ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Oct. 2002, p. 3156–3163 Vol. 46, No. 10
0066-4804/02/$04.00+0 DOI: 10.1128/AAC.46.10.3156–3163.2002

β-Lactamases in Ampicillin-Resistant *Escherichia coli* Isolates from Foods, Humans, and Healthy Animals

Laura Briñas, Myriam Zarazaga, Yolanda Sáenz, Fernanda Ruiz-Larrea, and Carmen Torres*

Area de Bioquímica y Biología Molecular, Universidad de La Rioja, 26006 Logroño, Spain

Received 17 January 2002/Returned for modification 23 April 2002/Accepted 9 July 2002

TEM-, SHV-, and OXA-type β-lactamases were studied by PCR with 124 ampicillin-resistant (AMP^r) *Escherichia coli* isolates recovered from foods of animal origin (n = 20) and feces of humans (n = 49) and healthy animals (n = 55). PCR showed that 103 isolates were positive for TEM and negative for SHV and OXA. Three *E. coli* isolates showed a positive reaction for OXA, and one showed a positive reaction for SHV. The remaining 17 *E. coli* isolates were negative for the three enzymes by PCR. Fifty-seven of the 103 bla_{TEM} amplicons were sequenced. Different molecular variants of bla_{TEM-1} were found in 52 isolates: bla_{TEM-1a} (n = 9), bla_{TEM-1b} (n = 36), bla_{TEM-1c} (n = 6), and bla_{TEM-1f} (n = 1). Four inhibitor-resistant TEM (IRT) β-lactamase-encoding genes were also detected: $bla_{TEM-30c}$ (IRT-2), $bla_{TEM-34b}$ (IRT-6), $bla_{TEM-40b}$ (IRT-11), and $bla_{TEM-51a}$ (IRT-15). A new bla_{TEM} gene, named $bla_{TEM-95b}$, which showed a mutation in amino acid 145 (P→A) was detected. It was found in a food isolate of chicken origin (AMP^r, amoxicillin-clavulanic acid susceptible). The promoter region in 24 bla_{TEM} amplicons was analyzed, and the weak *P3* promoter was found in 23 of them (bla_{TEM-1} in 20 amplicons and $bla_{TEM-51a}$, $bla_{TEM-30c}$, and $bla_{TEM-95b}$ in 1 amplicon each). The strong *Pa*/*Pb* promoter was found only in the $bla_{TEM-34b}$ gene. No extended-spectrum β-lactamases were detected. Mutations at position −42 or −32 in the *ampC* gene promoter were demonstrated in 4 of 10 *E. coli* isolates for which the cefoxitin MIC was ≥16 μg/ml. Different variants of bla_{TEM-1} and IRT bla_{TEM} genes were found among the AMP^r *E. coli* isolates from foods and the feces of humans and healthy animals, and a new gene, $bla_{TEM-95b}$ (*P3*), was detected.

Escherichia coli is one of the main causes of nosocomial infections in humans. *E. coli* is also a common inhabitant of the human and animal gut and is considered an indicator of fecal contamination in food. β-Lactams are widely used in human and veterinary medicine to treat human and animal infections (31). This widespread use of antibiotics could be associated with the selection of antibiotic resistance mechanisms in pathogenic and nonpathogenic isolates of *E. coli* (46).

Resistance to β-lactam antimicrobial agents in *E. coli* is primarily mediated by β-lactamases, which hydrolyze the β-lactam ring and thus inactivate the antibiotic (30). Many different β-lactamases have been described (9, 30, 31). Over 200 β-lactamases have been classified into four main groups and eight subgroups according to their functional and structural characteristics (9, 10). The classical TEM-1, TEM-2, and SHV-1 enzymes are the predominant plasmid-mediated β-lactamases of gram-negative rods. Six different nucleotide sequences, called bla_{TEM-1a}, bla_{TEM-1b}, bla_{TEM-1c}, bla_{TEM-1d}, bla_{TEM-1e}, and bla_{TEM-1f}, have been described to codify the same TEM-1 β-lactamase (15, 20, 21, 28, 47). Weak (*P3*) and strong (*P4* and *Pa*/*Pb*) promoters have been reported for bla_{TEM} genes (14, 20, 21, 28). Some variants of the TEM-1, TEM-2, and SHV-1 β-lactamases have emerged as a result of single amino acid substitutions in the sequences of the genes which render the extended-spectrum β-lactamases (ESBLs), which inactivate newer cephalosporins but which are still susceptible to β-lactamase inhibitors (e.g., clavulanic acid) (30).

β-Lactamase inhibitor-resistant strains emerged during the 1980s. Susceptibility to β-lactamase inhibitors could be affected in *E. coli* by different mechanisms. The most frequent one is the hyperproduction of classical β-lactamases or the synthesis of inhibitor-resistant TEM (IRT) β-lactamases by amino acid substitutions in TEM-1 or TEM-2. Other possible mechanisms are the hyperproduction of chromosomal AmpC β-lactamase (by gene amplification or the introduction of mutations at either the promoter or the attenuator of the structural gene) (11, 16, 25, 36, 37) and some types of OXA β-lactamases (30, 34), plasmidic cephalosporinase production (e.g., FOX) (2, 5, 19, 32, 40), or even changes in membrane permeability (33).

Multiple studies focused on the characterization of β-lactamases in human clinical *E. coli* isolates have been performed, but very few studies have been performed with *E. coli* isolates of other origins such as healthy animals or foods (23, 46), sick animals (6, 17; T. L. Teshager, L. Dominguez, M. A. Moreno, Y. Sáenz, M. Zarazaga, C. Torres, and S. Cardeñosa, Letter, Antimicrob. Agents Chemother. **44:**3483-3484, 2000), or healthy humans (8, 22, 42). The objective of this study was to characterize the types of β-lactamases produced by 124 ampicillin (AMP)-resistant (AMP^r) nonpathogenic *E. coli* isolates recovered from foods and from the feces of humans and healthy animals.

