ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Oct. 2004, p. 3996–4001 Vol. 48, No. 10
0066-4804/04/$08.00+0 DOI: 10.1128/AAC.48.10.3996–4001.2004

NOTES

Mechanisms of Resistance in Multiple-Antibiotic-Resistant *Escherichia coli* Strains of Human, Animal, and Food Origins

Yolanda Sáenz,[1] Laura Briñas,[1] Elena Domínguez,[1] Joaquim Ruiz,[2] Myriam Zarazaga,[1] Jordi Vila,[2] and Carmen Torres*[1]

Área Bioquímica y Biología Molecular, Universidad de La Rioja, Logroño,[1] and Servei de Microbiología, Institut Clínic d'Infeccions i Immunología, IDIBAPS, Hospital Clínic, Facultat de Medicina, Universitat de Barcelona, Barcelona,[2] Spain

Received 3 March 2004/Returned for modification 21 April 2004/Accepted 1 June 2004

Seventeen multiple-antibiotic-resistant nonpathogenic *Escherichia coli* strains of human, animal, and food origins showed a wide variety of antibiotic resistance genes, many of them carried by class 1 and class 2 integrons. Amino acid changes in MarR and mutations in *marO* were identified for 15 and 14 *E. coli* strains, respectively.

The emergence of *Escherichia coli* isolates with multiple-antibiotic-resistant phenotypes, involving coresistance to four or more unrelated families of antibiotics, has been previously reported and is considered a serious health concern (2, 5, 22). Transference of resistance determinants by mobile genetic elements including plasmids, transposons, and gene cassettes in integrons (4, 13) and the alteration in *mar* locus regulation (1, 2, 27) are important factors that can contribute to the increase in multiresistant bacteria.

In previous studies (7, 34), the antibiotic resistance profiles of 515 nonpathogenic *E. coli* isolates obtained from food products of animal origin ($n = 47$) and from fecal samples of healthy animals ($n = 177$) and humans ($n = 291$) were studied. Seventeen *E. coli* isolates from those groups (four from food, eight from animals, and five from humans) showed a multiple-antibiotic-resistant phenotype (resistance to nalidixic acid, ampicillin, rifampin, chloramphenicol, sulfamethoxazole, streptomycin [STR] and tetracycline). All 17 of these isolates were used in the present work to detect different mechanisms of antibiotic resistance and to study the antibiotic resistance genes inside integrons and the relevance of the *mar* locus in the multiple-antibiotic-resistant phenotype.

Additional susceptibilities to ciprofloxacin, amoxicillin-clavulanic acid, cefazolin, cefoxitin, ceftazidime, cefotaxime, ceftriaxone, imipenem, aztreonam, gentamicin (GEN), apramycin, tobramycin, kanamycin, and trimethoprim were determined by an agar dilution method (24).

The 17 *E. coli* isolates showed 16 unrelated pulsed-field gel electrophoresis (PFGE) patterns with the XbaI enzyme in experiments following the method of Gautom (9) (Fig. 1). Only strains Co71 and Co82 showed closely related patterns.

Analysis of antibiotic resistance mechanisms. The presence of antibiotic resistance genes in the 17 *E. coli* strains was analyzed by PCR, PCR-restriction fragment length polymorphism analysis, and sequencing (Table 1). Table 2 shows the resistance phenotypes and genes identified.

All strains were ampicillin resistant, and for eight of them, the minimal inhibitory concentration (MIC) of amoxicillin-clavulanic acid indicated intermediate resistance; no strain was resistant to the remaining β-lactams studied. The $bla_{\text{TEM-1a}}$ and $bla_{\text{TEM-1b}}$ genes were identified in 2 and 15 strains, respectively, whereas none of the bla_{SHV} and bla_{OXA} genes were found.

Four strains in which the *aac3-II* (found in one strain from a broiler) or *aac3-IV* gene (found in three strains from humans) was found were GEN resistant. The AAC(3)-IV enzyme modifies apramycin in addition to GEN. Apramycin is used exclusively in veterinary medicine, but the GEN-related chemical structure and the mobility of the *aac3-IV* gene inside plasmids may have contributed to the selection and dissemination of these strains in a human environment (17).

Eight of the seventeen *E. coli* strains were kanamycin resistant, and the *aphA1* and *aphA2* genes were detected in three and six strains, respectively. Both genes were found in one strain of food origin.

The following *aadA* genes were detected in 16 of the 17 STR-resistant strains: *aadA1* was found in 12 strains, *aadA2* was found in 5, and *aadA5* was found in 3 strains. The *aadA1* and *aadA2* genes were found together in four strains (two from pigs and two from humans). No STR resistance mechanism was detected in the Co53 strain, in which case other mechanisms of STR resistance, such as the production of APH(3′′)-I or APH(6)-I phosphoryltransferases (15, 35), cannot be excluded.

* Corresponding author. Mailing address: Área Bioquímica y Biología Molecular, Universidad de La Rioja, Madre de Dios 51, 26006 Logroño, Spain. Phone: 34-941-299750. Fax: 34-941-299721. E-mail: carmen.torres@daa.unirioja.es.

