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HORIZONTOL DISSEMINATION OF TEM- AND SHV-TYPE BETA-LACTAMASE GENES-CARRYING RESISTANCE PLASMIDS AMONGST CLINICAL ISOLATES OF *ENTEROBACTERIACEAE*

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ABSTRACT

The extended-spectrum β-lactamase (ESBL)-producing bacteria have been isolated at increasing frequency worldwide. Expression of ESBL is often associated with multidrug resistance and dissemination by resistance plasmids. During a two-month period in 2000, 133 clinical isolates of enterobacterial strains were randomly collected from outpatients and inpatients at a university hospital in Turkey. The ESBL producing strains were determined by double-disk synergy (DDS) testing. Twenty ESBL producing strains (15%) including *Escherichia coli* (n = 9), *Klebsiella pneumoniae* (n = 7), *Klebsiella oxytoca* (n = 2) and *Enterobacter aerogenes* (n = 2) were detected and further analyzed for their resistance transfer features, plasmid profile and nature of the resistance genes. Plasmid transfer assays were performed using broth mating techniques. TEM- and SHV- genes were analyzed by polymerase chain reaction (PCR) and hybridization using specific probes. *Eco*RI restriction enzyme analyses of R plasmids were used in the detection of epidemic plasmids. Fourteen plasmid profiles (A, B1, B2, C1, and C2 to L) were obtained with *Eco*RI restriction enzyme analysis. Most of these plasmids were detected to carry both TEM- and SHV-derived genes by PCR, and confirmed by localizing each gene by hybridization assay. Epidemiological evidence indicated that there was an apparent horizontal dissemination of conjugative R plasmids among multidrug-resistant enterobacterial genera and species in this hospital.

Key words: *Enterobacteriaceae*, horizontal spread, resistance plasmids, ESBL.

INTRODUCTION

Beta-lactam agents including penicillins, cephalosporins, monobactams and carbapenems are among the most frequently prescribed antibiotics worldwide (23). Bacterial resistance to β-lactam antibiotics is increasing throughout the world, mainly through the spread of plasmid-encoded extended-spectrum β-lactamases (ESBLs) (4). ESBLs were first recovered in *Klebsiella pneumoniae* and have spread to different genera of *Enterobacteriaceae*, which cause serious therapeutic problems in most developed and developing countries (17). Increasing rates of ESBL-producing *Enterobacteriaceae* isolates from either the community or the hospital setting have been reported (26).

After oxyimino-β-lactams came into clinical use, resistance to these agents through ESBLs appeared in *K. pneumoniae*, *Escherichia coli* strains and other genera. These pathogens have been shown to transfer this resistance to other bacteria via multi-resistant plasmids (16). Therefore, the ESBL genes are usually located on large conjugative plasmids, often carrying genes conferring resistance to aminoglycosides (19). Some are located within transposable elements which strongly facilitate their spread between DNA replicons, even between bacterial strains of different species (4,15). Resistance plasmids are transferred between bacterial strains by conjugation mechanism. The ability of resistance plasmids to transfer among different bacterial species by conjugation is of clinical importance, since

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they contribute to increased spread of antibiotic resistance in hospitalized patients (9).

Some authors have reported major single-strain outbreaks (12,24), whereas others emphasized the importance of plasmid transfer rather than the strain spread (3). The current study demonstrates that the R plasmids transferring and spreading the multidrug-resistance between different enterobacterial genera and species isolated from both hospitalized patients and outpatients in a 600-bed university hospital.