MATERIALS AND METHODS

Bacterial isolates. All 124 AMP^r *E. coli* isolates (MICs ≥ 32 μg/ml) recovered in a previous study (41) from samples of different origins (food products and fecal samples of humans and animals) were included in this work. The origins of the 124 AMP^r isolates were as follows (number of isolates): food products of chicken origin (n = 20), feces of healthy animals (broilers, n = 22; pigs, n = 20;

* Corresponding author. Mailing address: Area de Bioquímica y Biología Molecular, Universidad de La Rioja, Madre de Dios, 51, 26006 Logroño, Spain. Phone: 34-941299750. Fax: 34-941299721. E-mail: carmen.torres@daa.unirioja.es.

pets, bulls, or horses, $n = 13$), and human fecal samples (patients, $n = 40$; healthy volunteers, $n = 9$). The healthy human volunteers from whom *E. coli* isolates were recovered had not been treated with antibiotics for at least 3 months preceding isolation of *E. coli*. The *E. coli* isolates from patients were not implicated in any kind of infection, and they were considered part of the normal microflora in these individuals.

Antibiotic susceptibility. The antibiotic susceptibilities of the AMP^r *E. coli* isolates were analyzed by the NCCLS standard agar dilution method (35). The following antibiotics were tested: AMP, cefazolin, cefoxitin, cefotaxime, and ceftriaxone (Sigma Chemical Co., St. Louis, Mo.); amoxicillin-clavulanic acid (AMC) and ticarcillin (TIC; SmithKline Beecham, Madrid, Spain); ceftazidime (Glaxo, Madrid, Spain); imipenem (Merck Sharp & Dohme, Madrid, Spain); and aztreonam (Bristol-Myers Squibb, Madrid, Spain).

Detection of TEM, SHV, and OXA β-lactamase-encoding genes. Microorganisms were grown on brain heart infusion agar plates (Difco, Detroit, Mich.) for 24 h at 37°C, and one colony was resuspended in 500 μl of sterile distilled water. The cells were lysed by heating at 95°C for 10 min, and cellular debris was removed by centrifugation at 16,000 × *g* for 2 min. The supernatant was used as the source of template for PCRs. PCR amplifications of the bla_{TEM}, bla_{SHV}, and bla_{OXA} genes were carried out for screening purposes, as described previously (3, 39, 45). The following primers were used in these reactions: for bla_{TEM}, primers *tem*-F (5′-TTCTTGAAGACGAAAGGGC-3′) and *tem*-R (5′-ACGCTCAGTG GAACGAAAAC-3′); for bla_{SHV}, primers *shv*-F (5′-CACTCAAGGATGTATT GTG-3′) and *shv*-R (5′-TTAGCGTTGCCAGTGCTCG-3′); and for bla_{OXA}, primers *oxa*-F (5′-TTCAAGCCAAAGGCACGATAG-3′) and *oxa*-R (5′-TCC GAGTTGACTGCCGGGTTG-3′). The conditions used for these reactions were as indicated in previous papers (3, 39, 45). The sizes of the bla_{TEM}, bla_{SHV}, and bla_{OXA} amplicons were 1,150, 885, and 702 bp, respectively. Positive controls (*E. coli* C282 [bla_{TEM-1}], *E. coli* C321 [bla_{OXA-2}], and *E. coli* EC98/4453-2 [bla_{SHV-12}] [Teshager et al., letter, 2000]) as well as negative controls were included in each type of PCR. Fifty-seven bla_{TEM} amplicons from isolates showing different phenotypes of resistance to the AMC association were sequenced (both strands) with the Rhodamine Dye Terminator Cycle Sequencing kit and analyzed in an automatic DNA sequencer (ABI 310; Applied Biosystems). The same set of primers used in the PCR analysis was used for sequencing purposes. DNA and deduced amino acid sequences were compared with those previously described for bla_{TEM} genes (EMBL database and the website of G. Jacoby and K. Bush [http://www.lahey.org/studies/webt.htm]). The nomenclature used for the bla_{TEM} variants and promoters was the one proposed previously (21, 28).

The isolates were screened for ESBLs by the double disk diffusion method (30).

DNA sequence analysis of regulatory region of *ampC* gene. A 191-bp fragment of the promoter region of the *ampC* gene was amplified and sequenced by using primers *ampC*-F (5′-AATGGGTTTTCTACGGTCTG-3′) and *ampC*-R (5′-GG GCAGCAAATGTGGAGCAA-3′) (11) and the conditions described previously (11). The mutations in the promoter and attenuator regions were studied by comparing the sequences with the sequence of the same region in the *E. coli* K-12 *ampC* gene (24).

Nucleotide sequence accession number. The nucleotide sequence reported for $bla_{TEM-95b}$ is included in the EMBL database under accession number AJ308558.

RESULTS

MIC determination. Table 1 shows the MICs of the different β-lactams tested for the 124 AMP^r *E. coli* isolates from different origins included in this study (humans, 49 isolates; food and animals, 75 isolates). Most of the isolates (91%) were resistant to TIC (94% of the human isolates and 85% of the food and animal isolates). AMC resistance (MIC ≥ 32 μg/ml) was detected in 16% of the human isolates and 8% of the animal and food isolates. Thirty-five percent of the human isolates and 43% of the isolates of other origins were included in the intermediate category for AMC (MIC = 16 μg/ml). A cefoxitin MIC ≥16 μg/ml was observed for 10 isolates, 5 of which were from human samples (10%) and 5 of which were from the other origins (7%). Diminished susceptibility to extended-spectrum cephalosporins (MIC ≥ 2 μg/ml) was found for three isolates (one isolate each from a broiler, a pig, and a human patient).

Distribution of mechanisms of resistance. PCRs with primers specific for the bla_{TEM}, bla_{SHV}, and bla_{OXA} genes were performed with the 124 AMP^r isolates included this study, and the results are shown in Table 2. Positive PCR results for the bla_{TEM} gene and negative PCR results for the bla_{SHV} or bla_{OXA} gene were found for 103 of the 124 (83%) AMP^r *E. coli* isolates. An SHV-type β-lactamase was detected by PCR in one additional isolate that was negative by PCRs for both bla_{TEM} and bla_{OXA}. An OXA-type β-lactamase was detected in three other isolates that were negative by PCRs for both bla_{TEM} and bla_{SHV}. The PCRs for all genes were negative for the remaining 17 isolates. For 7 of these 17 *E. coli* isolates, as well as 3 isolates with *bla* genes, the cefoxitin MICs were ≥16 μg/ml (Table 1). No ESBLs were found by the double disk test in any of our *E. coli* isolates.