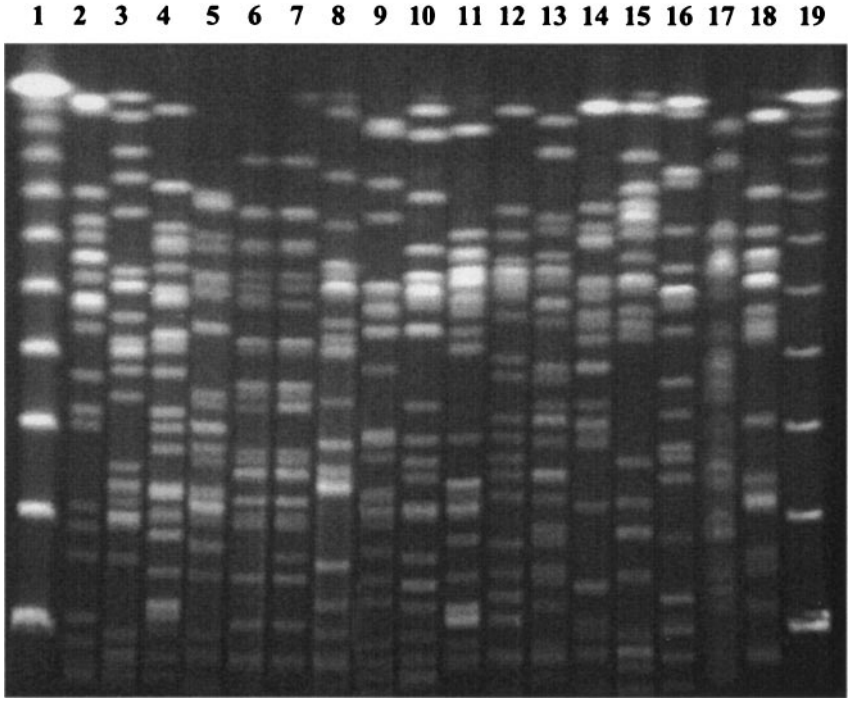

FIG. 1. PFGE patterns of XbaI-digested genomic DNA of the 17 multiresistant *E. coli* strains. Lanes: 1, λ ladder molecular size marker; 2, *E. coli* Co1; 3, *E. coli* Co19; 4, *E. coli* Co45; 5, *E. coli* Co53; 6, *E. coli* Co71; 7, *E. coli* Co82; 8, *E. coli* Co80; 9, *E. coli* Co110; 10, *E. coli* Co125; 11, *E. coli* Co177; 12, *E. coli* Co201; 13, *E. coli* Co227; 14, *E. coli* Co228; 15, *E. coli* Co232; 16, *E. coli* Co279; 17, *E. coli* Co354; 18, *E. coli* Co356; 19, λ ladder molecular size marker.

The *tetA* or *tetB* gene was found in all the strains (*tetA* was found in nine, and *tetB* was found in eight strains). No *tetC*, *tetD*, or *tetE* genes were detected. Chloramphenicol-acetyltransferase activity was demonstrated as previously described (8) in the seven strains for which the MICs of chloramphenicol were highest (≥128 μg/ml) (Table 2). The *cmlA* gene was detected in five additional strains (MICs of chloramphenicol, 32 to 64 μg/ml), while the *floR* gene was not found.

Fifteen *E. coli* strains were trimethoprim resistant, and the following *dfr* genes were identified by PCR-restriction fragment length polymorphism analysis (25): *dfrA1* was found in seven strains, *dfrA1* plus *dfrA12* was found in two, *dfrA1*-like plus *dfrA12* was found in one strain, *dfrA17* was found in three, and *dfrA12* was found in two strains. A new type of *dfrA* gene, called *dfrA1*-like, was found in the Co125 strain. Sequencing of the Co125 amplicon indicated a deduced amino acid substitution, Asn134Asp, in contrast to DHFRIa (Swiss-Prot accession number P00382).

The *sul1* and *sul2* genes were detected in 11 and 12 strains, respectively, and 8 of those strains showed both genes. These findings are in agreement with the high prevalence of these genes found in *Enterobacteriaceae* (18, 22). The *sul3* gene has recently been found in pathogenic *E. coli* isolates (10, 12, 29), being detected in six of our strains (one from a broiler, two from pigs, and three from humans). The mechanisms of quinolone resistance had been previously analyzed in these 17 strains (7, 33).

Integron analysis. Class 1 and class 2 integrons, the most frequently found in resistant bacteria (14, 23, 31, 32), were analyzed in all our strains. PCR amplification was used to detect class 1 and class 2 integrase genes, *intI1* and *intI2*, respectively, and the *qacEΔ1* gene. The variable regions (VR) of these integrons were studied by PCR and sequencing (Table 1). Twelve strains presented the *intI1* gene and four presented the *intI2* gene; one of these was *E. coli* Co125, which was positive for both genes.