MATERIALS AND METHODS

Patients and bacterial strains

One hundred and thirty-three enterobacterial Gram negative clinical isolates were randomly recovered from the various clinical specimens (including urine, tracheal aspirate, blood or cerebrospinal fluid) of the patients suffering from various infectious diseases. The samples obtained from the patients who admitted mainly to pediatrics and, intensive care units, surgical services, and the outpatient policlinics during a two-month interval from June to July 2000 at the 600-bed teaching hospital of Karadeniz Technical University, Trabzon, Turkey. Of 133 enterobacterial isolates, ESBL producing 20 strains, *E. coli* (n = 9), *K. pneumoniae* (n = 7), *K. oxytoca* (n = 2) and *Enterobacter aerogenes* (n = 2) were selected for further epidemiological study. All strains were identified at the species level using the Sceptor System (Becton-Dickinson Microbiology Systems, Sparks, MD, USA). The ESBL-producing clinical isolates were used as donors and *E. coli* K-12 strain J53-2 (F^- *met pro* Rif^s) as recipient in conjugation experiments. Plasmid DNA purified from *E. coli* strain V517 was used as plasmid marker (21). *E. coli* ATCC 25922 was used as control in susceptibility testing. *E. coli* 7604 and *E. coli* J53-2 strains served as TEM-1 and SHV-3 type of β-lactamase (carried on plasmid pUD18) positive controls, respectively, for double-disk synergy (DDS) testing and PCR assays.

Antimicrobial susceptibility testing

The susceptibility tests of the clinical strains were carried out by the Sceptor System (Becton-Dickinson Microbiology Systems, Sparks, MD, USA). Results were expressed as resistant (R), intermediate (I) or susceptible (S). Susceptibility of the transconjugants to the antibiotics was determined by standard disk diffusion method according to the Clinical and Laboratory Standards Institute (CLSI) guidelines (6). The following antibiotic disks (Oxoid, England) were used in susceptibility testing of the transconjugants: amoxicillin/clavulanate (10 μg/10 μg), cefazolin (30 μg), cefuroxime (30 μg), ceftazidime (30 μg), cefotaxime (30 μg); ceftriaxone (30 μg), cefepime (30 μg), aztreonam (30 μg), gentamicin (10 μg), tetracycline (30 μg) and trimethoprim-sulfamethoxazole (1.25 μg/23.75 μg).

Clinical isolates and the transconjugants were screened for ESBL production according to the CLSI criteria (6), and confirmed by the double-disk synergy (DDS) tests on Mueller-Hinton agar (Difco, USA) as described by Jarlier and colleagues (18).

Transfer of resistance and plasmid analysis

Conjugation assays were performed by broth mating method. Briefly, donor (the ESBL-producing clinical isolates) and recipient (*E. coli* J53-2) cells grown with agitation in Luria Bertani (LB) broth (1% tryptone, 0.5% yeast extract, 0.5% NaCl, pH 7.4) were mixed and incubated for 18 h at 35ºC. Transconjugants were selected on Methylene Blue agar (Oxoid, England) supplemented with 150 μg/mL of rifampin (Hoecst, Germany) and 100 μg/mL of ampicillin (Fisher Scientific, USA). The frequency of transfer was expressed relative to the number of donor cells.

Plasmid DNA was purified from clinical isolates and *E. coli* transconjugants by alkaline lysis method as previously described by Manniatis *et al*. (22), and digested with *Eco*R1 restriction endonuclease (MBI Fermentas, USA). Digested DNA fragments were separated by agarose gel electrophoresis, stained with 0.5μg/mL of ethidium bromide (Sigma, USA) and visualized with ultraviolet light. The approximate sizes of the large plasmids were estimated by comparison to plasmid marker (*E. coli* V517) (21) and confirmed by adding up the restriction fragments.

PCR, Southern blotting and Hybridization assay

The TEM and SHV type β-lactamase genes were amplified using the intragenic oligonucleotide primers as previously described by Arlet and Philippon (1). The primers used for bla_{TEM} were; OT1 (5'-TTGGGTGCACGAGTGGGTTA-3') and OT2 (5'-TAATTGTTGCCGGGAAGCTA-3'), to amplify a 504-bp fragment, and for bla_{SHV} genes, primers OS1 (5'-TCGGG CCGCGTAGGCATGAT-3' and OS2 (5'-AGCAGGGCGAC AATCCCGCG-3') were used to amplify a 626-bp fragment. The PCR products were analyzed by agarose (2%) gel electrophoresis and visualized under ultraviolet light.

