Applied and Environmental Microbiology, June 2010, p. 4118–4120 Vol. 76, No. 12
0099-2240/10/$12.00 doi:10.1128/AEM.02761-09

Genetic Detection of Extended-Spectrum β-Lactamase-Containing *Escherichia coli* Isolates from Birds of Prey from Serra da Estrela Natural Reserve in Portugal[▽]

Luís Pinto,[1,2,3,4] Hajer Radhouani,[1,2,3,4] Céline Coelho,[1,2,3,4] Paulo Martins da Costa,[5,6] Roméo Simões,[5] Ricardo M. L. Brandão,[7] Carmen Torres,[8] Gilberto Igrejas,[1,2] and Patrícia Poeta[3,4]*

Department of Genetics and Biotechnology, University of Trás-os-Montes and Alto Douro, Vila Real, Portugal[1]; Institute for Biotechnology and Bioengineering, Centre of Genetics and Biotechnology, University of Trás-os-Montes and Alto Douro, Vila Real, Portugal[2]; Centre of Studies of Animal and Veterinary Sciences, University of Trás-os-Montes and Alto Douro, Vila Real, Portugal[3]; Veterinary Science Department, University of Trás-os-Montes and Alto Douro, Vila Real, Portugal[4]; Abel Salazar Biomedical Sciences Institute, University of Oporto, Oporto, Portugal[5]; Interdisciplinary Center for Marine and Environmental Research, University of Oporto, Oporto, Portugal[6]; Center of Ecology, Monitoring and Recovery of Wild Animal, Gouveia, Portugal[7]; and Biochemistry and Molecular Biology Area, University of La Rioja, Logroño, Spain[8]

Received 13 November 2009/Accepted 12 April 2010

Extended-spectrum β-lactamase-containing *Escherichia coli* isolates were detected in 32 of 119 fecal samples (26.9%) from birds of prey at Serra da Estrela, and these isolates contained the following β-lactamases: CTX-M-1 ($n = 13$), CTX-M-1 plus TEM-1 ($n = 14$), CTX-M-1 plus TEM-20 ($n = 1$), SHV-5 ($n = 1$), SHV-5 plus TEM-1 ($n = 2$), and TEM-20 ($n = 1$).

The high and sometimes excessive use of antibiotics is directly related to the great spread and development of bacterial antibiotic resistance, a problem in public health nowadays. Thus, many studies have been done in order to understand the mechanisms of bacterial drug resistance, as many multidrug-resistant bacterial strains have been detected in domestic animals and humans (12). Recent studies from our research group have focused on wild animals, trying to understand how they acquire resistance to antibiotics without direct contact (11, 17, 20). One of the most clinically relevant antimicrobial resistance mechanisms is constituted by extended-spectrum β-lactamases (ESBLs) (3, 16) harbored by *Enterobacteriaceae*, such as *Escherichia coli*, commensals of the gastrointestinal tract of animals and humans, serving as a reservoir for resistances, namely, to β-lactam antibiotics (1, 15). Most ESBLs are derivatives of TEM or SHV enzymes, but a new family of plasmid-mediated ESBLs, CTX-M, has also been reported worldwide in *E. coli* and other *Enterobacteriaceae*. Different variants of the β-lactamase TEM, as in the case of TEM-1, are often reported in cases of plasmid-mediated β-lactamase resistance (2, 5, 8, 14, 21, 22). In this study, fecal samples from wild birds of prey at the Serra da Estrela Natural Reserve were analyzed in order to detect ESBL-containing *E. coli* isolates and characterize the type of ESBL-encoding genes and other associated resistance genes and the corresponding phylogenetic groups.

One hundred nineteen fecal samples from birds of prey were recovered from April to July 2008 and studied for the presence of ESBL-containing *E. coli* strains (Table 1). All the fecal samples were collected individually from each animal and obtained in collaboration with CERVAS (Center of Ecology, Collecting, Welcome and Handling of Wild Animals). This Center receives injured animals found in its natural environments, is located at the Serra da Estrela Natural Reserve, and belongs to the Portuguese Institute of the Nature Management. None of the animals had been previously fed by humans or had received antibiotics. Each bird that arrives at CERVAS