The VR of the class 1 integron was analyzed in the 12 *intI1*-positive strains, and the following gene cassette arrangements were detected (Table 3): *aadA1* (one strain), *dfrA1* plus *aadA1* (four strains), *dfrA1* plus *aadA1* and *dfrA12* plus *orfF* plus *aadA2* (two strains), *dfrA12* plus *orfF* plus *aadA2* (two strains), and *dfrA17* plus *aadA5* (three strains). Our *E. coli* strain Co125 gave unexpected results: the *intI1* PCR was positive, whereas no *qacEΔ1* or *sul1* genes and no PCR amplicon of the class 1 integron VR were detected. Thus, Co125 was studied in detail by PCR mapping. A 1,650-bp amplicon was obtained using the primers Int-F and AadA-R. Sequencing revealed the presence of the *dfrA12* plus *orfF* plus *aadA2* gene cassettes (Table 3). As in the case of our results, most class 1 integrons published are composed of two or more gene cassettes (12, 22, 40, 41).

A class 2 integron carrying the *dfrA1* plus *sat* plus *aadA1* gene cassettes was detected in four strains (three from foods and one from a pig). The *dfrA1* gene cassette detected in Co125 presented the Asn134Asp amino acid change, corresponding to the sequence of the *dfrA1*-like gene found in this strain (Table 3).

TABLE 1. Primers and annealing temperatures used in the PCR reactions carried out in this study

Primer name	Sequence (5′→3′)	Target gene(s) or region	PCR product size (bp)	Annealing temp (°C)	Reference
TEM-F	ATTCTTGAAGACGAAAGGGC	bla_{TEM}	1,150	60	3
TEM-R	ACGCTCAGTGGAACGAAAAC				
SHV-F	CACTCAAGGATGTATTGTG	bla_{SHV}	885	52	30
SHV-R	TTAGCGTTGCCAGTGCTCG				
OXA-F	ACACAATACATATCAACTTCGC	bla_{OXA}	813	61	36
OXA-R	AGTGTGTTTAGAATGGTGATC				
AacC1-F	ACCTACTCCCAACATCAGCC	*aac(3)-I*	169	60	37
AacC1-R	ATATAGATCTCACTACGCGC				
AacC2-F	ACTGTGATGGGATACGCGTC	*aac(3)-II*	237	60	37
AacC2-R	CTCCGTCAGCGTTTCAGCTA				
AacC3-F	CACAAGAACGTGGTCCGCTA	*aac(3)-III*	185	60	37
AacC3-R	AACAGGTAAGCATCCGCATC				
AacC4-F	CTTCAGGATGGCAAGTTGGT	*aac(3)-IV*	286	60	37
AacC4-R	TCATCTCGTTCTCCGCTCAT				
Ant(2′′)-F	ATGTTACGCAGCAGGGCAGTCG	*ant(2′′)*	187	55	38
Ant(2′′)-R	CGTCAGATCAATATCATCGTGC				
AphA1-F	ATGGGCTCGCGATAATGTC	*aphA1*	600	50	22
AphA1-R	CTCACCGAGGCAGTTCCAT				
AphA2-F	GAACAAGATGGATTGCACGC	*aphA2*	680	50	22
AphA2-R	GCTCTTCAGCAATATCACGG				
AadA-F	GCAGCGCAATGACATTCTTG	*aadA1* or *aadA2*	282	60	16
AadA-R	ATCCTTCGGCGCGATTTTG				20
TetA-F	GTAATTCTGAGCACTGTCGC	*tetA*	937	62	11
TetA-R	CTGCCTGGACAACATTGCTT				
TetB-F	CTCAGTATTCCAAGCCTTTG	*tetB*	416	57	11
TetB-R	CTAAGCACTTGTCTCCTGTT				
TetC-F	TCTAACAATGCGCTCATCGT	*tetC*	570	62	11
TetC-R	GGTTGAAGGCTCTCAAGGGC				
TetD-F	ATTACACTGCTGGACGCGAT	*tetD*	1,104	57	11
TetD-R	CTGATCAGCAGACAGATTGC				
TetE-F	GTGATGATGGCACTGGTCAT	*tetE*	1,179	62	11
TetE-R	CTCTGCTGTACATCGCTCTT				
CmlA-F	TGTCATTTACGGCATACTCG	*cmlA*	455	55	This study
CmlA-R	ATCAGGCATCCCATTCCCAT				
FloR1	CACGTTGAGCCTCTATAT	*floR*	868	55	26
FloR2	ATGCAGAAGTAGAACGCG				6
DfrIa-F	GTGAAACTATCACTAATGG	*dfrA1*, *dfrA5*, *dfrA15*, *dfrA15b*, *dfrA16*, *dfrA16b*	474	55	25
DfrIa-R	TTAACCCTTTTGCCAGATTT				
DfrIb-F	GAGCAGCTICTITTIAAAGC	*dfrA14*, *dfrA6*	393	60	25
DfrIb-R	TTAGCCCTTTIICCAATTTT				
DfrVII-F	TTGAAAATTTCATTGATT	*dfrA7*, *dfrA17*	474	55	25
DfrVII-R	TTAGCCTTTTTTCCAAATCT				
DfrII-F	GATCACGTGCGCAAGAAATC	*dfrB1*, *dfrB2*, *dfrB3*	141	60	25
DfrII-R	AAGCGCAGCCACAGGATAAAT				
DfrXII-F	GGTGSGCAGAAGATTTTTCGC	*dfrA12*, *dfrA13*	319	60	25
DfrXII-R	TGGGAAGAAGGCGTCACCCTC				
Sul-F	TGGTGACGGTGTTCGGCATTC	*sul1*	789	63	23
Sul-R	GCGAGGGTTTCCGAGAAGGTG				This study
Sul2-F	CGGCATCGTCAACATAACC	*sul2*	722	50	22
Sul2-R	GTGTGCGGATGAAGTCAG				
Sul3-F	CATTCTAGAAAACAGTCGTAGTTCG	*sul3*	990	51	29
Sul3-R	CATCTGCAGCTAACCTAGGGCTTTGGA				
IntI1-F	GGGTCAAGGATCTGGATTTCG	*intI1*	483	62	23
IntI1-R	ACATGGGTGTAAATCATCGTC				
IntI2-F	CACGGATATGCGACAAAAAGGT	*intI2*	788	62	23
IntI2-R	GTAGCAAACGAGTGACGAAATG				
Int-F	GGCATCCAAGCAGCAAG	Class 1 integron variable region	Variable	55	19
Int-R	AAGCAGACTTGACCTGA				
Hep-F	CGGGATCCCGGACGGCATGCACGATTTGTA	Class 2 integron variable region	Variable	60	39
Hep-R	GATGCCATCGCAAGTACGAG				
Qac-F	GGCTGGCTTTTTCTTGTTATCG	*qacEΔ1*	287	60	23
Qac-R	TGAGCCCCATACCTACAAAGC				
MarR-F	AGCTAGCCTTGCATCGCA	*marR* and *marO*	568	55	28
MarR-R	TACGGCAGGACTTTCTTAAGCA				