Two sets of agarose gels containing *Eco*RI-digested plasmid DNA fragments were transferred onto two separate nylon membranes (27). The TEM- and SHV-specific probes were generated with PCR using the intergenic primers and plasmid templates harboring bla_{TEM} (pUC18) and bla_{SHV} (pUD18) genes, respectively. The probes were separately labeled with digoxygenin as described by the manufacturer (Bohringer-Mannheim, Germany) and hybridized under high stringency conditions at 68ºC.

RESULTS

Antimicrobial susceptibility of isolates

Prevalence and distribution of clinical isolates of enterobacteria emerging in different hospital units are shown in Table 1. *Escherichia coli* was the most commonly isolated

Table 1. Prevalence and distribution of clinical isolates of enterobacteria emerging in different units of the hospital in a two month interval.

Isolate	No. of strains[1]						Total
	June 2000[2]			July 2000[2]			
	Inpatient ward	Outpatient policlinic	ICU	Inpatient ward	Outpatient policlinic	ICU	
Escherichia coli	10(1)	23(3)	2(1)	8(2)	16(1)	2(1)	61(9)
Klebsiella pneumoniae	5(3)	5(1)	1(1)	4	3(1)	2(1)	20(7)
Klebsiella oxytoca	6(2)	3	-	2	1	-	12(2)
Klebsiella ozaenae	5	2	-	2	1	-	10
Enterobacter aerogenes	2	2(1)	1	4	1(1)	-	10(2)
Enterobacter cloacae	-	3	-	-	1	-	4
Enterobacter sakazakii	-	-	1	1	-	-	2
Enterobacter agglomerans	-	-	-	1	-	-	1
Proteus mirabilis	-	5	-	1	3	-	9
Proteus vulgaris	-	1	-	-	1	-	2
Serratia marcescens	1	-	1	-	-	-	2
Total	29(6)	44(5)	6(2)	23(2)	27(3)	4(2)	133(20)
% of ESBL-producer		16.4			12.9		15.0

[1] Parentheses indicate number of ESBL-producing strains.
[2] ICU, intensive care unit.

pathogen followed by *Klebsiella* spp. and *Enterobacter* spp. Antimicrobial susceptibility profiles and β-lactamase types of ESBL-producing isolates are summarized in Table 2. The susceptibility results indicated that nearly all isolates were resistant to piperacillin but susceptible to imipenem, cefotetan and amikacin. Seven isolates were resistant to nitrofurantoin whereas all isolates except for *Enterobacter aerogenes* strain TRE164 were susceptible to ciprofloxacin. The only effective antimicrobial among the non-β-lactam antibiotics to all strains seems to be an aminoglycoside antibiotic, amikacin.

ESBL production and plasmid transfer

Twenty of the 133 isolates, which represents 15% of the enterobacterial strains, produced ESBL and showed a typical positive synergy test between cefotaxime, ceftazidime, aztreonam or cefepime and clavulanic acid, but were susceptible to imipenem and cefotetan, consistent with an ESBL production. The ESBL-gene carrying strains represented with a ratio of 16.4% and 12.9% in the months June and July, respectively (Table 1). All ESBL-producing strains including 10 *E. coli*, 6 *K. pneumoniae*, 2 *K. oxytoca* and 2 *E. aerogenes* were able to transfer their ESBL genes to a recipient *E. coli* strain J53-2 by conjugation at a transfer frequency of $5x10^{-8}$ to 10^{-4}. Co-transfer of gentamicin, tetracycline or trimethoprim/sulfamethoxazole resistance occurred in three cases (Table 3). Most of the transconjugants showed similar antibiotic resistance profile as donors.

Isolates carrying ESBL determinants were obtained most frequently from urine samples in both hospital and community patients, six inpatients and seven outpatients (Table 2). The remaining samples were obtained from respiratory tract infections (four inpatients), bloodstream infections (one inpatient), and cerebrospinal infections (one inpatient). Among the hospitalized patients, half of the ESBL-producing isolates were detected in pediatric-associated wards (Table 2).

