Two novel CMY-2-type β-lactamases encountered in clinical Escherichia coli isolates

Vera Manageiro¹,², Eugénia Ferreira¹, Margarida Pinto³,⁶, Fernando Fonseca⁴,⁷, Mónica Ferreira¹, Richard Bonnet⁵ and Manuela Caniça¹*

Abstract

Background: Chromosomally encoded AmpC β-lactamases may be acquired by transmissible plasmids which consequently can disseminate into bacteria lacking or poorly expressing a chromosomal blaAmpC gene. Nowadays, these plasmid-mediated AmpC β-lactamases are found in different bacterial species, namely Enterobacteriaceae, which typically do not express these types of β-lactamase such as Klebsiella spp. or Escherichia coli. This study was performed to characterize two E. coli isolates collected in two different Portuguese hospitals, both carrying a novel CMY-2-type β-lactamase-encoding gene.

Findings: Both isolates, INSRA1169 and INSRA3413, and their respective transformants, were non-susceptible to amoxicillin, amoxicillin plus clavulanic acid, cephalothin, cefoxitin, ceftazidime and cefotaxime, but susceptible to cefepime and imipenem, and presented evidence of synergy between cloxacillin and cefoxitin and/or ceftazidime. The genetic characterization of both isolates revealed the presence of blaCMY-46 and blaCMY-50 genes, respectively, and the following three resistance-encoding regions: a Citrobacter freundii chromosome-type structure encompassing a blc-sugE-blaCMY-2-type-ampR platform; a sul1-type class 1 integron with two antibiotic resistance gene cassettes (dfrA1 and aadA1); and a truncated mercury resistance operon.

Conclusions: This study describes two new blaCMY-2-type genes in E. coli isolates, located within a C. freundii-derived fragment, which may suggest their mobilization through mobile genetic elements. The presence of the three different resistance regions in these isolates, with diverse genetic determinants of resistance and mobile elements, may further contribute to the emergence and spread of these genes, both at a chromosomal or/and plasmid level.

Keywords: β-lactamase, Resistance regions, Genetic environment, Escherichia coli

Background

AmpC β-lactamases, along with Class A β-lactamases, are a major group of clinically important enzymes [1,2]. They belong to class C according to the Ambler classification and to group 1 following the functional classification of Bush-Jacoby [3,4], whose prevalence is increasing worldwide [1]; these β-lactamases are associated with infections caused by pathogenic Gram-negative bacteria, particularly Escherichia coli and Klebsiella pneumoniae. The identification of isolates containing plasmid-mediated AmpC-β-lactamase (PMAβ) is epidemiologically and clinically relevant due to the limitations of treatment options [5].

AmpC enzymes hydrolyse amino- and ureidopenicillins, and cephamycins (cefotaxin and cefotetan) and, at a low level, oximinocephalosporins (ceftazidime, cefotaxime, and ceftriaxone) and aztreonam: they are not inhibited by β-lactamase inhibitors such as clavulanic acid [1]. AmpC-producing isolates are susceptible to carbapenems and to zwitterionic cephalosporins (cefeipime and ceplifore).

In this study, we performed the phenotypic and molecular characterization of two new CMY-2-types (designated CMY-46 and CMY-50), both encoded by probably chromosomal inducible ampC genes, produced by two clinical E. coli isolates. The genetic environment of blaCMY-46 and blaCMY-50 was also investigated.
Methods

Bacterial isolate collection
Two clinical E. coli strains (INSRA1169 and INSRA3413) were isolated, in 1999, from urine samples of two patients of 77 years and 7 months old, in two different hospitals in Portugal. E. coli DH5α (pBK-CMY-2) strain was used as control for antimicrobial susceptibility tests.