TABLE 2. Phenotypes and mechanisms of antibiotic resistance detected in the 17 multiresistant *E. coli* strains of this study

E. coli strain (origin)[a]	Phenotype of resistance[b]	Mechanisms of resistance			
		Resistance genes detected	Position(s) of amino acid change(s) in:		CAT[c]
			GyrA	ParC	
Co1 (F)	Nal Cip Amp Kan Str Rif Tet Chl Tmp Smx	bla_{TEM1b}, *aphA1*, *aphA2*, *aadA1*, *tetB*, *dfrA1*, *sul2*	83 + 87	80	
Co19 (F)	Nal Amp Kan Str Rif Tet Chl Tmp Smx	bla_{TEM1b}, *aphA2*, *aadA1*, *tetB*, *dfrA1*, *sul2*	83		
Co45 (F)	Nal Cip Amp Amc[d] Kan Str Rif Tet Chl Tmp Smx	bla_{TEM1b}, *aphA2*, *aadA1*, *tetA*, *dfrA1*, *sul2*	83 + 87	80	
Co53 (F)	Nal Amp Amc[d] Kan Str Rif Tet Chl Smx	bla_{TEM1b}, *aphA2*, *tetB*, *sul2*	83		
Co71 (B)	Nal Cip Amp Kan Str Rif Tet Chl Tmp Smx	bla_{TEM1b}, *aphA1*, *aadA5*, *tetB*, *dfrA17*, *sul1*, *sul2*	83 + 87	80	+
Co80 (B)	Nal Amp Gen Tob[d] Kan Str Rif Tet Chl Smx	bla_{TEM1b}, *aac(3)-II, aphA2, aadA1, tetB, sul1, sul2*	83		+
Co82 (B)	Nal Cip Amp Kan Str Rif Tet Chl Tmp Smx	bla_{TEM1b}, *aphA1*, *aadA5*, *tetB*, *dfrA17*, *sul1*, *sul2*	83 + 87	80	+
Co110 (B)	Nal Amp Amc[d] Str Rif Tet Chl Tmp Smx	bla_{TEM1a}, *aadA1*, *tetA*, *cmlA*, *dfrA1*, *sul1*, *sul3*	83		
Co125 (P)	Nal Cip Amp Str Rif Tet Chl Tmp Smx	bla_{TEM1b}, *aadA1*, *aadA2*, *tetA*, *cmlA*, *dfrA12*, *dfrA1*-like, *sul3*	83 + 87	80	
Co279 (P)	Nal Amp Amc[d] Str Rif Tet Chl Tmp Smx	bla_{TEM1b}, *aadA1*, *aadA2*, *tetB*, *cmlA*, *dfrA12*, *sul3*	83		
Co177 (D)	Nal Amp Amc[d] Str Rif Tet Chl Tmp Smx	bla_{TEM1b}, *aadA1*, *tetA*, *dfrA1*, *sul1*, *sul2*	83		+
Co201 (D)	Nal Amp Amc[d] Str Rif Tet Chl Tmp Smx	bla_{TEM1b}, *aadA1*, *tetA*, *dfrA1*, *sul1*, *sul2*	83		+
Co227 (H)	Nal Amp Amc[d] Gen Apr Tob Str Rif Tet Chl Tmp Smx	bla_{TEM1b}, *aac(3)*-IV, *aadA1*, *aadA2*, *tetA*, *cmlA*, *dfrA1*, *dfrA12*, *sul1*, *sul2*, *sul3*	83		
Co228 (H)	Nal Amp Amc[d] Gen Apr Tob Kan Str Rif Tet Chl Tmp Smx	bla_{TEM1a}, *aac(3)*-IV, *aphA2*, *aadA2*, *tetA*, *cmlA*, *dfrA12*, *sul1*, *sul3*	83		
Co232 (H)	Nal Cip Amp Str Rif Tet Chl Tmp Smx	bla_{TEM1b}, *aadA1*, *tetA*, *dfrA1*, *sul1*, *sul2*	83 + 87	80 + 84	+
Co354 (H)	Nal Cip Amp Gen Apr Tob Str Rif Tet Chl[d] Tmp Smx	bla_{TEM1b}, *aac(3)*-IV, *aadA1*, *aadA2*, *tetA*, *dfrA1*, *dfrA12*, *sul1*, *sul3*	83 + 87	80	
Co356 (H)	Nal Cip Amp Str Rif Tet Chl Tmp Smx	bla_{TEM1b}, *aadA5*, *tetB*, *dfrA17*, *sul1*, *sul2*	83 + 84	80 + 108	+