Fifty-seven of the 103 bla_{TEM} amplicons detected were sequenced. These 57 amplicons corresponded to the following *E. coli* isolates: (i) most of the isolates for which the AMC MIC was ≥16 μg/ml (intermediate or resistant phenotype; 48 of 54 isolates in which the bla_{TEM} gene was detected by PCR) and (ii) a group of isolates for which the AMC MIC was ≤8 μg/ml (susceptible phenotype; 9 of 49 isolates in which the bla_{TEM} gene was detected by PCR). Table 3 shows the bla_{TEM} sequences obtained. The bla_{TEM-1} gene was found in 52 of the 57 sequences studied (91%). Different molecular variants of bla_{TEM-1} were detected in the analysis of the nucleotide sequences of the structural gene and the promoter: bla_{TEM-1a}, bla_{TEM-1b}, bla_{TEM-1c}, and bla_{TEM-1f}. Neither bla_{TEM-1d} nor bla_{TEM-1e} variants were found. The most frequent molecular variant was bla_{TEM-1b} (69%), followed by bla_{TEM-1a} (17%). Curiously, bla_{TEM-1c} was mostly found in isolates from healthy humans (four of the six isolates in which this bla_{TEM} molecular variant was detected), and bla_{TEM-1f} was found in only one isolate recovered from a human patient. Other types of bla_{TEM} genes were identified (Table 3) in the remaining 5 of the 57 amplicons sequenced: $bla_{TEM-30c}$ (which encodes the TEM-30 β-lactamase, also named the IRT-2 β-lactamase), $bla_{TEM-34b}$ (which encodes the TEM-34 β-lactamase, also named the IRT-6 β-lactamase), $bla_{TEM-40b}$ (which encodes the TEM-40 β-lactamase, also named the IRT-11 β-lactamase), $bla_{TEM-51a}$ (which encodes the TEM-51 β-lactamase, also named the IRT-15 β-lactamase), and a new bla_{TEM} gene not previously described (see below). These four IRT β-lactamases showed a single amino acid substitution either at position 69 (TEM-34, Met→Val; TEM-40, Met→Ile) or at position 244 (TEM-30, Arg→Ser; TEM-51, Arg→His) and were detected in isolates recovered from a healthy pig (TEM-51) or from human feces (TEM-30, TEM-34, and TEM-40). The sequences of each of these IRT β-lactamase genes were obtained by sequencing both strands of the amplicons from two or three independent PCRs.

A new bla_{TEM} gene was detected in this study and was named $bla_{TEM-95b}$. It was found in an *E. coli* isolate (*E. coli* Co52) recovered from a food sample. A comparison of the nucleotide and amino acid sequences of the new $bla_{TEM-95b}$ gene and those of the bla_{TEM-1a} and bla_{TEM-1b} genes is shown in Table 4. The gene encoding $bla_{TEM-95b}$ shows a mutation at nucleotide 635 (C→G) (numbering is according to Sutcliffe [47]), causing a change in amino acid 145 (proline→alanine) (numbering is according to Ambler and Coulson [1]). Both

TABLE 1. Susceptibilities to different β-lactams of the 124 AMP^r *E. coli* isolates included in the study

Antibiotic and origin[a]	No. of isolates for which MIC (μg/ml) was:														
	≤0.015	0.03	0.06	0.125	0.25	0.5	1	2	4	8	16	32	64	128	≥256
Ampicillin															
Humans												2			47
Animals or food												7	3	3	62
Ticarcillin															
Humans										2		1			46
Animals or food							2	1	1	2		1	1	3	64
AMC															
Humans									4	20	17	5	3		
Animals or food									14	23	32	2	4		
Cefazolin															
Humans							2	25	13	6	2	1			
Animals or food						1	7	32	28	3			2		2
Cefoxitin															
Humans							1		23	21	2	1	1		
Animals or food							2	7	22	38	1		1	4[b]	
Ceftazidime															
Humans			2	12	32	1	1			1					
Animals or food	1		10	19	31	12		1		1					
Cefotaxime															
Humans		1	22	20	3	2			1						
Animals or food	1	14	21	19	13	5		1	1						
Ceftriaxone															
Humans	2	19	19	7	1			1							
Animals or food	8	27	27	11			2								
Imipenem															
Humans		4[c]	1	22	21	1									
Animals or food				16	54	5									
Aztreonam															
Humans		19	17	10	1	1			1						
Animals or food	5	24	29	14	1		1		1						

[a] Humans, $n = 49$; Animals or food $n = 75$.
[b] MIC, ≥128 μg/ml.
[c] MIC, ≤0.03 μg/ml.

strands of the amplicons from three independent PCRs were sequenced to ascribe this sequence, and identical results were obtained in all three cases. This sequence is included in the EMBL database under accession number AJ308558. The MICs of β-lactam antibiotics for *E. coli* Co52 are as follows: AMP and TIC, >256 μg/ml; AMC, 8/4 μg/ml; cefazolin, 4 μg/ml; cefoxitin, 8 μg/ml; ceftazidime, 0.5 μg/ml; cefotaxime and imipenem, 0.25 μg/ml; and ceftriaxone and aztreonam, 0.125 μg/ml. As can be observed, *E. coli* Co52 shows a phenotype of resistance similar to that conferred in *E. coli* by the expression of a TEM-1-type β-lactamase.

The promoter region was analyzed in 24 bla_{TEM} amplicons in which the electropherogram allowed us a clear reading of the entire region. The weak *P3* promoter was found in 23 of these sequences. The AMC MICs for all isolates with bla_{TEM} genes and the *P3* promoter were within the range of 8 to 32 μg/ml (for 17 of these isolates the AMC MIC was 16 μg/ml). Of the series of 23 isolates with the *P3* promoter, 20 of them had the $bla_{\text{TEM-1}}$ gene, 1 had the $bla_{\text{TEM-51a}}$ gene, and other 2 had the $bla_{\text{TEM-30c}}$ and the $bla_{\text{TEM-95b}}$ genes, respectively. The strong *Pa*/*Pb* promoter was found in only one isolate with the $bla_{\text{TEM-34b}}$ gene, which encodes an IRT β-lactamase (the AMC MIC for this isolate was 64 μg/ml) (Table 3). The *Pa*/*Pb* promoter was identified by a mutation at nucleotide position 32 (C→T) of the bla_{TEM} promoter region.