PCR detection of *bla*$_{TEM}$ *and bla*$_{SHV}$ genes, plasmid profile and hybridization

Multiple β-lactamase genes were identified in 20 strains by TEM- and SHV-specific PCR. Five strains contained single β-lactamase gene, *bla*$_{SHV}$. Twelve strains harbored both *bla*$_{TEM}$ and *bla*$_{SHV}$ genes whereas three strains had neither *bla*$_{TEM}$ nor *bla*$_{SHV}$ genes (Table 2). The sizes of the plasmids recovered from the transconjugants ranged from about 7 to over 80 kb (Fig. 1 and Table 3).

The conjugative plasmids showed 14 different *Eco*RI restriction enzyme profiles (A, B1, B2, C1, C2, D, E, F, G, H, I, J, K, and L) (Fig. 2A). The first seven profiles (A to E) were harbored by more than one strain, confirming them to be epidemic plasmids, and the others (F to L) were determined as nonepidemic as they depicted different digestion patterns and sporadic emergence.

Two *K. pneumoniae* isolates, TRE025 and TRE037, were cultured from tracheal aspirate from two unrelated patients. One

Table 2. Epidemiological characteristics and antimicrobial susceptibilities of ESBL producing *Enterobacteriaceae* isolates.

Isolate	Species[1]	Date isolated (m/d/y)	Sample[2]	Unit[3]	*bla* gene[4]	Result of susceptibility test by automated system[5]												
						PIP	AMC	CAZ	CTX	ATM	CTT	IPM	CIP	F	CN	AK	TOB	TE	SXT
TRE025	Kpneu.	06/13/00	TA	P-ICU	TEM, SHV	R	R	R	R	R	S	S	S	S	R	S	R	R	R
TRE037	Kpneu.	06/16/00	U	P	TEM, SHV	R	I	R	I	R	S	S	S	S	S	S	R	R	R
TRE040	Kpneu.	06/20/00	U	P	TEM, SHV	R	R	R	I	S	S	S	S	S	S	S	S	S	R
TRE044	Kpneu.	06/20/00	U	P	TEM, SHV	R	I	R	R	R	S	S	S	S	R	S	R	R	R
TRE056	E. coli	06/21/00	U	PP	Neither	ND	S	R	R	ND	S	S	S	S	S	S	S	R	R
TRE063	Koxy.	06/22/00	U	P	TEM, SHV	ND	I	R	I	R	S	S	S	S	R	S	R	S	R
TRE066	E. coli	06/23/00	U	IM	SHV	R	I	R	I	R	S	S	S	S	S	S	S	R	R
TRE073	E. coli	06/26/00	B	P-ICU	SHV	R	R	R	R	R	S	S	S	S	R	S	I	R	R
TRE082	Koxy	06/26/00	U	P	TEM, SHV	R	R	R	R	R	S	S	S	S	R	S	R	I	R
TRE083	Kpneu.	06/26/00	U	PP	Neither	R	S	ND	ND	R	S	S	S	S	R	S	R	S	S
TRE088	Eaero.	06/27/00	U	PP	TEM, SHV	ND	R	ND	ND	R	S	S	S	R	R	S	S	I	S
TRE090	E. coli	06/27/00	U	PP	TEM, SHV	ND	S	ND	ND	R	S	S	S	I	R	S	S	R	S
TRE092	E. coli	06/27/00	U	PP	Neither	R	S	R	S	R	S	S	S	S	S	S	S	R	R
TRE146	Kpneu.	07/10/00	U	PP	SHV	R	R	R	S	S	S	S	S	S	S	S	S	R	R
TRE153	E. coli	07/12/00	TA	P-ICU	TEM, SHV	R	R	R	R	R	S	S	S	R	R	S	R	R	R
TRE154	E. coli	07/12/00	CSF	P	TEM, SHV	R	R	R	R	R	S	S	S	R	S	S	S	R	S
TRE157	E. coli	07/13/00	U	PP	SHV	R	R	R	R	S	S	S	S	R	S	S	S	I	S
TRE160	E. coli	07/17/00	U	P	SHV	ND	R	S	S	S	S	S	S	I	S	S	S	S	R
TRE164	Eaero.	07/18/00	TA	ID	TEM, SHV	R	R	R	I	R	S	S	R	I	R	S	R	S	R
TRE165	Kpneu.	07/18/00	TA	P-ICU	TEM, SHV	R	I	R	R	R	S	S	S	S	R	S	R	R	R