[a] F, food; B, broiler; P, pig; D, dog; H, human.
[b] Nal, nalidixic acid; Cip, ciprofloxacin; Amp, ampicillin; Amc, amoxicillin-clavulanic acid; Gen, gentamicin; Apr, apramycin; Tob, tobramycin; Kan, kanamycin; Str, streptomycin; Rif, rifampin; Tet, tetracycline; Chl, chloramphenicol; Tmp, trimethoprim; Smx, sulfamethoxazole.
[c] CAT, chloramphenicol-acetyl-transferase enzymatic activity. +, CAT was detected for the strain indicated.
[d] Resistance to the drug indicated is in the intermediate category according to NCCLS standards for the corresponding strain.

Analysis of the *mar* locus. Another mechanism contributing to a multiple-antibiotic-resistant phenotype is associated with *mar* locus regulation (1, 2, 5). Amino acid changes in MarR and the nucleotide mutations in the operator-promoter region *marO* were studied for all strains by PCR, sequencing, and comparison with the GenBank sequence found under accession number M96235 and corresponding to the *mar* regulon (Table 4). Fifteen strains showed Gly103Ser and Tyr137His substitutions in MarR, which had been found also in resistant clinical strains (27). Note that position 103 is inside the conserved region (between amino acids 92 and 104 in MarR) and may be important for binding with the region corresponding to *marO* (1). However, other authors have considered that these substitutions could correspond to genotypic variations in *marR* without loss of repressor activity (27). Another amino acid change in MarR, Leu36Gln, was found in only one strain in our study; this is the first time that this substitution has been reported. Further studies are necessary to relate this substitution to antibiotic resistance.

Regarding nucleotide mutations in *marO*, 14 strains showed the previously reported A1332C transversion (27) together with amino acid substitutions in MarR at positions 103 and 137. We identified other nucleotide mutations (at positions 1331, 1370, 1375, 1379, and 1414) not previously found in the literature. MarA is known to activate the *marRAB* operon binding the "marbox" region in *marO*, but the adjacent region (nucleotides 1329 to 1346) also plays a role in binding and increases this transcriptional activation (21). Indeed, the mutations found at positions 1331 and 1332 are located inside this adjacent region, but additional studies should determine their effect on MarA activity. We found no differences in resistance phenotype between the strains with and without these mutations.

Our results show a wide variety of resistance genes in multiresistant nonpathogenic *E. coli* strains from humans, animals, and food products. Therefore, this normal flora may play a key role as an acceptor and donor of transmissible antimicrobial resistance mechanisms. The inclusion of some resistance genes

TABLE 3. Analysis of class 1 and class 2 integrons and their resistance gene cassettes in the 17 multiresistant *E. coli* strains