TABLE 1. Animal species in which ESBL-positive *E. coli* isolates were recovered

Animal species	No. of tested animals	No. of animals with ESBL-positive *E. coli* isolates
Common buzzard (*Buteo buteo*)	23	11
Common barn owl (*Tyto alba*)	8	4
Eurasian tawny owl (*Strix aluco*)	10	2
Booted eagle (*Hieraaetus pennatus*)	11	5
Montagu's harrier (*Circus pygargus*)	5	2
Black kite (*Milvus migrans*)	16	2
Eurasian black vulture (*Aegypius monachus*)	2	1
Bonelli's eagle (*Hieraaetus fasciatus*)	2	1
Eurasian eagle owl (*Bubo bubo*)	8	2
Common raven (*Corvus corax*)	3	2
Other species[a]	31	0

[a] Northern goshawk (*Accipiter gentilis*; $n = 5$); European scops owl (*Otus scops*; $n = 5$); Eurasian sparrowhawk (*Accipiter nisus*; $n = 4$); common crane (*Grus grus*; $n = 2$); honey buzzard (*Pernis apivorus*; $n = 2$); carrion crow (*Corvus corone*; $n = 2$); yellow-legged gull (*Larus cachinnans*; $n = 2$); little owl (*Athene noctua*; $n = 2$); Eurasian marsh harrier (*Circus aeruginosus*; $n = 1$); Egyptian vulture (*Neophron percnopterus*; $n = 1$); common kestrel (*Falco tinnunculus*; $n = 1$); azure-winged magpie (*Cyanopica cyanus*; $n = 1$); European magpie (*Pica pica*; $n = 1$); common swift (*Apus apus*; $n = 1$); and red kite (*Milvus milvus*; $n = 1$).

* Corresponding author. Mailing address: Department of Veterinary Sciences, University of Trás-os-Montes and Alto Douro, Vila Real, Portugal. Phone: 351-259350466. Fax: 351-259350629. E-mail: ppoeta@utad.pt.

▽ Published ahead of print on 23 April 2010.

TABLE 2. Characteristics of the ESBL-positive fecal *E. coli* isolates recovered from birds of prey