[1] E. coli, *Escherichia coli*; Kpneu., *Klebsiella pneumoniae*, Koxy., *Klebsiella oxytoca*; Eaero., *Enterobacter aerogenes*.
[2] TA, tracheal aspirate; U, urine; B, blood; CSF, cerebrospinal fluid.
[3] P-ICU, pediatrics intensive care unit; P, pediatrics ward; PP, pediatrics outpatient policlinic; IM, internal medicine ward; ID, infectious diseases ward.
[4] *bla*, β-lactamase gene.
[5] PIP, piperacillin; AMC, amoxicillin/clavulanate; CAZ, ceftazidime; CTX, cefotaxime; ATM, aztreonam; CTT, cefotetan; IPM, imipenem; CIP, ciprofloxacin; F, nitrofurantoin; CN, gentamicin; AK, amikacin; TOB, tobramycin; TE, tetracycline; SXT, trimethoprim/sulfamethoxazole; ND, not done; R, resistant; I, intermediate; S, susceptible.

of the samples was recovered from a patient who suffered from a respiratory infection in pediatrics-intensive care unit (P-ICU) in July, while the other one was from the urine sample of a patient with urinary tract infection (UTI) in a pediatric surgical ward in June. *Eco*RI restriction analyses showed that these strains harbored two very similar plasmids (Fig 1, profile A).

E. aerogenes TRE088 and *E. coli* TRE090 strains were isolated from urine specimens of two unrelated outpatients, both with UTI, in June. Both strains harbored two very similar plasmids (Fig. 1, profile B2). Similarly, *K. oxytoca* TRE063 and *E. aerogenes* TRE164 strains were isolated from urine samples of a patient with UTI in the pediatric surgical ward in June, and from tracheal aspirate of another patient with respiratory infection in the infectious diseases ward in July, respectively. They carried profile B1 plasmids. The *Eco*RI restriction pattern of profile B1 plasmid consisted of eight fragments. An additional fragment was observed in B2 profile which indicates the presence of the additional small plasmid (Fig. 1 and Fig 2A). *E. coli* TRE153 and *K. pneumoniae* TRE044 strains were isolated from tracheal aspirate of a patient with lung infection in P-ICU in June, and from urine of another patient with UTI in the infant ward in July, respectively. These two different bacteria harbored a large conjugative plasmid with about 87 kb in size (Fig. 1, profile C2).

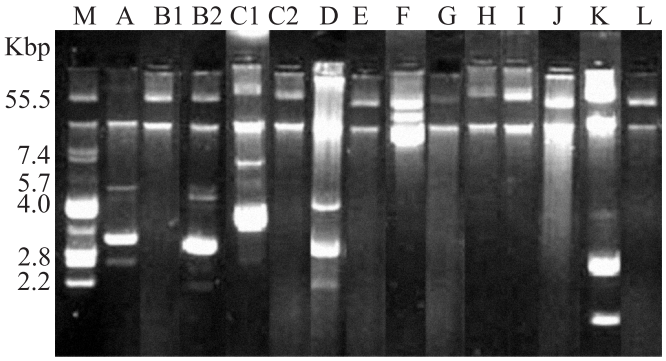

Figure 1. Agarose gel electrophoresis of R plasmids purified from the R^+ transconjugants. M, plasmid marker (*E. coli* V517); A to E, epidemic plasmids; F to L, nonepidemic plasmids.

Table 3. Plasmid and resistance profile of ransconjugants.

