%, respectively (4,5). The rate of fecal carriage was documented as 15.2% in Turkey (6). Most ESBLs are derivatives of TEM, SHV, CTX-M, OXA, CMY, IMP and VIM type enzymes. The previous studies cite that among community-acquired ESBL, CTX-M is of major concern since it spreads worldwide by plasmids from a few incompatibility groups (7). Plasmid-mediated quinolone resistance has three different resistance mechanisms. The first is qnr with A, S, B, C, D allele; the second is aac-(6)-lb, a variant of aminoglycoside acetyltransferase; the third is QepA, responsible for quinolone extrusion.

We investigated the distribution of genes that encode TEM, SHV, and CTX-M through polymerase...
chain reaction (PCR). Subsequently, we detected distribution of qnrA, qnrB, qnrC, qnrS and aac-(6)-Ib genes in CTX-M positive strains in patients undergoing transrectal needle prostate biopsy (TRNBP).

Patients and Methods

Fecal samples from 400 patients who underwent TRNBP to be screened for the presence of ESBL at various centers (Marmara University, School of Medicine, Department of Infectious Diseases and Urology, Taksim Training and Research Hospital Department of Urology, Göztepe Training and Research Hospital, Department of Infectious Diseases, Acibadem Hospital, Department of Infectious Diseases) between October 2008 and February 2010.

Microbiologic studies

Amies transport medium from centers participating in the study were vortexed in 2 cc brain heart infusion broth and placed into an incubator for 24 h at 37°C. Each sample was inoculated with two different MacConkey agar plates, supplemented with either 1 μg/ml ceftazidime or 1 μg/ml cefotaxime (Becton Dickinson, Sigma-Aldrich). Culture media was kept in an incubator for 48 h at 37°C and on detecting morphology of the growth; they were passed onto the eosin methylene blue agar (EMB) media plates. Pink colored colonies growing on the EMB media plates were stored in cryobank (AbtekBiologicals) beads at −20°C for further identification.

Mueller-Hinton agar (MHA) media was used for ESBL analysis during the modified disc (combined disc) test. Escherichia coli ATCC 25922 was used as the positive control and Klebsiella pneumoniae ATCC 700603 as the negative control. Isolates were subjected to CLSI-recommended 5% bloody agar for the combined disc synergy test. McFarland was set to 0.5, and then planted on the plates containing MHA. Ceftazidime (30 μl), cefotaxime (30 μl), ceftazidime/clavulanic acid (30/10 μl) and cefotaxime/clavulanic acid (30 μl/10) discs were placed on plates at room temperature for 15 min. Cefotaxime was chosen because CTXM enzyme is more effective on cefotaxime than on ceftazidime. Plates were incubated for 18-24 h at 35°C. ESBL appears to be produced when the inhibition zone is raised by ≥ 5 mm toward the cefotaxime plus clavulanic acid or ceftazidime plus clavulanic acid disc, in comparison with the cefotaxime or ceftazidime disc alone (CLSI, M100-S18, Wayne, PA, USA, 2008). Detection of ESBL positive strains was followed by defining the types and antibiogram tests using the VITEK system. We identified aminoglycoside and quinolone resistance by antibiogram tests using the VITEK II automated system.

DNA extraction

DNA was obtained from bacteria using the QIAGEN DNA isolation kit to detect Beta-lactamase genes. PCR mixture (45 μl) was added on the bacterial DNA (5 μl) and loaded into the PCR machine (Perkin ElmerCetus, DNA ThermalCycler 480). The machine was programmed according to the primer used. The required environment and primers were obtained for the PCR reaction. Woodford method was used for analysis subgroups of CTX-M. Positive control isolates were acquired from Coque et al. (8). PCR products were analyzed by agarose gel (2% agarose) electrophoresis (1 h 90V) and ethidium bromide staining. Multiplex PCR of blaCTX-M gene for Groups I, II, III and IV was carried out as described by Pitout et al. (9). Positive control isolates were used for each CTX-M subgroup. Screening of the qnrA, qnrB, qnrC, qnrS, aac-(6)-Ib genes were completed through multiplex PCR (10,11).