Antimicrobial susceptibility tests
Minimal inhibitory concentrations were determined by a microdilution method according to guidelines of the French Society of Microbiology (SFM 2013, http://www.sfm-microbiologie.org) against seven β-lactams, alone or in combination with clavulanic acid, and against ciprofloxacin, gentamicin and trimethoprim. Isolates non-susceptible to one third-generation cephalosporin, cefoxitin and/or exhibiting synergy with boronic acid and/or cloxacillin, were considered as presumptive AmpC producers. Imipenem and clavulanic acid were used in order to identify induction effect of AmpC [1,6]. Disks of inducing agents (imipenem 10 μg and amoxicillin plus clavulanic acid 25 + 10 μg) and disks of cephalosporins (cefotaxime 30 μg and cefazidime 30 μg) were placed on Mueller–Hinton agar plates, 20 mm apart. Positive induction was demonstrated by the antagonism effect surrounding the cephalosporin disks adjacent to the inducers.

Isoelectric point determination
β-Lactamases were characterized by isoelectric focusing of ultrasonicated bacterial extracts with the control strains expressing pl 5.2, 5.6, 7.6, 9.0, 9.2, as previously described [7].

Molecular characterization of ampC and ESBL-encoding genes
The presence of acquired ampC (blaCMY, blaMOX, blaFOX, bla_LKT, bla_ACT, bla_MIR, bla_DHA, bla_MOR, bla_ACC) and blaESBL genes (bla_TEM, bla_SHV, bla_OXA-1-type, blaCTX-M) was investigated by multiplex PCR assays with primers and conditions as described elsewhere [7-10], and those from Table 1. Controls were included in all assays.

Gene transfer experiments
Transferrability of the blaCMY genes was attempted by both broth mating-out assays and electroporation. Conjugation experiments were performed at 37°C, using recipient strains E. coli C600 RifR, StrR and E. coli J53 NaN3 R, according to the antibiotic susceptibilities of the clinical isolates used as donor. Transconjugants were selected on MacConkey agar plates containing 250 μg/ml of rifampicin, 160 μg/ml of streptomycin or 160 μg/ml of sodium azide plus 10 μg/ml of cefoxitin. Plasmid DNA was extracted from clinical strains, using the Wizard Plus Midipreps DNA Purification kit (Promega), and used to transform electrocompetent E. coli DH5α ΔampC by electroporation, as previously described [7]. Transformants were selected on Luria broth medium containing 10 μg/ml of cefoxitin.

Cloning experiments
The blaCMY-2, blaCMY-46 and blaCMY-50 genes were amplified with iProof™ High-Fidelity DNA Polymerase (Bio-Rad, Hercules, CA), using primers from Table 1. Amplicons (1169 bp) were ligated in the Smal site of the phagemid pBK-CMV (Stratagene) downstream of its inducible lacZ promoter and transformed into electrocompetent E. coli DH5α ΔampC cells. A gene Pulser II apparatus (Bio-Rad, Hercules, CA) was used for standard electroporation techniques, as previously described [7]. Recombinant bacteria were selected on LB agar plates containing 10 μg/ml of cefoxitin.

Genetic background characterization
The presence of class 1 integrons was determined in both isolates through PCR amplification of the integrase-specific intI1 gene with the same specific primers and conditions as reported previously [11] (Table 1). PCR-mapping and sequencing of the genetic environment of blaCMY-46 and blaCMY-50 was performed using primers targeting genes known for promoting antibiotic resistance and integrons (Table 1). Sequence alignments and generation of resistance cassette contigs were performed using BioNumerics (Applied Maths). Gene identity was confirmed at the NCBI website (http://www.ncbi.nlm.nih.gov/).

Findings
The two clinical E. coli isolates INSRA1169 and INSRA3413 were resistant to amoxicillin, amoxicillin plus clavulanic acid, cephalothin, cefoxitin, cefazidime, cefotaxime, gentamicin and trimethoprim, but susceptible to cefepime and imipenem (Table 2). INSRA1169 was also nonsusceptible to ciprofloxacin. Synergy between cloxacillin and cefoxitin plus cefotaxime and boronic acid, along with the absence of synergy between extended-spectrum cephalosporins and clavulanic acid, suggest that the resistance to extended-spectrum cephalosporins was mediated by the overproduction of AmpC β-lactamases. The resistance phenotype was not transferable, neither in conjugation assays with E. coli C600 as a recipient, or in transformation assays by electroporation of plasmid-DNA preparations into E. coli DH5α. This might suggest a chromosomal location of AmpC-encoding genes.