Isolate no.	Animal species	Resistance pattern to β-lactam antibiotics[a]	β-Lactamase-encoding genes detected	Resistance pattern to non-β-lactam antibiotics[b]	Resistance genes for non-β-lactam antibiotics	Integron-related genes	Phylogenetic group
B1	*Buteo buteo*	AMP-CTX	$bla_{CTX-M-1}$ plus bla_{TEM-20}	CIP-NAL-TET-STR-SXT	*tetA*, *sul2*	*intI1*	B1
B2	*Buteo buteo*	AMP-CTX-ATM	$bla_{CTX-M-1}$ plus bla_{TEM-1D}	CIP-NAL-TET-SXT	*tetA*, *sul2*	*intI1*	B1
B3	*Buteo buteo*	AMP-CTX	$bla_{CTX-M-1}$	NAL-SXT	*sul2*, *sul3*	*intI1*	B1
B9	*Buteo buteo*	AMP-CTX-ATM	$bla_{CTX-M-1}$ plus bla_{TEM-1b}	CIP-NAL-TET-SXT	*tetA*, *sul2*	*intI1*	B1
B10	*Buteo buteo*	AMP-CTX	$bla_{CTX-M-1}$ plus bla_{TEM-1b}	CIP-NAL-TET-STR-SXT	*tetA*, *sul2*	*intI1*	B1
B14	*Buteo buteo*	AMP-CTX-ATM	$bla_{CTX-M-1}$ plus bla_{TEM-1D}	NAL-TET-STR-CHL-SXT	*tetA*, *sul2*	*intI1*	A
B15	*Buteo buteo*	AMP-CTX	$bla_{CTX-M-1}$ plus bla_{TEM-1D}	NAL-TET-STR-SXT	*aadA*, *tetA*, *sul2*	*intI1*, *intI2*	A
B17	*Buteo buteo*	AMP-CTX	$bla_{CTX-M-1}$	CIP-NAL-STR-SXT	*sul2*, *sul3*	*intI1*	B2
B18	*Buteo buteo*	AMP-CTX-CAZ	bla_{TEM-20}	NAL-TET-STR-SXT-KAN	*tetA*, *sul2*	*intI1*	B1
B19	*Buteo buteo*	AMP-CTX	$bla_{CTX-M-1}$	CIP-NAL-STR-SXT	*sul2*, *sul3*	*intI1*	B2
B24	*Buteo buteo*	AMP-CTX	$bla_{CTX-M-1}$	CIP-NAL-TET-SXT	*tetA*, *sul3*		B2
H6	*Hieraaetus pennatus*	AMP-CTX	$bla_{CTX-M-1}$	NAL-TET-SXT	*sul2*, *sul3*	*intI1*	B1
H7	*Hieraaetus pennatus*	AMP-CTX	bla_{CTXM-1}	TET-SXT	*sul2*	*intI1*	A
H8	*Hieraaetus pennatus*	AMP-CTX	$bla_{CTX-M-1}$ plus bla_{TEM-1b}	CIP-NAL-TET-SXT	*tetA*, *sul2*	*intI1*	B1
H11	*Hieraaetus pennatus*	AMP-CTX	$bla_{CTX-M-1}$ plus bla_{TEM-1d}	CIP- NAL-TET-SXT	*tetA*, *sul2*		A
H12	*Hieraaetus pennatus*	AMP-CTX	$bla_{CTX-M-1}$ plus bla_{TEM-1d}	CIP- NAL-TET-SXT	*tetA*, *sul2*		A
T4	*Tyto alba*	AMP-CTX-ATM	$bla_{CTX-M-1}$ plus bla_{TEM-1d}	NAL-TET-STR-SXT	*aadA*, *tetA*, *sul2*	*intI1*, *intI2*	A
T28	*Tyto alba*	AMP-CTX-AMC	$bla_{CTX-M-1}$ plus bla_{TEM-1b}	NAL-TET-STR-SXT	*aadA*, *tetA*, *sul1*	*intI1*	B2
T29	*Tyto alba*	AMP-CTX-ATM-CAZ-AMC	bla_{SHV-5}	CIP-NAL-TET-STR-CHL- SXT-KAN-TOB-GEN	*aadA*, *sul1*	*intI1*	A
T30	*Tyto alba*	AMP-CTX-ATM	$bla_{CTX-M-1}$ plus bla_{TEM-1b}	TET-STR-CHL-SXT-KAN	*aadA*, *sul1*	*intI1*, *intI2*	B1
S5	*Strix aluco*	AMP-CTX	$bla_{CTX-M-1}$	NAL-TET-SXT	*sul2*, *sul3*	*intI1*	B1
S20	*Strix aluco*	AMP-CTX	$bla_{CTX-M-1}$	CIP-NAL-STR-SXT	*sul2*, *sul3*		B2
M13	*Milvus migrans*	AMP-CTX	$bla_{CTX-M-1}$ plus bla_{TEM-1b}	CIP- NAL-TET-STR-SXT	*sul1*, *sul2*		B1
M21	*Milvus migrans*	AMP-CTX-ATM	$bla_{CTX-M-1}$	NAL-SXT	*sul3*	*intI1*	B1
C22	*Circus pygargus*	AMP-CTX	$bla_{CTX-M-1}$ plus bla_{TEM-1b}	NAL-TET-STR-SXT	*tetA*		B2
C23	*Circus pygargus*	AMP-CTX	$bla_{CTX-M-1}$	NAL-TET-SXT	*sul3*	*intI1*	A
BU26	*Bubo bubo*	AMP-CTX	$bla_{CTX-M-1}$	CIP- NAL-SXT	*sul3*	*intI1*	B2
BU27	*Bubo bubo*	AMP-CTX	$bla_{CTX-M-1}$	CIP-NAL-SXT	*sul3*	*intI1*	B2
CO31	*Corvus corax*	AMP-CTX-ATM	bla_{SHV-5} plus bla_{TEM-1b}	TET-STR-CHL-SXT	*cmlA*, *tetA*, *sul3*	*intI1*	B1
CO32	*Corvus corax*	AMP-CTX-ATM-CAZ	bla_{SHV-5} plus bla_{TEM-1b}	TET- STR-CHL-SXT	*cmlA*, *aadA*, *tetA*, *sul3*	*intI1*	B1
A16	*Aegypius monachus*	AMP-CTX	$bla_{CTX-M-1}$ plus bla_{TEM-1b}	CIP-NAL-STR-CHL-SXT	*sul1*, *sul2*	*intI1*	B1
HI25	*Hieraaetus fasciatus*	AMP-CTX	$bla_{CTX-M-1}$	CIP-NAL-SXT	*sul3*	*intI1*	B2