In this study, the cefoxitin MICs for 10 *E. coli* isolates were ≥16 μg/ml (4 isolates from foods, 2 isolates from broilers, and 4 isolates from humans) (Table 5). A bla_{TEM} gene was detected by PCR in 3 of these 10 isolates. Amplification of the *ampC* gene promoter, including the −35 and −10 boxes as well as the attenuator, was carried out for all 10 isolates by using specific primers. The expected 191-bp fragment was obtained for six of these isolates. A negative PCR result was obtained for the remaining four isolates (the cefoxitin MIC was >64 μg/ml for all of them); these four isolates proved to be unrelated when they were studied by the pulsed-field gel electrophoresis methodology (data not shown). The six *ampC* amplicons detected were sequenced, and mutations were analyzed

TABLE 2. PCR detection of *bla* genes in the 124 AMP *E. coli* isolates included in this study

Origin and AMC resistance phenotype of isolates			No. of isolates in which *bla* genes for the following were detected by PCR:		
Origin (no.)	Phenotype[a]	No. of isolates	TEM	SHV	OXA
Broiler (22)	S	15	12	0	0
	I	6	5	0	1
	R	1	0	0	0
Food (20)	S	10	5	1	1
	I	5	4	0	0
	R	5	1	0	0
Pig (20)	S	9	5	0	1
	I	11	11	0	0
	R	0	0	0	0
Pet, bull, or horse (13)	S	3	3	0	0
	I	10	10	0	0
	R	0	0	0	0
Human patient (40)	S	23	23	0	0
	I	11	11	0	0
	R	6	6	0	0
Healthy human (9)	S	1	1	0	0
	I	6	6	0	0
	R	2	0	0	0

[a] S, susceptible; I, intermediate; R, resistant.

by comparing the nucleotide sequences with the nucleotide sequence of the same region in the *E. coli* K-12 *ampC* gene (24) (Table 5). Two promoters (*E. coli* Co100 and A99; cefoxitin MICs = 64 μg/ml) showed point mutations at positions −42, −18, −1, and +58. Two promoters (*E. coli* Co313 and Co321; cefoxitin MICs = 16 μg/ml) showed mutations at positions −32 and −28. The last two promoters (*E. coli* Co64 and Co210; cefoxitin MICs = 16 to 32 μg/ml) did not show mutations in the *ampC* promoter (Table 5). Mutations at position −32 or −42 were observed in four *E. coli* isolates recovered from three human samples and one broiler. The amplification of the *ampC* promoter was also performed with four additional *E. coli* isolates from our series, for which the cefoxitin MICs were 2 to 8 μg/ml (Table 5). Three point mutations (at positions −18, −1, and +58) were detected in two promoters (*E. coli* Co51 and Co61; cefoxitin MICs = 8 μg/ml). Two point mutations were detected (positions −28 and +17) in another *ampC* promoter (*E. coli* Co183; cefoxitin MIC = 2 μg/ml). No mutations were found in the last promoter (*E. coli* Co170; cefoxitin MIC = 2 μg/ml).

TABLE 4. Nucleotide mutation and amino acid substitution in the new β-lactamase-encoding gene $bla_{TEM-95b}$ compared to the sequences of bla_{TEM-1a} and bla_{TEM-1b}

bla_{TEM} gene (promoter)	Base at the indicated nucleotide position[a] (amino acid position)[b] in the:						
	Promoter region			Coding region			
	32	162	175	226 (6)	436 (78)	604 (134)	635 (145)
bla_{TEM-1a} (*P3*)	C	G	A	C	C	G	C (Pro)
bla_{TEM-1b} (*P3*)	C	G	G	T	T	T	C
$bla_{TEM-95b}$ (*P3*)	C	G	G	T	T	T	G (Ala)

[a] Numbered according to Sutcliffe (47).
[b] Numbered according to Ambler and Coulson (1).

The correlation between the β-lactam resistance phenotypes and the mechanism of resistance detected in our 124 AMP^r *E. coli* isolates are shown in Table 6. The following were the most frequent resistance patterns observed. Isolates of phenotype I were resistant to AMP and TIC but susceptible to the other β-lactams tested (48% of the isolates). Classic TEM and SHV

TABLE 3. Types of bla_{TEM} genes detected in 57 AMP^r *E. coli* isolates in this study

Origin and AMC resistance phenotypes of isolates			bla_{TEM} genes types				
Origin	Phenotype[a]	No. of isolates	No. of isolates with the following bla_{TEM-1} molecular variant:				Other bla_{TEM} genes[b] (promoter)
			bla_{TEM-1a}	bla_{TEM-1b}	bla_{TEM-1c}	bla_{TEM-1f}	
Broiler	S	8	1	7			
	I	3	1	2			
Food	S	1					$bla_{TEM-95b}$ (*P3*)[c]
	I	4	1	3			
	R	1		1			
Pig	I	10		8	1		$bla_{TEM-51a}$ (*P3*)
Pet, bull, or horse	I	9	1	7	1		
Healthy human	I	5		1	4		
Human patient	I	10	5	5			
	R	6		2		1	$bla_{TEM-30c}$ (*P3*), $bla_{TEM-34b}$ (*Pa*/*Pb*), $bla_{TEM-40b}$

[a] S, susceptible; I, intermediate; R, resistant.
[b] Each of these bla_{TEM} genes was detected in a single *E. coli* isolate.
[c] New bla_{TEM} gene found in this study (EMBL database accession number AJ308558).

TABLE 5. Mutations found in the promoter and/or attenuator region of the *ampC* genes of 14 *E. coli* isolates analyzed in this study for which cefoxitin MICs were different

E. coli isolate	Origin	Cefoxitin MIC (μg/ml)	*bla* gene detected	Positions of mutations at the *ampC* promoter[a]
Co13	Food	>64	None	ND[b]
Co22	Food	>64	None	ND
Co23	Food	>64	None	ND
Co25	Food	>64	None	ND
Co100[c]	Broiler	64	None	−42, −18, −1, +58
A99[c]	Human patient	64	bla_{TEM}	−42, −18, −1, +58
Co210	Human patient	32	bla_{TEM}	None
Co64	Broiler	16	bla_{TEM}	None
Co313	Healthy human	16	None	−32, −28
Co321	Healthy human	16	None	−32, −28
Co51	Food	8	bla_{OXA}	−18, −1, +58
Co61	Broiler	8	bla_{OXA}	−18, −1, +58
Co183	Dog	2	bla_{TEM}	−28, +17
Co170[d]	Pig	2	None	None

[a] Mutations with respect to the *E. coli* K-12 sequence (24).
[b] ND, not determined. No PCR amplification was obtained with the primers for the *ampC* promoter.
[c] This isolate shows a diminished susceptibility to extended-spectrum cephalosporins.
[d] The ampicillin MIC for this isolate is 2 μg/ml.