Transformants, obtained after cloning of DNA amplicons of INSRA1169 and INSRA3413 in pBK-CMV plasmid vector (Figure 1a), showed a resistance phenotype similar to that of clinical isolates. However, they were susceptible to ciprofloxacin, gentamicin and trimethoprim, like the control strain E. coli DH5α (pBK-CMY-2)
isolates and (pBK-CMY-50) were transformants of TTACGGAACTGATTTCATG TCGTCAGTTATTGCAGC 1169 / PCR + Seq.

Δ and ’ (pBK-CMY-46) >2048 >2048 1024 32 4 8 0.25 64 0.5

bla gene (encoding sugE DH5 bla CCTTCGAGGCGGGTATC CCGATACCCGCCTCGAAG Seq.

revealed the presence of the CGTCCAATCTGCCATAGTG GTAGGGGAACAACTGGTCG Seq.

AmpC gene. The chromosome-mediated CTX FEP FOX IMP CIP GEN TMP bla gene in the PCR product GCCAGGTCTTGAGTATCGTC CATGTAATTGAGTCAGCGTATC 363 / PCR + Seq.

was the recipient strain. INSRA1169 (harboring genes was 3CTCAGTTGACCACCACGAAC GAATGCCAATAGCCGTTACGAC 920 / PCR + Seq.

region was identical to the sequence flanking the segment in both E. coli gene (encoding E. coli c TCTTTCTCCCCTTGCAGCG CACCTTGTCGAACAGCCCA Variable / PCR + Seq.

was the usual transcriptional regulator of ampC genes was observed in an opposite direction of transcription (Figure 1a). Upstream, an ampR gene encoding the usual phenotypic induction method. This cmY-46 and CMY-50 β-lactamases did not confer resistance to cefepime and conferred low level of resistance to ceftazidime and cefotaxime, which suggests that, in contrast to extended-spectrum AmpCs, they have moderate or no extended-spectrum activity (Table 2) [15-18].

The study of sequences surrounding bla_CMY-46 and bla_CMY-50 revealed the presence of the blc gene (encoding an outer membrane lipoprotein) and the sugE gene (encoding a small MDR protein responsible for resistance to quaternary ammonium compounds) downstream of their open reading frames (Figure 1a). Upstream, an ampR gene encoding the usual transcriptional regulator of ampC genes was observed in an opposite direction of transcription (Figure 1a). Upstream, an ampR gene encoding the usual transcriptional regulator of ampC genes was observed in an opposite direction of transcription (Figure 1a).

indeed, PCR revealed the absence of blaESBL genes plus the presence of chromosomal E. coli ampC-type gene; the sequence of cloned DNA fragments identified two new CMY-2-like genes, which were not related to chromosome-mediated E. coli AmpC gene. The chromosomal location of such genes has only been observed in Salmonella spp. and Proteus mirabilis isolates [12-14].

The deduced amino acid sequences confirmed that the new genes encoding the β-lactamases CMY-46 (in INSRA1169) and CMY-50 (in INSRA3413), which were new variants of CMY-2, differed by 9 and 13 amino acid substitutions, respectively (Table 3). Two of these mutations (Q193K plus P208A for CMY-46 and N194S plus T226N for CMY-50) are in the region of the active Ser64. Substitutions in this region have been linked to the extension of extended-spectrum activity [15]. However, CMY-46 and CMY-50 β-lactamases did not confer resistance to cefepime and conferred low level of resistance to ceftazidime and cefotaxime, which suggests that, in contrast to extended-spectrum AmpCs, they have moderate or no extended-spectrum activity (Table 2) [15-18].