E. coli strain (origin)[a]	Class 1 integron				Class 2 integron	
	Detection of[b]:		Variable region amplified by PCR		Detection of *intI2* gene[b]	Variable region amplified by PCR
	intI1	*qacEΔ1 sulI*	Size of amplicons obtained (bp)	Genes included in cassettes		Size of amplicons obtained (bp) / Genes included in cassettes
Co1 (F)	−	−	NAD[c]		+	2,000 / *dfrA1* + *sat* + *aadA1*
Co19 (F)	−	−	NAD		+	2,000 / *dfrA1* + *sat* + *aadA1*
Co45 (F)	−	−	NAD		+	2,000 / *dfrA1* + *sat* + *aadA1*
Co53 (F)	−	−	NAD		−	NAD
Co71 (B)	+	+	1,700	*dfrA17* + *aadA5*	−	NAD
Co80 (B)	+	+	1,000	*aadA1*	−	NAD
Co82 (B)	+	+	1,700	*dfrA17* + *aadA5*	−	NAD
Co110 (B)	+	+	1,500	*dfrA1* + *aadA1*	−	NAD
Co125 (P)	+	−	NAD	*dfrA12* + *orfF* + *aadA2*[d]	+	2,000 / *dfrA1*-like + *sat* + *aadA1*
Co279 (P)	−	−	NAD		−	NAD
Co177 (D)	+	+	1,500	*dfrA1* + *aadA1*	−	NAD
Co201 (D)	+	+	1,500	*dfrA1* + *aadA1*	−	NAD
Co227 (H)	+	+	1,500, 2,000	*dfrA1* + *aadA1*, *dfrA12* + *orfF* + *aadA2*	−	NAD
Co228 (H)	+	+	2,000	*dfrA12* + *orfF* + *aadA2*	−	NAD
Co232 (H)	+	+	1,500	*dfrA1* + *aadA1*	−	NAD
Co354 (H)	+	+	1,500, 2,000	*dfrA1* + *aadA1*, *dfrA12* + *orfF* + *aadA2*	−	NAD
Co356 (H)	+	+	1,700	*dfrA17* + *aadA5*	−	NAD

[a] F, food; B, broiler; P, pig; D, dog; H, human.
[b] +, detected; −, not detected.
[c] NAD, No amplicon was detected.
[d] These gene cassettes were detected by sequencing of amplicons obtained with the primers Int-F and AadA-R.

TABLE 4. Analysis of amino acid changes in MarR protein and nucleotide mutations in *marO* region of 17 multiresistant *E. coli* strains[a]

E. coli strain(s)	Amino acid changes in MarR	Nucleotide mutation(s) in *marO*
Co53, Co356	None	None
Co177	Gly103Ser, Tyr137His	None
Co279	Gly103Ser, Tyr137His	A1332C
Co1	Gly103Ser, Tyr137His	A1332C, A1331G
Co110, Co232, Co354	Gly103Ser, Tyr137His	A1332C, C1370T
Co201, Co227, Co228	Gly103Ser, Tyr137His	A1332C, C1375T
Co71, Co82, Co125	Gly103Ser, Tyr137His	A1332C, C1379T
Co19, Co80	Gly103Ser, Tyr137His	A1332C, C1414T
Co45	Leu36Gln, Gly103Ser, Tyr137His	A1332C

[a] The sequence found under GenBank accession number M96235 was used as a reference.

inside integrons constitutes an effective means to spread antibiotic resistance among bacteria from different ecosystems. Moreover, different amino acid changes in MarR and mutations in *marO* were found, possibly contributing to the multiple antibiotic resistance phenotype.

This work has been partially supported by grants from the Fondo de Investigaciones Sanitarias (FIS 01/973) and the Consejería de Educación del Gobierno de La Rioja (ACPI2001/04) of Spain. Y. Sáenz has a fellowship from the University of La Rioja (FPI-UR-00/72785864).

We are grateful to R. Del Campo for critical review of the manuscript.