Transconjugant	Size (kbp) of plasmid(s)	Plasmid profile	Selected resistance phenotype[1]	Presence of ESBL	*bla* gene	Frequency of transfer (cfu/recipient)
R^+ (pTRE025)	73, 3	A	CAZ, CTX, ATM	Yes	TEM, SHV	$5x10^{-8}$
R^+ (pTRE037)	73, 3	A	CAZ, CTX, ATM	Yes	TEM, SHV	10^{-8}
R^+ (pTRE063)	70.3	B1	CAZ, ATM, CN	Yes	TEM, SHV	10^{-7}
R^+ (pTRE164)	70.3	B1	CAZ, ATM, CN	Yes	TEM, SHV	$4x10^{-7}$
R^+ (pTRE090)	70.3, 3	B2	CAZ, ATM, CN	Yes	TEM, SHV	10^{-7}
R^+ (pTRE088)	70.3, 3	B2	CAZ, ATM, CN	Yes	TEM, SHV	10^{-7}
R^+ (pTRE165)	87, 4	C1	AMC, CAZ, CTX, ATM, CN, TE	Yes	TEM, SHV	$7x10^{-7}$
R^+ (pTRE044)	87	C2	AMC, CAZ, CTX, ATM, CN, TE	Yes	TEM, SHV	10^{-7}
R^+ (pTRE153)	87	C2	AMC, CAZ, CTX, ATM, CN, TE	Yes	TEM, SHV	10^{-7}
R^+ (pTRE083)	6.7	D	CAZ, ATM	Yes	Neither	$3x0^{-7}$
R^+ (pTRE092)	6.7	D	CAZ, ATM	Yes	Neither	$5x10^{-7}$
R^+ (pTRE040)	27.4	E	AMC, CAZ, CTX, SXT	Yes	SHV	10^{-7}
R^+ (pTRE073)	27.4	E	AMC, CAZ, CTX, SXT	Yes	SHV	10^{-8}
R^+ (pTRE154)	48.5	F	CAZ, CTX, ATM	Yes	TEM, SHV	10^{-7}
R^+ (pTRE082)	39	G	AMC, CAZ, CTX, ATM, CN, SXT	Yes	TEM, SHV	10^{-8}
R^+ (pTRE146)	58.6	H	AMC, CAZ, SXT	Yes	SHV	$4x10^{-5}$
R^+ (pTRE160)	62.5	I	AMC, CAZ	Yes	SHV	10^{-4}
R^+ (pTRE066)	53.3	J	CAZ	Yes	SHV	$6x10^{-7}$
R^+ (pTRE157)	73	K	AMC, CAZ	Yes	SHV	$3x10^{-6}$
R^+ (pTRE056)	30	L	CTX, CAZ	Yes	Neither	10^{-6}

[1] For abbreviations, see footnote to Table 2.

Surprisingly, *K. pneumoniae* TRE165 isolated from tracheal aspirate of a patient with respiratory infection from P-ICU carried a small plasmid in addition to a large plasmid of about 87 kb (Fig. 1, profile C1). Plasmid profile C1 consists of seven fragments, one of which belongs to the additional small plasmid (Fig. 1 and Fig. 2A). Remaining plasmid profiles (F to L) were determined as nonepidemic plasmids due to different restriction fragment patterns.

The presence of TEM- or SHV-derived β-lactamase genes was also confirmed by hybridization of *Eco*RI restriction digested conjugative plasmids with $bla_{\text{TEM-1}}$ (Fig. 2B) and $bla_{\text{SHV-3}}$-specific probes (Fig. 2C). Both probes hybridized to both epidemic and non-epidemic plasmids digested with *Eco*RI enzyme.

DISCUSSION

Extended-spectrum β-lactamase-producing organisms can be introduced into ICUs. Epidemics of infection from ICUs to other parts of the hospital have been well documented (13). It has been reported that most enterobacteria are responsible for the dissemination of multidrug resistance plasmids in the hospital environment (11). Plasmid-encoded ESBLs often contain resistance determinants for other classes of antimicrobial agents and are readily transmissible from strain-to-strain and between different species of enteric Gram-negative bacilli (8,12). During two months, ESBL-producing enterobacterial genera were mainly isolated from different patients hospitalized in the P-ICU, infant ward and from the pediatrics outpatient policlinics. The patients hospitalized in pediatrics-associated wards were at increased risk of infection because of young age and prolonged period of hospitalization involving various invasive therapeutic and diagnostic procedures. Such factors have been reported to contribute to infection caused by ESBL-producing bacteria (23). Several epidemics have been detected among neonates, elderly patients and even outpatients (2).