The study of sequences surrounding bla_CMY-46 and bla_CMY-50 revealed the presence of the blc gene (encoding an outer membrane lipoprotein) and the sugE gene (encoding a small MDR protein responsible for resistance to quaternary ammonium compounds) downstream of their open reading frames (Figure 1a). Upstream, an ampR gene encoding the usual transcriptional regulator of ampC genes was observed in an opposite direction of transcription (Figure 1a). Upstream, an ampR gene encoding the usual transcriptional regulator of ampC genes was observed in an opposite direction of transcription (Figure 1a). Upstream, an ampR gene encoding the usual transcriptional regulator of ampC genes was observed in an opposite direction of transcription (Figure 1a). Upstream, an ampR gene encoding the usual transcriptional regulator of ampC genes was observed in an opposite direction of transcription (Figure 1a). Upstream, an ampR gene encoding the usual transcriptional regulator of ampC genes was observed in an opposite direction of transcription (Figure 1a). Upstream, an ampR gene encoding the usual transcriptional regulator of ampC genes was observed in an opposite direction of transcription (Figure 1a). Upstream, an ampR gene encoding the usual transcriptional regulator of ampC genes was observed in an opposite direction of transcription (Figure 1a).

Table 1 Primers, drawn in this study, used for PCR amplification and sequencing of PMAβ genes and for PCR mapping of blaCMY-46 and blaCMY-50

| Gene (s) | Primer Sequence (5’ → 3’) | PCR product (bp) / Method |
|----------|---------------------------|--------------------------|
| Forward  | Reverse                   |                           |
| blaCMY-46 | TTAACGTAAGTGATTTGATCG    | 1169 / PCR + Seq.         |
| arf513   | TAGCTTGTTAGTAGTCTG         | 363 / PCR + Seq.          |
| fadB - fadD | CGCAGTGACCCACCGAGG        | 920 / PCR + Seq.          |
| fadB - ampR | TGGCCGACCTGACGGCCAA      | 1214 / PCR + Seq.         |
| ampR - blaCMY-50 | TGGCCGACCTGACGGCCAA | 1660 / PCR + Seq.         |
| blaCMY-46 - sugE | TGGCCGACCTGACGGCCAA | Variable / PCR + Seq.     |
| merA - urf2 | TTTCCCTACTGAGATGG         | Seq.                     |
| merA - merA | TCCTTTCCCTTGTGCACG        | Seq.                     |
| merD | CCGATCGCTGGTGATGC          | Seq.                     |
| merD | CCGATCGCTGGTGATGC          | Seq.                     |
| merA | CGTAACCGCCCGGTATC          | Seq.                     |
| merD | CCGATCGCTGGTGATGC          | Seq.                     |
| merA | CGTAACCGCCCGGTATC          | Seq.                     |
| merD | CCGATCGCTGGTGATGC          | Seq.                     |

*Method used for screening and/or identification of genes: M-PCR, Multiplex-PCR; Seq, sequencing; PCR + Seq, PCR + Seq, PCR and sequencing.

Table 2 MICs of antibiotics for CMY-46- and CMY-50-producing E. coli isolates and E. coli transformants and recipients

| E. coli strain | MICs (μg/ml) |
|---------------|-------------|
|               | AMX | AMC | CF | CAZ | CCAZ | CTX | FEP | FOX | IMP | CIP | GEN | TMP |
| DHE5a ΔampC   | 8   | 8   | 8  | 0.25 | 0.125 | 0.06 | 0.03 | 4   | 0.25 | ≤0.125 | $0.125$ | $0.25$ |
| DHE5a (pBK-CMY-2) | >2048 | >2048 | 1024 | 32 | 16 | 8 | 0.25 | 64 | 0.5 | ≤0.125 | 1 | ≤0.25 |
| INSRA1169 (CMY-46 + TEM-1) | >2048 | >2048 | 1024 | 16 | 4 | 4 | 0.032 | 64 | 2 | 4 | >128 | >128 |
| DHE5a (pBK-CMY-46) | >2048 | >2048 | 1024 | 32 | 4 | 8 | 0.25 | 64 | 0.5 | ≤0.125 | 2 | ≤0.25 |
| INSRA3413 (CMY-50) | >2048 | >2048 | 1024 | 16 | 8 | 4 | 0.25 | 64 | 2 | ≤0.125 | 64 | 64 |
| DHE5a (pBK-CMY-50) | >2048 | >2048 | 1024 | 32 | 8 | 4 | 0.25 | 64 | 0.5 | ≤0.125 | 0.5 | ≤0.25 |

*E. coli ECDHE5a (pBK-CMY-2) was control strain; E. coli DHE5a (pBK-CMY-46) and E. coli DHE5a (pBK-CMY-50) were transformants of E. coli INSRA1169 (harboring CMY-46 and TEM-1 enzymes) and E. coli INSRA3413 (harboring CMY-50 enzyme), respectively; E. coli ECDHE5a was the recipient strain.