[a] AMP, ampicillin; AMC, amoxicillin-clavulanic acid; ATM, aztreonam.
[b] GEN, gentamicin; TOB, tobramycin; STR, streptomycin; TET, tetracycline; SXT, sulfamethoxazole-trimethoprim; NAL, nalidixic acid; CIP, ciprofloxacin; CHL, chloramphenicol; KAN, kanamycin.

is placed in an individual cage to be treated and released back into the environment. Sampling was performed by recovering the fecal material in the cloaca by using a sterile swab. They were immediately transferred to peptone water and manipulated during the first 24 h of arrival to the laboratory. Samples were seeded in Levine agar supplemented with cefotaxime (2 mg/liter), and colonies with typical *E. coli* morphology were selected, identified by classical biochemical methods and by the API 20E system (bioMérieux, La Balme Les Grottes, France), and further studied. Susceptibility to 16 antibiotics (ampicillin, amoxicillin plus clavulanic acid [AMC], cefoxitin, cefotaxime [CTX], ceftazidime [CAZ], aztreonam [ATM], imipenem, gentamicin, amikacin, tobramycin, streptomycin, nalidixic acid, ciprofloxacin, sulfamethoxazole-trimethoprim, tetracycline, and chloramphenicol) was tested by the disc diffusion method (7) for all recovered *E. coli* isolates. *E. coli* ATCC 25922 was used as a quality control strain. The double-disc diffusion test (CTX, CAZ, and ATM in the presence or absence of AMC) was performed to detect ESBL production (7).

The presence of genes encoding TEM, SHV, OXA, CTX-M, and CMY type β-lactamases was studied by specific PCRs (17). All obtained amplicons were sequenced on both strands, and sequences were compared with those included in the GenBank database and at the Lahey Clinic website (http://www.lahey.org/Studies/) to identify the β-lactamase genes.

Non-β-lactamase genes [*tetA*/*tetB*, *aadA*, *aac*(3)-II/*aac*(3)-IV, *aac*(6′), *cmlA*, and *sul1*/*sul2*/*sul3*, associated with tetracycline, streptomycin, gentamicin, amikacin, chloramphenicol, and trimethoprim-sulfamethoxazole resistance, respectively] were also studied by PCR (17). The presence of the genes *intI1* and *intI2*, encoding class 1 and 2 integrases, respectively, was studied by PCR. Positive and negative controls from the bacterial collection of the University of Trás-os-Montes and Alto Douro were used in all assays. The ESBL-positive isolates were classified into one of the four main phylogenetic groups, A, B1, B2, and D, by PCR as described previously based on the presence or absence of the *chuA*, *yjaA*, or tspE4.C2 gene (6).

E. coli isolates were detected in Levine-CTX plates for 32 of the 119 samples (26.9%) from wild birds of prey at the Serra da Estrela Natural Reserve (Table 1). All 32 isolates recovered in this survey resulted in a positive ESBL screening test, which also indicated a resistant phenotype to CTX and/or CAZ. The β-lactamase genes detected in these isolates were as follows: $bla_{CTX-M-1}$ ($n = 13$), $bla_{CTX-M-1}$ plus bla_{TEM-1b} ($n = 8$),

$bla_{CTX\text{-}M\text{-}1}$ plus $bla_{TEM\text{-}1d}$ ($n = 6$), $bla_{CTX\text{-}M\text{-}1}$ plus $bla_{TEM\text{-}20}$ ($n = 1$), $bla_{SHV\text{-}5}$ plus $bla_{TEM\text{-}1b}$ ($n = 2$), $bla_{SHV\text{-}5}$ ($n = 1$), and $bla_{TEM\text{-}20}$ ($n = 1$) (Table 2). The $bla_{CTX\text{-}M\text{-}1}$ gene was found in most of our ESBL-positive *E. coli* isolates ($n = 28$); this ESBL was also reported in studies of healthy poultry (13) and by our research group in healthy pets (9) and wild animals (10, 17). It is interesting to underline that approximately half of the $bla_{CTX\text{-}M\text{-}1}$-containing *E. coli* isolates also harbored a variant of a bla_{TEM} gene, concretely $bla_{TEM\text{-}1b}$, $bla_{TEM\text{-}1d}$, or $bla_{TEM\text{-}20}$. The $bla_{TEM\text{-}1b}$ gene is the most frequent variant found in β-lactam-resistant *E. coli* isolates of food, humans, and healthy animals or in wild boars in other studies (4, 18). The $bla_{TEM\text{-}1d}$ genetic variant is relatively infrequent, although it has been detected in some other studies (19). Two ESBL-containing *E. coli* isolates were positive for the presence of $bla_{TEM\text{-}20}$, and another three isolates carried the $bla_{SHV\text{-}5}$ gene. Most ESBL-positive *E. coli* isolates of this study were classified into the A or B1 phylogenetic group (72%), although nine isolates which harbored $bla_{CTX\text{-}M\text{-}1}$ with or without $bla_{TEM\text{-}1b}$ were included in the B2 phylogenetic group. The predominance of CTX-M-1-producing isolates belonging to phylogenetic group B2 is of great concern, as, in fact, this phylogenetic group often carries virulence determinants that are less frequently present in other phylogenetic groups. Similar results were found in wild boars by our research group (18).