β-lactamases were found in isolates of phenotype I, although a new TEM-type β-lactamase (TEM-95) was also detected in this group. Isolates of phenotype II were resistant to AMP and TIC and resistant or of intermediate susceptibility to AMC, in conjunction with susceptibility to the other β-lactams tested (41% of the isolates). The main mechanism of resistance found in isolates of phenotype II was the production of the TEM-1 β-lactamase, although different types of IRT enzymes (TEM-30, TEM-34, TEM-40, and TEM-51) were also detected. Six other phenotypes of β-lactam resistance were found in 12% of the isolates, and different mechanisms of resistance were demonstrated (Table 6). The two *E. coli* isolates from this study for which the ceftazidime and cefotaxime MICs were in the range of 4 to 8 μg/ml (diminished susceptibility) comprised the phenotype VI isolates. These isolates were recovered from a broiler (*E. coli* Co100) and from a human fecal sample (*E. coli* A99).

DISCUSSION

As shown in Table 6, almost half (48%) of the AMP^r *E. coli* isolates recovered from foods and fecal samples of humans and healthy animals showed a β-lactam resistance phenotype that included resistance only to aminopenicillins (AMP and TIC) and not to other β-lactams or β-lactamase inhibitors (phenotype I), and 41% of the isolates showed the AMP^r TIC^r AMC^{i,r} phenotype (phenotype II). Phenotype I (AMP^r TIC^r) was less frequently found (15%) in a study performed by other investigators with consecutive AMP^r *E. coli* isolates of human clinical origin (49), and a large group of the isolates in that series (62%) showed the AMP^r TIC^r AMC^r phenotype. The widespread use of β-lactams alone or in combination with β-lactamase inhibitors in hospitals could account for the higher frequency of phenotype II among clinical isolates.

It has been shown in this study that the most frequent mech-

TABLE 6. Phenotypes of β-lactam resistance and β-lactamases identified in 124 AMP^r *E. coli* isolates of different origins

Resistance phenotype	No. (%) of isolates	Phenotype of β-lactam resistance[a]								Mechanism detected (no. of isolates)
		AMP	TIC	AMC	FOX	CAZ	CTX	IMP	ATM	
I	59 (48)	R	R	S	S	S	S	S	S	TEM[b] (39), TEM-1 (8), TEM-95 (1), SHV[b] (1), negative[c] (9), OXA[b] (1)
II	51 (41)	R	R	R, I	S	S	S	S	S	TEM-1 (42), TEM[b] (4), TEM-51 (1), TEM-30 (1), TEM-34 (1), TEM-40 (1), OXA[b] (1)
III	6 (4.8)	R	S	R, I	R, I	S	S	S	S	AmpC[c,d] (2), AmpC[c,e] (4)
IV[f]	2 (1.6)	R	R	I	R, I	S	S	S	S	TEM-1 (1), TEM[b] (1)
V	2 (1.6)	R	S	I	S	S	S	S	S	TEM-1 (1), OXA[b] (1)
VI[g]	2 (1.6)	R	R, I	R	R	S↓	S↓	S	S↓	TEM-1 + AmpC[d] (1), AmpC[c,d] (1)
VII[h]	1 (0.8)	R	R	S	S	S↓	S↓	S	S	Negative[c] (1)
VIII	1 (0.8)	R	I	S	S	S	S	S	S	TEM[b] (1)

[a] AMP, ampicillin; TIC, ticarcillin, AMC, amoxicillin-clavulanic acid; FOX, cefoxitin; CAZ, ceftazidime; CTX, cefotaxime; IMP, imipenem; ATM, aztreonam; S, susceptible, S↓, diminished susceptibility; I, intermediate susceptibility; R, resistant.
[b] This result was obtained by PCR but not by sequencing.
[c] PCRs for TEM, SHV, and OXA were negative.
[d] A point mutation was detected at either position −32 or −42 in the *ampC* promoter.
[e] Putative mechanism of resistance.
[f] Other mechanism of cefoxitin resistance could be involved, such as through a efflux pump or permeability.
[g] For the two *E. coli* isolates with this phenotype (Co100 and A99), cefotaxime, ceftazidime and aztreonam MICs were in the range of 4 to 8 μg/ml.
[h] For the single *E. coli* isolate with this phenotype (Co129), the cefotaxime and ceftazidime MICs were 2 μg/ml.

anism of AMP resistance in *E. coli* isolates recovered from foods and feces of healthy animals and humans was by a TEM-type β-lactamase (83%). SHV- or OXA-type β-lactamases were detected in only a few isolates (3%). A bla_{TEM-1} gene was found in 91% of the bla_{TEM} amplicons sequenced. We also found a number of molecular variants: bla_{TEM-1a}, bla_{TEM-1b}, bla_{TEM-1c}, and bla_{TEM-1f}. The most frequent variant among our isolates was bla_{TEM-1b} (69%). The first description of the bla_{TEM-1b} molecular variant was provided by Chen and Clowes (15), although it was called *blaT1b*. Goussard and Courvalin (20) completed the sequence of this variant. Recently, a large variety of other bla_{TEM} genes have been reported: bla_{TEM-1c} (21) and bla_{TEM-1d}, bla_{TEM-1e}, and bla_{TEM-1f} (28). We found bla_{TEM-1c} in *E. coli* isolates recovered from animals (one isolate from a pig and one isolate from a dog) and from healthy humans (four isolates). The bla_{TEM-1c} gene was previously described in a clinical *E. coli* isolate (21). In our study the bla_{TEM-1f} gene was found in only one nonpathogenic AMP^r *E. coli* isolate recovered from the feces of a human patient. The bla_{TEM-1f} gene was very recently described (29) in human clinical *E. coli* isolates. Very few studies on the molecular characterization of the bla_{TEM} genes in isolates of food or animal origin have been performed to date. The reason why some molecular variants of the bla_{TEM} genes are mainly found in human *E. coli* isolates and not in animal isolates could be linked to the use of different β-lactams in humans and animals or, probably, to a lack of studies with animal isolates. More extensive studies with animal *E. coli* isolates are required to obtain conclusive results.