REFERENCES

1. **Alekshun, M. N., and S. B. Levy.** 1997. Regulation of chromosomally mediated multiple antibiotic resistance: the *mar* regulon. Antimicrob. Agents Chemother. **41:**2067–2075.
2. **Ariza, R. R., S. P. Cohen, N. Bachhawat, S. B. Levy, and B. Demple.** 1994. Repressor mutations in the *marRAB* operon that activate oxidative stress genes and multiple antibiotic resistance in *Escherichia coli*. J. Bacteriol. **176:**143–148.
3. **Belaaouaj, A., C. Lapoumeroulie, M. M. Caniça, G. Vedel, P. Névot, R. Krishnamoorthy, and G. Paul.** 1994. Nucleotide sequences of the genes coding for the TEM-like β-lactamases IRT-1 and IRT-2 (formerly called TRI-1 and TRI-2). FEMS Microbiol. Lett. **120:**75–80.
4. **Carattoli, A.** 2001. Importance of integrons in the diffusion of resistance. Vet. Res. **32:**243–259.
5. **Cohen, S. P., L. M. McMurry, D. C. Hooper, J. S. Wolfson, and S. B. Levy.** 1989. Cross-resistance to fluoroquinolones in multiple-antibiotic-resistant (Mar) *Escherichia coli* selected by tetracycline or chloramphenicol: decreased drug accumulation associated with membrane changes in addition to OmpF reduction. Antimicrob. Agents Chemother. **33:**1318–1325.
6. **del Cerro, A., S. M. Soto, and M. C. Mendoza.** 2003. Virulence and antimicrobial-resistance gene profiles determined by PCR-based procedures for *Salmonella* isolated from samples of animal origin. Food Microbiol. **20:**431–438.
7. **Domínguez, E., M. Zarazaga, Y. Sáenz, L. Briñas, and C. Torres.** 2002. Mechanisms of antibiotic resistance in *Escherichia coli* isolates obtained from healthy children in Spain. Microb. Drug Resist. **8:**321–327.
8. **Gallardo, F., J. Ruiz, F. Marco, K. J. Towner, and J. Vila.** 1999. Increase in incidence of resistance to ampicillin, chloramphenicol and trimethoprim in clinical isolates of *Salmonella* serotype Typhimurium with investigation of molecular epidemiology and mechanisms of resistance. J. Med. Microbiol. **48:**367–374.
9. **Gautom, R. K.** 1997. Rapid pulsed-field gel electrophoresis protocol for typing of *Escherichia coli* O157:H7 and other gram-negative organisms in 1 day. J. Clin. Microbiol. **35:**2977–2980.
10. **Grape, M., L. Sundstrom, and G. Kronvall.** 2003. Sulphonamide resistance gene *sul3* found in *Escherichia coli* isolates from human sources. J. Antimicrob. Chemother. **52:**1022–1024.
11. **Guardabassi, L., L. Dijkshoorn, J.-M. Collard, J. E. Olsen, and A. Dalsgaard.** 2000. Distribution and in-vitro transfer of tetracycline resistance determinants in clinical and aquatic *Acinetobacter* strains. J. Med. Microbiol. **49:**929–936.
12. **Guerra, B., E. Junker, A. Schroeter, B. Malorny, S. Lehmann, and R. Helmuth.** 2003. Phenotypic and genotypic characterization of antimicrobial