The common phenotype of resistance to multiple antibiotics among our ESBL-producing isolates is probably due to the coexistence of $bla_{CTX\text{-}M\text{-}1}$ with other antibiotic resistance genes in the same plasmid, contributing to maintain ESBL-producing populations under different antibiotic selective pressures. With this study, it was possible to detect and characterize ESBL-producing *E. coli* isolates from birds of prey at the Serra da Estrela Natural Reserve in Portugal, with two types of ESBLs detected as CTX-M-1 and SHV-5. However, we cannot exclude the possibility that these wild animals had been exposed to fecal material of farm animals or even that of humans. These facts might be involved in the acquisition and dissemination of antibiotic-resistant bacteria even in the absence of direct antibiotic pressure and might explain the presence of ESBL-positive *E. coli* isolates. However, more studies should be performed to better understand the role of these animals in the spread of this type of resistance.

We thank CERVAS, Portugal, for its contribution to the collection of samples.

REFERENCES

1. **Blanc, V., R. Mesa, M. Saco, S. Lavilla, G. Prats, E. Miró, F. Navarro, P. Cortés, and M. Llagostera.** 2006. ESBL- and plasmidic class C β-lactamase-producing *Escherichia coli* strains isolated from poultry, pig and rabbit farms. Vet. Microbiol. **118:**299–304.
2. **Bouchillon, S. K., B. M. Johnson, D. J. Hoban, J. L. Johnson, M. J. Dowzicky, D. H. Wu, M. A. Visalli, and P. A. Bradford.** 2004. Determining incidence of extended-spectrum β-lactamase producing *Enterobacteriaceae*, vancomycin-resistant *Enterococcus faecium* and methicillin-resistant *Staphylococcus aureus* in 38 centres from 17 countries: the PEARLS study 2001–2002. Int. J. Antimicrob. Agents **24:**119–124.
3. **Bradford, P. A.** 2001. Extended-spectrum β-lactamases in the 21st century: characterization, epidemiology, and detection of this important resistance threat. Clin. Microbiol. Rev. **14:**933–951.
4. **Briñas, L., M. A. Moreno, T. Teshager, Y. Sáenz, M. C. Porrero, L. Domínguez, and C. Torres.** 2005. Monitoring and characterization of extended-spectrum β-lactamases in *Escherichia coli* strains from healthy and sick animals in Spain, in the year 2003. Antimicrob. Agents Chemother. **49:**1262–1264.
5. **Bush, K., G. Jacoby, and A. Medeiros.** 1995. A functional classification scheme for β-lactamases and its correlation with molecular structure. Antimicrob. Agents Chemother. **39:**1211–1233.
6. **Clermont, O., S. Bonacorsi, and E. Bingen.** 2000. Rapid and simple determination of the *Escherichia coli* phylogenetic group. Appl. Environ. Microbiol. **66:**4555–4558.
7. **Clinical and Laboratory Standards Institute.** 2007. Performance standards for antimicrobial susceptibility testing; 17th informational supplement, M100-S17. Clinical and Laboratory Standards Institute, Wayne, PA.
8. **Coque, T. M., A. Novais, A. Carattoli, L. Poirel, J. Pitout, L. Peixe, F. Baquero, R. Cantón, and P. Nordmann.** 2008. Dissemination of clonally related *Escherichia coli* strains expressing extended-spectrum β-lactamase CTX-M-15. Emerg. Infect. Dis. **14:**195–200.
9. **Costa, D., P. Poeta, L. Briñas, Y. Sáenz, J. Rodrigues, and C. Torres.** 2004. Detection of CTX-M-1 and TEM-52 beta-lactamases in *Escherichia coli* strains from healthy pets in Portugal. J. Antimicrob. Chemother. **54:**960–961.