The same plasmid in *E. coli*, *K. oxytoca* or *E. aerogenes* strains of unrelated origin is an important finding in regards to the possibility of horizontal spread of the resistance plasmid from one unit to entire hospital. Some ESBL outbreaks have been attributed to the dissemination of plasmids among strains of the family *Enterobacteriaceae* (19). In other case, spread of a given ESBL in a single environment has been reported due to the occurrence of the same gene within unrelated plasmids (30). Although plasmid dissemination has been well documented (2,9), the spread of an epidemic strain remains the most

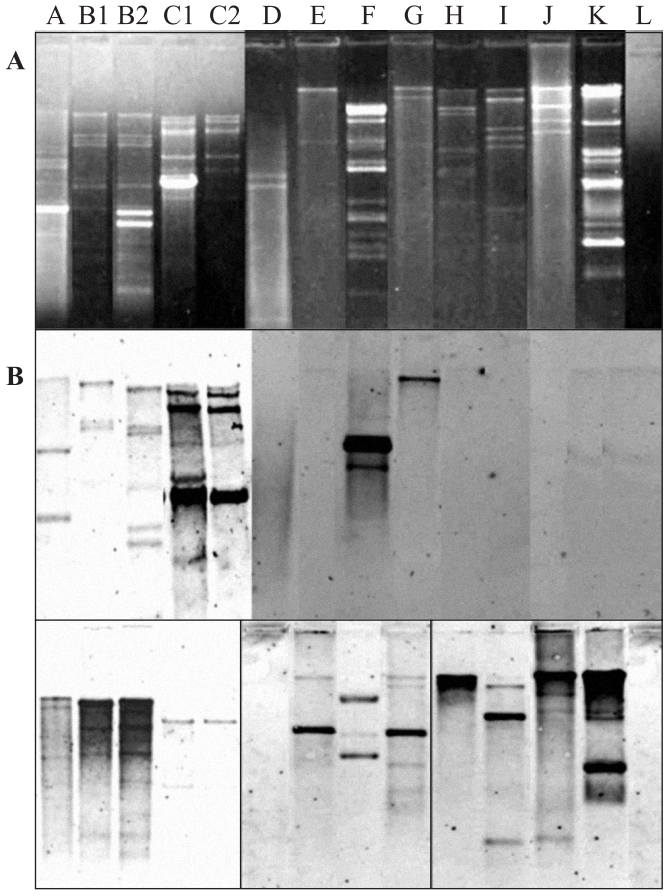

Figure 2. *Eco*RI-digested epidemic (A to E) and non epidemic (F to L) R plasmids (A); hybridized with bla_{TEM-1} probe (B); and with bla_{SHV-3} probe (C).

commonly reported mechanism of ESBL dissemination (13). In this study, detection of very similar, if not identical, R plasmids carried by different enterobacterial genera, isolated from different patients in different times, indicates that the plasmid transfer is quite frequent in the hospital environment.

Discovery of novel transposons encoding an ESBL on a self-transmissible plasmid in a hospital where large quantities of extended-spectrum β-lactam antibiotics are used raises concern for the increased spread of resistance to these groups of antibiotics (15). According to the hospital records regarding β-lactam use in a five month interval in the year 2000 (involving the period of present study), the amount of amoxicillin/clavulanate, ceftazidime, cefotaxime, ceftriaxone, cefepime and aztreonam uses were ca. 0.234, 0.065, 0.008, 0.036, 0.239, 0.053 and 0.002 kg/day/all wards, respectively (data not shown). It is likely that excessive use of antimicrobials in the healthcare environment resulted in the selective increase of R plasmid bearing isolates belonging to different genera of *Enterobacteriaceae*. It has been reported that constant selective pressure exerted by β-lactams is a risk factor for selection of resistant ESBL-producing strains (14). Similarly, it was also confirmed that genetically different strains could acquire the same plasmid(s), either randomly or specifically due to antibiotic therapy, thus leading to plasmid epidemics (25).