The weak *P3* promoter was found in all 20 bla_{TEM-1} genes (molecular variants bla_{TEM-1a}, bla_{TEM-1b}, bla_{TEM-1c}, and bla_{TEM-1f}) analyzed for their promoter sequences. The *P3* promoter corresponding to molecular variants bla_{TEM-1a}, bla_{TEM-1b}, bla_{TEM-1c}, bla_{TEM-1d}, and bla_{TEM-1e} has previously been found in *E. coli* isolates (15, 20, 21, 29, 47). Nevertheless, the strong *P4* promoter was detected in the only studies in which an *E. coli* isolate containing the bla_{TEM-1f} gene was described (28, 29).

In our study, the *bla* genes encoding IRT-type β-lactamases were found in only four isolates recovered from one pig fecal sample ($bla_{TEM-51a}$ [*P3*]) and three fecal samples from human patients ($bla_{TEM-30c}$ [*P3*], $bla_{TEM-34b}$ [*Pa*/*Pb*], and $bla_{TEM-40b}$). These IRT β-lactamases showed a single amino acid substitution at either position 69 (TEM-34 and TEM-40) or position 244 (TEM-30 and TEM-51). The bla_{TEM-34} and bla_{TEM-40} genes were described for the first time in 1994 and 1995, respectively, from clinical *E. coli* isolates (44, 52). Variants $bla_{TEM-34b}$ (*Pa*/*Pb*) and $bla_{TEM-40b}$ found in our study have previously been reported by Leflon-Guibout et al. (28) in clinical *E. coli* isolates. The TEM-30 β-lactamase was first described by Vedel et al. (50), and its sequence was published 2 years later (3). The $bla_{TEM-30c}$ variant did not appear in the studies in which the bla_{TEM-30} gene was reported by using the new nomenclature (28). The TEM-51 β-lactamase in an *E. coli* clinical isolate was described for the first time in 1997 by Bret et al. (7). In that paper the molecular variant was not mentioned, but from a comparison of its sequence with those of all the variants, it can be concluded that it corresponds to $bla_{TEM-51c}$ or $bla_{TEM-51d}$. To our knowledge, our paper represents the first description of the variants $bla_{TEM-30c}$ (*P3*) and $bla_{TEM-51a}$ (*P3*). Leflon-Guibout et al. (28) reported that the IRT-encoding genes show strong promoters most of the time, whereas in our study two of the three IRT-encoding genes studied showed weak promoters and only one showed the *Pa*/*Pb* strong promoter (TEM-34).

The new $bla_{TEM-95b}$ (*P3*) gene found in this study showed one mutation at position 635, which led to the change of the amino acid proline-145 to an alanine. The roles played by specific mutations in the action spectra of TEM β-lactamases were analyzed previously and described in different papers (4, 13, 27, 43, 48). The new $bla_{TEM-95b}$ (*P3*) gene renders a β-lactam resistance phenotype similar to those of other isolates in this study with a TEM-1-type β-lactamase. In fact, the isolate was resistant only to aminopenicillins (AMP and TIC) but was susceptible to the other β-lactams tested. Amino acid 145 is positioned at the beginning of the 5α helix. This change could affect the tertiary structure, but according to our results, this substitution does not affect the inhibition profile. None of the important substitutions found in the IRT variants (amino acid positions 69, 165, 182, 244, 275, and 276) occurs at position 145 (4, 13, 27, 48). The purification of this new enzyme is in process in our laboratory.

For 10 of the *E. coli* isolates tested the cefoxitin MIC was ≥16 μg/ml. The hyperproduction of the AmpC chromosomal β-lactamase might be a possible mechanism of cefoxitin resistance. The mutations at positions −42 and −32 of the *ampC* promoter region were previously reported to be important in increasing the level of *ampC* transcription (11, 12, 26, 36, 37, 38). Among our isolates, four *E. coli* isolates for which the cefoxitin MICs were within the range of 16 to 64 μg/ml showed mutations at position −42 or −32. Mutations at both positions were not found together in the same isolate in our study, and similar results were documented previously (11, 37). Jaurin and Grundström (24) reported that a mutation at position +24 was important to increase the level of transcription due to the modification of the attenuator loop structure. This specific mutation was not found in our isolates. We detected three mutations (at positions −18, −1, and +58) in two isolates for which the cefoxitin MIC was 8 μg/ml. These three changes are frequent in resistant isolates but are usually combined with the mutation at position −42 (11, 37). As expected, no mutation was found in an *E. coli* isolate for which the cefoxitin MIC was 2 μg/ml. Nevertheless, in another isolate for which the MIC was the same, two mutations were identified (at positions −28 and +17). According to our results, it might seem that these mutations are not related to an increase in the level of *ampC* transcription. In some of our isolates with resistance or diminished susceptibility to cefoxitin and in which no mechanisms of cefoxitin resistance were identified (phenotype IV of Table 6), other mechanisms of resistance could be involved, such as the presence of plasmidic AmpC β-lactamases (2, 5, 18, 19, 32, 40, 51) and even the presence of efflux pumps or altered porins (33).

It is important to emphasize the spread of different variants of *bla* genes, including IRT β-lactamase-encoding genes, among nonpathogenic *E. coli* isolates recovered from foods or from the intestinal environments of humans and healthy animals. Further research should be carried out to study in depth the distribution and evolution of the *bla* genes in isolates from different ecosystems.

ACKNOWLEDGMENTS

This work was partly supported by a grant from the Fondo de Investigaciones Sanitarias of Spain (grant FIS 01/973) and by grants of the Consejería de Educación (grant ACPI/2001/04) and the Consejería de Salud del Gobierno de La Rioja of Spain. Laura Briñas has a fellowship from the Gobierno de La Rioja of Spain.