Statistical analysis

The software STATA version 11.0 was used for all analysis. Univariate variables with a P < 0.05 were considered to be statistically significant. Multiple logistic regression analysis was performed for further multivariate examination for factors that were regarded as noteworthy in the univariate analysis. Multiple logistic regression analysis was completed according to the backward selection criteria.

Results

ESBL-PE strains were detected in 80 (18.75%) fecal samples from 400 patients. In five patients, the ESBL-PE stemmed from two different strains. The detected strains were E. coli in 75 (94%) of the cases and K. pneumoniae in the remaining 5 (6%).

About 80 (20%) ESBL-PE strains were detected in 400 fecal samples. Of the 80 isolates, the PCR revealed blaSHV gene in 12, blaTEM in 46 and blaCTX-M in 79. All three genes were present in eight isolates (Figure 1). Further analysis of ESBL-PE isolates that were tested with blaCTX-M-group specific primers revealed all of them to be positive for blaCTX-M-1 group and for blaCTX-M-15 specific primers. Four isolates were also positive for blaCTX-M-9-14 group.

ESBL-PE showed co-resistance to other antibiotic classes; quinolone resistance was observed
in 67 (83.7%) isolates, aminoglycoside resistance in 52 (65%), and both quinolone and aminoglycoside resistance in 46 (57.5%). The presence of \( qnrA \), \( qnrB \), \( qnrC \), \( qnrS \) and \( aac-(6)-Ib \) genes was also discovered in ESBL-PE \( blaCTX-M-15 \) isolates. \( qnrA \) and \( qnrC \) were not detected in any of the isolates. A number of isolates carrying \( qnrB \), \( qnrS \) and \( aac-(6)-Ib \) genes were detected as 7 (8.8%), 11 (14%) and 60 (76%), respectively.

**Discussion**

In the study, we found the fecal carriage rate of ESBL-PE (20%) to be much higher than that of in Tunisia (7.3%), another Mediterranean country (12). Fecal carriage rate of ESBL-PE (16.7%, 15.6%) from other studies is similar to our conclusions (13,14).

CTX-M enzyme-producing \( E. \ coli \) is the most prevalent form of community-acquired ESBL-PE (2). We also discovered a higher number of \( E. \ coli \) strains than \( K. \ pneumoniae \) in ESBL-PE strains with CTX-M enzyme.

During the past decade, a dominant enzyme in ESBL-PE shifted from TEM and SHV toward CTX-M, both in hospital and community-acquired isolates (15,16). CTX-M types differ according to geographical regions. Limited data collected from Turkey show that the predominant subtype of CTX-M is group 1 enzymes, most of which are CTX-M-15 (17). The epidemic of CTX-M poses a threat to community health. Encountering the enzyme, especially in patients with no previous history of hospitalization, is interesting and it is one of the reasons why the situation poses a threat to the community. Genetic factors easing the mobilization of \( blaCTX-M \) gene such as plasmids, insertions, integrons, and transposons may be the reason for the high prevalence of ESBL and \( blaCTX-M \) gene in fecal samples. The fast spread of CTX-M-15 among the community is one of its most grave characteristics. It is asserted that the plasmid Inc-F plays a crucial role in the speedy dissemination of CTX-M-15. A study conducted in Turkey discovered dissemination of plasmids Inc FI and Inc FII, as well as community-acquired ESBL-PE with the enzyme CTX-M-15 at hospitals (18). We must be aware of the genetic subtype of ESBL-PE with the enzyme CTX-M-15 as it can spread swiftly throughout the community.