TEM- or SHV-gene carrying isolates were detected by PCR and confirmed the genes localizing on the conjugative plasmids by hybridization assays. Detection of ESBL genes should be confirmed only by sequencing of the PCR products because the genes detected by PCR or hybridization could be the non-ESBL variants TEM-1, TEM-2 or SHV-1. SHV β-lactamase genes are actually present in all *K. pneumoniae* strains, but once they were transferred by conjugation, and the transconjugant isolates were resistant to extended spectrum β-lactam antibiotics, these β-lactamases should be indeed ESBLs. However, all families of β-lactamases should be probed for the epidemiological importance of β-lactamase genes in Europe, especially the OXA (7) and PER (29) family of enzymes that have been prevalent at other sites in Turkey. Recently, Tasli and Bahar (28) reported that 52.7% TEM, 74.3% SHV, and 32.4% TEM and SHV genes in clinical isolates of *Enterobacteriaceae* were determined by PCR from western Turkey, and TEM-1, SHV-2, SHV-5 and SHV-12 type enzymes were found to be predominant.

The suitable β-lactam choice seems to be imipenem in treatment of serious infectious diseases caused by enterobacteria in our hospital. However, it is known that the heavy use of carbapenems may favor the selection of *Stenotrophomonas maltophilia* which is naturally resistant to these drugs (20). Moreover, epidemiological typing of this nonfermenting Gram-negative organism revealed that three small outbreaks occurred from June 2000 to December 2001 in this hospital (5).

The presence of a plasmid-mediated resistance to extended-spectrum β-lactams and to aminoglycosides and capable of intra- and inter-generic spread among enterobacteria are the most important part of our experience in this hospital.

In conclusion, the results of the epidemiological analysis reported here reflect that the multidrug-resistant bacteria can continuously prompt to disseminate antibiotic resistance genes to other clinical pathogens under the constant selective pressure arising from the excess β-lactam usage in the hospital unless effective and strict precautions are taken. Antibiotic policies that restrict the use of cephalosporins, as well as the aminoglycosides, even though the strains were susceptible to amikacin, may be required in addition to infection control policies in this hospital.

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RESUMO

Disseminação horizontal de plasmídios de resistência contendo genes de beta-lactamase dos tipos TEM e SHV entre isolados clínicos de *Enterobacteriaceae*

O isolamento de bactérias produtoras de beta-lactamases de espectro expandido (ESBL) está aumentando no mundo todo. Freqüentemente, a expressão de ESBL está associada com resistência a múltiplas drogas e disseminação por plasmídios de resistência. Durante um período de dois meses em 2000, 133 isolados clínicos de cepas de enterobactérias foram obtidos aleatoriamente de pacientes internos e externos de um hospital universitário na Turquia. As cepas produtoras de ESBL foram identificadas pelo teste de sinergia em disco-duplo (DDS). Foram detectadas vinte cepas produtoras de ESBL, entre as quais Escherichia coli (n=9), Klebsiella pneumoniae (n=7), Klebsiella oxytoca (n=2) e Enterobacter aerogenes (n=2), que foram posteriormente analisadas quanto a suas características de transferência de resistência, perfil plasmidial e natureza dos genes de resistência. Os testes de transferência de plasmídios foram realizados empregando técnicas de conjugação em caldo. Os genes TEM e SHV foram analisados pela reação da polimerase em cadeia (PCR) e hibridização com sondas especificas. A detecção de plasmídios epidêmicos foi feita por análise dos plasmídios R com a enzima de restrição *Eco*RI. Através desta análise, foram obtidos catorze perfis plasmidiais (A, B1, B2, C1 e C2 até L).Observou-se pela PCR que a maioria dos plasmidios carregavam genes derivados de TEM e SHV, confirmados através da detecção dos genes pelos testes de hibridização. As evidencias epidemiológicas indicaram que havia uma aparente transferência horizontal dos plasmídios R conjugativos entre as enterobactérias multiresistentes neste hospital.

Palavras-chave: *Enterobacteriaceae,* transferência horizontal, ESBL, plasmídios de resistência

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