REFERENCES

1. **Ambler, R. P., and A. F. W. Coulson.** 1991. A standard numbering scheme for the class A β-lactamases. Biochem. J. **276:**269–272.
2. **Bauernfeind, A., S. Wagner, R. Jungwirth, I. Schneider, and D. Meyer.** 1997. A novel class C β-lactamase (FOX-2) in *Escherichia coli* conferring resistance to cephamycins. Antimicrob. Agents Chemother. **41:**2041–2046.
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. **Bonomo, R. A., and L. B. Rice.** 1999. Inhibitor resistant class A beta-lactamases. Frontiers Biosci. **4:**34–41.
5. **Bou, G., A. Oliver, M. Ojeda, C. Monzón, and J. Martínez-Beltrán.** 2000. Molecular characterization of FOX-4, a new AmpC-type plasmid-mediated β-lactamase from an *Escherichia coli* strain isolated in Spain. Antimicrob. Agents Chemother. **44:**2549–2553.
6. **Bradford, P. A., P. J. Petersen, I. M. Fingerman, and D. G. White.** 1999. Characterization of extended-spectrum cephalosporin resistance in *E. coli* isolates associated with bovine calf diarrhoeal disease. J. Antimicrob. Chemother. **44:**607–610.
7. **Bret, L., E. B. Chaïbi, C. Chanal-Claris, D. Sirot, R. Labia, and J. Sirot.** 1997. Inhibitor-resistant TEM (IRT) β-lactamases with different substitutions at position 244. Antimicrob. Agents Chemother. **41:**2547–2549.
8. **Burman, L. G., S. Haeggman, M. Kuistila, K. Tullus, and P. Huovinen.** 1992. Epidemiology of plasmid-mediated beta-lactamases in enterobacteria Swedish neonatal wards and relation to antimicrobial therapy. Antimicrob. Agents Chemother. **36:**989–992.
9. **Bush, K., G. A. Jacoby, and A. A. Medeiros.** 1995. A functional classification scheme for beta-lactamases and its correlation with molecular structures. Antimicrob. Agents Chemother. **39:**1211–1233.
10. **Bush, K., and G. A. Jacoby.** 1997. Nomenclature of TEM β-lactamases. J. Antimicrob. Chemother. **39:**1–3.
11. **Caroff, N., E. Espaze, I. Bérard, H. Richet, and A. Reynaud.** 1999. Mutations in the *ampC* promoter of *Escherichia coli* isolates resistant to oxyiminocephalosporins without extended spectrum β-lactamase production. FEMS Microbiol. Lett. **173:**459–465.
12. **Caroff, N., E. Espaze, D. Gautreau, H. Richet, and A. Reynaud.** 2000. Analysis of the effects of −42 and −32 *ampC* promoter in clinical isolates of *Escherichia coli* hyperproducing AmpC. J. Antimicrob. Chemother. **45:**783–788.
13. **Chaïbi, E. B., D. Sirot, G. Paul, and R. Labia.** 1999. Inhibitor resistant TEM β-lactamases: phenotypic, genetic and biochemical characteristics. J. Antimicrob. Chemother. **43:**447–458.
14. **Chen, S. T., and R. C. Clowes.** 1984. Two improved promoter sequences for the beta-lactamase expression arising from a single base-pair substitution. Nucleic Acids Res. **12:**3219–3234.
15. **Chen, S. T., and R. C. Clowes.** 1987. Variations between the nucleotide sequences of Tn*1*, Tn*2*, and Tn*3* and expression of beta-lactamase in *Pseudomonas aeruginosa* and *Escherichia coli*. J. Bacteriol. **169:**913–916.
16. **Edlund, T., T. Grundstrom, and S. Normark.** 1977. Isolation and characterization of DNA repetitions carrying the chromosomal β-lactamase gene of *Escherichia coli* K12. Mol. Gen. Genet. **173:**115–125.
17. **Féria, C., E. Ferreira, J. D. Correia, J. Goncalves, and M. Canica.** 2002. Patterns and mechanisms of resistance to β-lactams and β-lactamase inhibitors in uropathogenic *Escherichia coli* isolates from dogs in Portugal. J. Antimicrob. Chemother. **49:**77–85.
18. **Gazouli, M., L. S. Tzouvelekis, A. C. Vatopoulos, and E. Tzelepi.** 1998. Transferable class C β-lactamases in *Escherichia coli* strains isolated in Greek hospitals and characterization of two enzyme variants (LAT-3 and LAT-4) closely related to *Citrobacter freundii* AmpC β-lactamase. J. Antimicrob. Chemother. **42:**419–425.
19. **Gonzalez Leiza, M., J. C. Pérez-Díaz, J. Ayala, J. M. Casellas, J. Martinez-Beltrán, K. Bush, and F. Baquero.** 1994. Gene sequence and biochemical characterization of FOX-1 from *Klebsiella pneumoniae*, a new AmpC-type plasmid-mediated β-lactamase with two molecular variants. Antimicrob. Agents Chemother. **38:**2150–2157.
20. **Goussard, S., and P. Courvalin.** 1991. Sequence of the genes *blaT-1B* and *blaT-2*. Gene **102:**71–73.
21. **Goussard, S., and P. Courvalin.** 1999. Updated sequence information for TEM β-lactamase genes. Antimicrob. Agents Chemother. **43:**367–370.
22. **Gulay, Z., M. Bicmen, S. G. Amyes, and N. Yulug.** 2000. Beta-lactamase patterns and betalactam/clavulanic acid resistance in *Escherichia coli* isolated from fecal samples from healthy volunteers. J. Chemother. **12:**208–215.
23. **Hunter, J. E. B., J. E. Corkill, A. G. McLennan, J. N. Fletcher, and C. A. Hart.** 1993. Plasmid encoded β-lactamases resistant to inhibition by clavulanic acid produced by calf faecal coliforms. Res. Vet. Sci. **55:**367–370.
24. **Jaurin, B., and T. Grundström.** 1981. AmpC cephalosporinase of *Escherichia coli* K-12 has a different evolutionary origin from that of β-lactamases of the penicillinase type. Proc. Natl. Acad. Sci. USA **78:**4897–4901.
25. **Jaurin, B., T. Grundström, T. Edlund, and S. Normak.** 1981. The *E. coli* β-lactamase attenuator mediates growth-dependent regulation. Nature **290:**221–225.
26. **Jaurin, B., T. Grundström, and S. Normak.** 1982. Sequence elements determining *ampC* promoter strength in *E. coli.* EMBO J. **1:**875–881.
27. **Knox, J.** 1995. Extended-spectrum and inhibitor-resistant TEM-type β-lactamases: mutations, specificity, and three-dimensional structure. Antimicrob. Agents Chemother. **39:**2593–2601.
28. **Leflon-Guibout, V., B. Heym, and M.-H. Nicholas-Chanoine.** 2000. Updated sequence information and proposed nomenclature for bla_{TEM} genes and their promoters. Antimicrob. Agents Chemother. **44:**3232–3234.
29. **Leflon-Guibout, V., V. Speldooren, B. Heym, and M.-H. Nicholas-Chanoine.** 2000. Epidemiological survey of amoxicillin-clavulanate resistance and corresponding molecular mechanisms in *Escherichia coli* isolates in France: new genetic features of bla_{TEM} genes. Antimicrob. Agents Chemother. **44:**2709–2714.
30. **Livermore, D. M.** 1995. β-Lactamases in laboratory and clinical resistance. Clin. Microbiol. Rev. **8:**557–584.
31. **Livermore, D. M.** 1998. Beta-lactamase-mediated resistance and opportunities for its control. J. Antimicrob. Agents **41**(Suppl. D)**:**25–41.
32. **Marchese, A., A. Guillaume, G. C. Schito, P. H. Lagrange, and A. Philippon.** 1998. Characterization of FOX-3, an AmpC-type plasmid-mediated β-lactamase from an Italian isolate of *Klebsiella oxytoca*. Antimicrob. Agents Chemother. **42:**464–467.
33. **Martinez-Martinez, L., M. C. Conejo, A. Pascual, S. Hernández-Allés, S. Ballesta, E. Ramírez de Arellano-Ramos, V. J. Benedí, and E. J. Perea.** 2000. Activities of imipenem and cephalosporins against clonally related strains of *Escherichia coli* hyperproducing chromosomal β-lactamases and showing altered porin profiles. Antimicrob. Agents Chemother. **44:**2534–2536.
34. **Naas, T., and P. Nordmann.** 1999. OXA-type β-lactamases. Curr. Pharm. Design **5:**865–879.
35. **National Committee for Clinical Laboratory Standards.** 2000. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. NCCLS document M7-A5. National Committee for Clinical Laboratory Standards, Wayne, Pa.
36. **Nelson, E. C., and B. G. Elisha.** 1999. Molecular basis of AmpC hyperproduction in clinical isolates of *Escherichia coli*. Antimicrob. Agents Chemother. **43:**957–959.
37. **Olsson, O., S. Bergstrom, and S. Normark.** 1982. Identification of a novel AmpC beta-lactamase promoter in a clinical isolate of *Escherichia coli*. EMBO J. **1:**1411–1416.
38. **Olsson, O., S. Bergstrom, F. P. Lindberg, and S. Normark.** 1983. AmpC beta-lactamase hyperproduction in *Escherichia coli*: natural ampicillin resistance generated by horizontal chromosomal DNA transfer from *Shigella*. Proc. Natl. Acad. Sci. USA **80:**7556–7560.
39. **Pitout, J. 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.
40. **Queenan, A. M., S. Jenkins, and K. Bush.** 2001. Cloning and biochemical characterization of FOX-5, an AmpC-type plasmid-encoded β-lactamase from a New York City *Klebsiella pneumoniae* clinical isolate. Antimicrob. Agents Chemother. **45:**3189–3194.
41. **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.
42. **Shanahan, P. M., C. J. Thomson, and S. G. Amyes.** 1995. Beta-lactam resistance in normal faecal flora from South Africa. Epidemiol. Infect. **115:**243–253.
43. **Sideraki, V., W. Huang, T. Palzkill, and H. F. Gilbert.** 2001. A secondary drug resistance mutation of TEM-1 β-lactamases that suppresses misfolding and aggregation. Proc. Natl. Acad. Sci. USA **98:**283–288.
44. **Stapleton, P., P. J. Wu, A. King, K. Shannon, G. French, and I. Phillips.** 1995. Incidence and mechanisms of resistance to the combination of amoxicillin and clavulanic acid in *Escherichia coli*. Antimicrob. Agents Chemother. **39:**2478–2483.
45. **Steward, C. D., J. K. Rasheed, S. K. Hubert, J. W. Biddle, P. M. Raney, G. J. Anderson, P. P. Williams, K. L. Britain, 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.