Ubiquitous co-resistance to fluoroquinolones and to aminoglycosides among ESBL-PE, most probably caused by the carriage of multiple resistance genes in the plasmids coding ESBLs, was also established in our study (19). A significant relationship between \( qnr \) and \( aac-(6)-Ib \) genes and high rates of quinolone and aminoglycoside co-resistance has been identified (20). Four main \( qnr \) genes of \( qnrA \), \( qnrB \), \( qnrC \), and \( qnrS \) are present (21). In the study, 88 patients had a history of previous quinolone consumption within the last 2 months. ESBL-PE was detected in 29 (49.2%) of these patients. In this subset of patients, the likely reason of co-resistance is thought to be the presence of \( qnr \) and \( aac-(6)-Ib \) genes. The study proves that exposure to quinolones is significantly associated with ESBL-PE colonization (odds ratio: 3.5, \( P < 0.001 \), confidence interval: 1.94-6.37). This is one of the reasons why we think \( qnr \) and \( aac-(6)-Ib \) genes need to be studied further. The first study about the presence of \( qnr \) in Turkey was conducted by Gülay et al. in blood culture samples (22). Another study reports \( qnrB1 \) gene of 0.4%, \( aac-(6)-Ib \) gene of 78%, but no \( qnrA \) or \( qnrS \) gene (23).

The study concluded the highest ever prevalence in the fecal carriage rate of ESBL-PE in the literature. CTX-M-15 enzyme is the most common among community-acquired ESBL-PE in Turkey. The cotransmission of \( qnr \) with \( aac-(6)-Ib \) and ESBLs causes the formation of multidrug resistance in Enterobacteriaceae, and these genes permeate the county to a great extent. We believe dissemination of ESBL-PE is associated with uncontrolled antibiotic consumption. Most of the medical laboratories in our country do not screen ESBL production. Appropriate antibiotic therapy and detection of ESBLs in laboratories need to be implemented to
prevent dissemination of these organisms and resistance genes.

Acknowledgments
We thank Theresa Coque for providing control strains for CTX-M subgroups.