10. **Costa, D., P. Poeta, Y. Sáenz, L. Vinué, B. Rojo-Bezares, A. Jouini, M. Zarazaga, J. Rodrigues, and C. Torres.** 2007. Detection of *Escherichia coli* harbouring extended-spectrum β-lactamases of the CTX-M, TEM and SHV classes in faecal samples of wild animals in Portugal. J. Antimicrob. Chemother. **59:**1311–1312.
11. **Costa, D., P. Poeta, Y. Sáenz, L. Vinué, A. C. Coelho, M. Matos, B. R. Bezares, J. Rodrigues, and C. Torres.** 2008. Mechanisms of antibiotic resistance in *Escherichia coli* isolates recovered from wild animals. Microb. Drug Resist. **14:**71–77.
12. **Duo, M., S. Hou, and D. Ren.** 2008. Identifying *Escherichia coli* genes involved in intrinsic multidrug resistance. Appl. Environ. Biotechnol. **81:**731–741.
13. **Girlich, D., L. Poirel, A. Carattoli, I. Kempf, M. Lartigue, A. Bertini, and P. Nordmann.** 2007. Extended-spectrum β-lactamase CTX-M-1 in *Escherichia coli* isolates from healthy poultry in France. Appl. Environ. Microbiol. **73:**4681–4685.
14. **Livermore, D. M., R. Canton, M. Gniadkowski, P. Nordmann, G. M. Rossolini, G. Arlet, J. Ayala, T. M. Coque, I. Kern-Zdanowicz, F. Luzzaro, L. Poirel, and N. Woodford.** 2007. CTX-M: changing the face of ESBLs in Europe. J. Antimicrob. Chemother. **59:**165–174.
15. **Mendonça, N., J. Leitão, V. Managueiro, E. Ferreira, the Antimicrobial Resistance Surveillance Program in Portugal, and M. Caniça.** 2007. Spread of extended-spectrum β-lactamase CTX-M-producing *Escherichia coli* clinical isolates in community and nosocomial environments in Portugal. Antimicrob. Agents Chemother. **51:**1946–1955.
16. **Nordmann, P.** 1998. Trends in β-lactam resistance among *Enterobacteriaceae*. Clin. Infect. Dis. Suppl. **1:**S100–S106.
17. **Poeta, P., H. Radhouani, G. Igrejas, A. Gonçalves, C. Carvalho, J. Rodrigues, L. Vinué, S. Somalo, and C. Torres.** 2008. Seagulls of the Berlengas natural reserve of Portugal as carriers of fecal *Escherichia coli* harboring CTX-M and TEM extended-spectrum β-lactamases. Appl. Environ. Microbiol. **74:**7439–7441.
18. **Poeta, P., H. Radhouani, L. Pinto, A. Martinho, V. Rego, R. Rodrigues, A. Gonçalves, J. Rodrigues, V. Estepa, C. Torres, and G. Igrejas.** 2009. Wild boars as reservoirs of extended spectrum β-lactamases *Escherichia coli* isolates of the A, B1 and B2 phylogenetic groups. J. Basic Microbiol. [Epub ahead of print.]
19. **Pomba-Féria, C., and M. Caniça.** 2003. A novel sequence framework (blaTEM-1G) encoding the parental TEM-1 beta-lactamase. FEMS Microbiol. Lett. **22:**177–180.
20. **Radhouani, H., L. Pinto, C. Coelho, A. Gonçalves, R. Sargo, C. Torres, G. Igrejas, and P. Poeta.** 2009. Detection of *Escherichia coli* harbouring extended-spectrum β-lactamases of the CTX-M and TEM classes in faecal samples of common buzzards (*Buteo buteo*). J. Antimicrob. Chemother. [Epub ahead of print.]
21. **Shah, A. A., F. Hasan, S. Ahmed, and A. Hameed.** 2004. Characteristics, epidemiology and clinical importance of emerging strains of Gram-negative bacilli producing extended-spectrum β-lactamases. Res. Microbiol. **155:**409–421.
22. **Tzouvelekis, L. S., E. Tzelepi, P. T. Tassios, and N. J. Legakis.** 2000. CTX-M-type β-lactamases: an emerging group of extended-spectrum enzymes. Int. J. Antimicrob. Agents **14:**137–142.