46. **Sunde, M., and H. Sorum.** 1999. Characterization of integrons in *Escherichia coli* of the normal intestinal flora of swine. Microb. Drug Resist. **5:**279–287.
47. **Sutcliffe, J. G.** 1978. Nucleotide sequence of the ampicillin resistance gene of *Escherichia coli* plasmid pBR322. Proc. Natl. Acad. Sci. USA **75:**3737–3741.
48. **Vakulenko, S. B., B. Geryk, L. P. Kotra, S. Mobashery, and S. A. Lerner.** 1998. Selection and characterization of β-lactam–β-lactamase inactivator-resistant mutants following PCR mutagenesis of the TEM-1 β-lactamase gene. Antimicrob. Agents Chemother. **42:**1542–1548.
49. **Vanjak, D., C. Muller-Serieys, B. Picard, E. Bergogne-Berezin, and N. Lambert-Zechovsky.** 1995. Activity of beta-lactamase inhibitor combinations on *Escherichia coli* isolates exhibiting various patterns of resistance to beta-lactam agents. Eur. J. Clin. Microbiol. Infect. Dis. **14:**972–978.
50. **Vedel, G., A. Belaaouaj, L. Gilly, R. Labia, A. Philippon, P. Nevot, and G. Paul.** 1992. Clinical isolates of *Escherichia coli* producing TRI β-lactamases: novel TEM-enzymes conferring resistance to β-lactamase inhibitors. J. Antimicrob. Chemother. **30:**449–462.
51. **Winokur, P. L., D. L. Vonstein, L. J. Hoffman, E. K. Uhlenhopp, and G. V. Doern.** 2001. Evidence for transfer of CMY-2 AmpC β-lactamase plasmids between *Escherichia coli* and *Salmonella* isolates from food animals and humans. Antimicrob. Agents Chemother. **45:**2716–2722.
52. **Zhou, X. Y., F. Bordon, D. Sirot, M.-D. Kitzis, and L. Gutmann.** 1994. Emergence of clinical isolates of *Escherichia coli* producing TEM-1 derivatives or an OXA-1 β-lactamase conferring resistance to β-lactamase inhibitors. Antimicrob. Agents Chemother. **38:**1085–1089.