References
1. Pitout JD, Nordmann P, Laupland KB, Poirel L. Emergence of Enterobacteriaceae producing extended-spectrum beta-lactamases (ESBLs) in the community. J Antimicrob Chemother. 2005;56:52-9.
2. Woerther PL, Angebault C, Jacquier H, Hugede HC, Janssens AC, Sayadi S, et al. Massive increase, spread, and exchange of extended spectrum beta-lactamase-encoding genes among intestinal Enterobacteriaceae in hospitalized children with severe acute malnutrition in Niger. Clin Infect Dis. 2011;53:677-85.
3. Herindrainy P, Randrianarina F, Ratovoson R, Ratsimia Hariniana E, Buissin Y, Genel N, et al. Rectal carriage of extended-spectrum beta-lactamase-producing gram-negative bacilli in community settings in Madagascar. PLoS One. 2011;6:e22738.
4. Valverde A, Coque TM, Sánchez-Moreno MP, Rollán A, Baquero F, Cantón R. Dramatic increase in prevalence of fecal carriage of extended-spectrum beta-lactamase-producing Enterobacteriaceae during nonoutbreak situations in Spain. J Clin Microbiol. 2004;42:4769-75.
5. Mfinarini LA, Palazzo IC, Darini AL. Prevalence of community-occurring extended spectrum beta-lactamase-producing Enterobacteriaceae in Brazil. Curr Microbiol. 2007;54:335-41.
6. Kurt OA, Karaman S, Togan T. Risk factors for fecal carriage of extended-spectrum beta-lactamase producing Escherichia coli and Klebsiella spp. in the community. Turk J Med Sci. 2007;31:131-8.
7. Coque TM, Novais A, Carattoli A, Poirel L, Pitout J, Peixe L, et al. Dissemination of clonally related Escherichia coli strains expressing extended-spectrum beta-lactamase CTX-M-15. Emerg Infect Dis. 2008;14:195-200.
8. Coque TM, Oliver A, Pérez-Díaz JC, Baquero F, Cantón R. Genes encoding TEM-4, SHV-2, and CTX-M-10 extended-spectrum beta-lactamases are carried by multiple Klebsiella pneumoniae clones in a single hospital (Madrid, 1989 to 2000). Antimicrob Agents Chemother. 2002;46:500-10.
9. Pitout JD, Hossain A, Hanson ND. Phenotypic and molecular detection of CTX-M-beta-lactamases produced by Escherichia coli and Klebsiella spp. J Clin Microbiol. 2004;42:5715-21.
10. Cattoir V PL, Rotimi V, Soussy CJ, Nordmann P. Multiplex PCR for detection of plasmid mediated quinolone resistance qnr genes in ESBL-producing enterobacterial isolates. J Antimicrob Chemother. 2007;60:394-7.
11. Kim HB, Park CH, Kim CJ, Kim EC, Jacoby GA, Hooper DC. Prevalence of plasmid-mediated quinolone resistance determinants over a 9-year period. Antimicrob Agents Chemother. 2009;53:639-45.
12. Ben Sallem R, Ben Slama K, Esteva V, Jouini A, Gharsa H, Klibi N, et al. Prevalence and characterisation of extended-spectrum beta-lactamase (ESBL)-producing Escherichia coli isolates in healthy volunteers in Tunisia. Eur J Clin Microbiol Infect Dis. 2012;31:1511-6.
13. Valverde A, Grill F, Coque TM, Pintado V, Baquero F, Cantón R, et al. High rate of intestinal colonization with extended-spectrum-beta-lactamase-producing organisms in household contacts of infected community patients. J Antimicrob Chemother. 2008;62:1142-9.
14. Rodríguez-Baño J, López-Cerero L, Navarro MD, Díaz de Alba P, Pascual A. Faecal carriage of extended-spectrum beta-lactamase-producing Escherichia coli: Prevalence, risk factors and molecular epidemiology. J Antimicrob Chemother. 2008;62:1142-9.
15. Pitout JD, Hanson ND, Church DL, Laupland KB. Population-based laboratory surveillance for Escherichia coli producing extended-spectrum beta-lactamases: Importance of community isolates with blaCTX-M genes. Clin Infect Dis Off Publ Infect Dis Soc Am. 2004;38:1736-41.
16. Paterson DL, Hujer KM, Hujer AM, Yeiser B, Bonomo MD, Rice LB, et al. Extended-spectrum beta-lactamases in Klebsiella pneumoniae bloodstream isolates from seven countries: Dominance and widespread prevalence of SHV- and CTX-M-type beta-lactamases. Antimicrob Agents Chemother. 2003;47:3554-60.
17. Gur D, Gulyaz Z, Akan OA, Aktas Z, Kayacan CB, Cakici O, et al. Resistance to newer beta-lactams and related ESBL types in gram-negative nosocomial isolates in Turkish hospitals: Results of the multicentre HITIT study. Mikrobiyoloji Bulteni. 2008;42:537-44.
18. Gonullu N, Aktas Z, Kayacan CB, Salcioglu M, Carattoli A, Yong DE, et al. Dissemination of CTX-M-15 beta-lactamase genes carried on Inc FI and FII plasmids among clinical isolates of Escherichia coli in a university hospital in Istanbul, Turkey. J Clin Microbiol. 2008;46:1110-2.
19. Lautenbach E, Fishman NO, Bilker WB, Castiglioni A, Metlay JP, Edelstein PH, et al. Risk factors for fluoroquinolone resistance in nosocomial Escherichia coli and Klebsiella pneumoniae infections. Arch Intern Med. 2002;162:2469-77.
20. Shin SY, Kwon KC, Park JW, Song JH, Ko YH, Sung JY,

Disease and Molecular Medicine - www.dismolmed.org
et al. Characteristics of aac(6’)-Ib-cr gene in extended-spectrum beta-lactamase-producing Escherichia coli and Klebsiella pneumoniae isolated from Chungnam area. Korean J Lab Med. 2009;29:541-50.
21. Jacoby G, Cattoir V, Hooper D, Martínez-Martínez L, Nordmann P, Pascual A, et al. Qnr gene nomenclature. Antimicrob Agents Chemother. 2008;52:2297-9.
22. Oktem IM, Gulay Z, Bicmen M, Gur D. qnrA prevalence in extended-spectrum beta-lactamase-positive Enterobacteriaceae isolates from Turkey. Jpn J Infect Dis. 2008;61:13-7.
23. Poirel L, DGu L, Minarini U, Arslan P. Nordmann on Behalf of the Turkish Study Group on ESBL/Qnr Relationships. London: 18th ECCMID/26th ICC, Posters; 2008.