resistance in German *Escherichia coli* isolates from cattle, swine and poultry. J. Antimicrob. Chemother. **52:**489–492.
13. **Hall, R. M., and C. M. Collis.** 1995. Mobile gene cassettes and integrons: capture and spread of genes by site-specific recombination. Mol. Microbiol. **15:**593–600.
14. **Hall, R. M., and C. M. Collis.** 1998. Antibiotic resistance in gram-negative bacteria: the role of gene cassettes and integrons. Drug Resist. Updates **1:**109–119.
15. **Heinzel, P., O. Werbitzky, J. Distler, and W. Peipersberg.** 1988. A second streptomycin resistance gene from *Streptomyces griseus* codes for streptomycin-3′′-phosphotransferase. Arch. Microbiol. **150:**184–192.
16. **Hollingshead, S., and D. Vapnek.** 1985. Nucleotide sequence analysis of the gene encoding a streptomycin/spectinomycin adenylyltransferase. Plasmid **13:**17–30.
17. **Hunter, J. E. B., J. C. Shelley, J. R. Walton, C. A. Hart, and M. Bennett.** 1992. Apramycin resistance plasmids in *Escherichia coli*: possible transfer to *Salmonella typhimurium* in calves. Epidemiol. Infect. **108:**271–278.
18. **Huovinen, P.** 2001. Resistance to trimethoprim-sulfamethoxazole. Clin. Infect. Dis. **32:**1608–1614.
19. **Lévesque, C., and P. H. Roy.** 1993. PCR analysis of integrons, p. 590–594. *In* D. H. Persing, T. F. Smith, F. C. Tenover, and T. J. White (ed.), Diagnostic molecular microbiology. American Society for Microbiology, Washington, D.C.
20. **Madsen, L., F. M. Aarestrup, and J. E. Olsen.** 2000. Characterisation of streptomycin resistance determinants in Danish isolates of *Salmonella* Typhimurium. Vet. Microbiol. **75:**73–82.
21. **Martin, R. G., P. S. Nyantakyi, and J. L. Rosner.** 1995. Regulation of the multiple antibiotic resistance (*mar*) regulon by *marORA* sequences in *Escherichia coli*. J. Bacteriol. **177:**4176–4178.
22. **Maynard, C., J. M. Fairbrother, S. Bekal, F. Sanschagrin, R. C. Levesque, R. Brousseau, L. Masson, S. Larivière, and J. Harel.** 2003. Antimicrobial resistance genes in enterotoxigenic *Escherichia coli* O149:K91 isolates obtained over a 23-year period from pigs. Antimicrob. Agents Chemother. **47:**3214–3221.
23. **Mazel, D., B. Dychinco, V. A. Webb, and J. Davies.** 2000. Antibiotic resistance in the ECOR collection: integrons and identification of a novel *aad* gene. Antimicrob. Agents Chemother. **44:**1568–1574.
24. **National Committee for Clinical Laboratory Standards.** 2002. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. Approved standard M7-A5. National Committee for Clinical Laboratory Standards, Wayne, Pa.
25. **Navia, M. M., J. Ruiz, J. Sánchez-Céspedes, and J. Vila.** 2003. Detection of dihydrofolate reductase genes by PCR and RFLP. Diagn. Microb. Infect. Dis. **46:**295–298.
26. **Ng, L.-K., M. R. Mulvey, I. Martin, G. A. Peters, and W. Johnson.** 1999. Genetic characterization of antimicrobial resistance in Canadian isolates of *Salmonella* serovar Typhimurium DT104. Antimicrob. Agents Chemother. **43:**3018–3021.
27. **Oethinger, M., I. Podglajen, W. V. Kern, and S. B. Levy.** 1998. Overexpression of the *marA* or *soxS* regulatory gene in clinical topoisomerase mutants of *Escherichia coli*. Antimicrob. Agents Chemother. **42:**2089–2094.
28. **Park, Y.-H., J.-H. Yoo, D.-H. Huh, Y.-K. Cho, J.-H. Choi, and W.-S. Shin.** 1998. Molecular analysis of fluoroquinolone-resistance in *Escherichia coli* on the aspect of gyrase and multiple antibiotic resistance (*mar*) genes. Yonsei Med. J. **39:**534–540.
29. **Perreten, V., and P. Boerlin.** 2003. A new sulfonamide resistance gene (*sul3*) in *Escherichia coli* is widespread in the pig population of Switzerland. Antimicrob. Agents Chemother. **47:**1169–1172.
30. **Pitout, J. D. D., K. S. Thomson, N. D. Hanson, A. F. Ehrhardt, E. S. Moland, and C. C. Sanders.** 1998. β-Lactamases responsible for resistance to expanded-spectrum cephalosporins in *Klebsiella pneumoniae*, *Escherichia coli*, and *Proteus mirabilis* isolates recovered in South Africa. Antimicrob. Agents Chemother. **42:**1350–1354.
31. **Recchia, G. D., and R. M. Hall.** 1995. Gene cassettes: a new class of mobile element. Microbiology **141:**3015–3027.
32. **Sabaté, M., and G. Prats.** 2002. Estructura y función de los integrones. Enferm. Infecc. Microbiol. Clin. **20:**341–345.
33. **Sáenz, Y., M. Zarazaga, L. Briñas, F. Ruiz-Larrea, and C. Torres.** 2003. Mutations in *gyrA* and *parC* genes in nalidixic acid-resistant *Escherichia coli* strains obtained from food products, humans and animals. J. Antimicrob. Chemother. **51:**1001–1005.
34. **Sáenz, Y., M. Zarazaga, L. Briñas, M. Lantero, F. Ruiz-Larrea, and C. Torres.** 2001. Antibiotic resistance in *Escherichia coli* isolates obtained from animals, foods and humans in Spain. Int. J. Antimicrob. Agents. **18:**353–358.
35. **Scholz, P., V. Haring, B. Wittmann-Liebold, K. Ashman, M. Bagdasarian, and E. Scherzinger.** 1989. Complete nucleotide sequence and gene organization of the broad-host-range plasmid RSF1010. Gene **75:**271–288.
36. **Steward, C. D., J. K. Rasheed, S. K. Hubert, J. W. Biddle, P. M. Raney, G. J. Anderson, P. P. Williams, K. L. Brittain, A. Oliver, J. E. McGowan, Jr., and F. C. Tenover.** 2001. Characterization of clinical isolates of *Klebsiella pneumoniae* from 19 laboratories using the National Committee for Clinical Laboratory Standards extended-spectrum β-lactamase detection methods. J. Clin. Microbiol. **39:**2864–2872.
37. **van de Klundert, J. A. M., and J. S. Vliegenthart.** 1993. PCR detection of genes coding for aminoglycoside-modifying enzymes, p. 547–552. *In* D. H. Persing, T. F. Smith, F. C. Tenover, and T. J. White (ed.), Diagnostic molecular microbiology. American Society for Microbiology, Washington, D.C.
38. **Vanhoof, R., J. Content, E. Van Bossuyt, L. Dewit, and E. Hannecart-Pokorni.** 1992. Identification of the *aadB* gene coding for the aminoglycoside-2′′-O-nucleotidyltransferase, ANT (2′′), by means of the polymerase chain reaction. J. Antimicrob. Chemother. **29:**365–374.
39. **White, P. A., C. J. McIver, and W. D. Rawlinson.** 2001. Integrons and gene cassettes in the *Enterobacteriaceae*. Antimicrob. Agents Chemother. **45:**2658–2661.
40. **White, P. A., C. J. McIver, Y. Deng, and W. D. Rawlinson.** 2000. Characterization of two new gene cassettes, *aadA5* and *dfrA17*. FEMS Microbiol. Lett. **182:**265–269.
41. **Yu, H. S., J. C. Lee, H. Y. Kang, D. W. Ro, J. Y. Chung, Y. S. Jeong, S. H. Tae, C. H. Choi, E. Y. Lee, S. Y. Seol, Y. C. Lee, and D. T. Cho.** 2003. Changes in gene cassettes of class 1 integrons among *Escherichia coli* isolates from urine specimens collected in Korea during the last two decades. J. Clin. Microbiol. **41:**5429–5433.