*AMX, amoxicillin; AMC, amoxicillin-clavulanic acid; CF, cephalothin; CAZ, ceftazidime; CCAZ, ceftazidime-clavulanic acid; CTX, cefotaxime; FEP, cefepime; FOX, cefoxitin; IMP, imipenem; CIP, ciprofloxacin; GEN, gentamicin and TMP, trimethoprim.

*Clavulanic acid, at fixed concentration of 2 μg/ml.
positions related to AmpR function [20,21]. The promoter regions of our blaCMY-type and ampR genes harbored no sequence element associated with increased strength of the promoter [20,21]. In addition, the frdD, frdC, and frdB genes that are usually adjacent to ampC-ampR in the C. freundii chromosome were not identified in the sequences flanking blaCMY-46 or blaCMY-50.

Class 1 integrons, also detected in INSRA1169 and INSRA3413 (Figure 1b), comprised the integrase-encoding gene intI1, two gene cassettes, aacA1 and dfrA1, and qacEΔ1 plus sul1, which were probably responsible for the observed resistances to trimethoprim and aminoglycosides. We also found a truncated mercury resistance operon (Figure 1c), which was previously reported as belonging to a “kan” region that included a kanamycin resistance gene [22]. This finding is of concern since mercury resistance may help to promote antibiotic resistance through indirect selection [23].

In summary, this study describes two new blaCMY-type genes located within a C. freundii-derived fragment. Considering that CMY-type β-lactamases, detected in E. coli, are derived from the C. freundii chromosomal AmpC [1] and that chromosome-derived genes are usually mobilized by MGE [24,25], the presence of three

Table 3 Comparison of amino acid substitutions of two new CMY-type β-lactamases

| PMAβ | Amino acid at position no.* | pl | Accession Number |
|------|-----------------------------|----|------------------|
|      | 3   | 5   | 9   | 0   | 2   | 2   | 3   | 2   | 5   | 4   | 6   | 3   | 3   | 4   | 0   | 3   | 4   | 8   | 8   | 6   | 2   | 3   | 3   | 1   | 8   | | X91840 |
| CMY-2 | V   | Q   | A   | R   | D   | R   | H   | T   | K   | T   | Q   | N   | D   | P   | A   | H   | A   | R   | V   | 9.0 | |
| CMY-46 | T   | S   | T   | R   | A   | K   | A   | V   | R   | 9.2 | FNS56186 |
| CMY-50 | I   | E   | R   | S   | E   | T   | R   | Q   | S   | N   | E   | C   | E   | A   | 9.2 | FN645444 |

*Numbering according to Bauernfeind et al., 1996 [19].
resistance regions with diverse resistance determinants and MGE in this study, suggests the dynamics of bacteria in the transference of antibiotic resistance. In addition, they might also trigger the future emergence and spread of these resistant determinants both at a chromosomal or/and plasmid level.

Availability of supporting data
The data set supporting the results of this article is included within the article.

Abbreviations
PMAβ: Plasmid-mediated AmpC-β-lactamase; SFM: French Society of Microbiology; MDR: Multidrug-resistance; MGE: Mobile genetic elements; ESAC: Extended-spectrum AmpC cephalosporinases; ESBL: Extended-spectrum β-lactamase.

Competing interests
The authors declare that they have no competing interests.

Authors’ contributions
VM performed the experiments, data interpretation and drafted the manuscript; EF and MF took part in the experiments; MP and FF worked on microbiology and clinical data; RB participated in data interpretation and reviewing of the manuscript; and MC conceived the study, and contributed to data interpretation and reviewing of the manuscript. All authors read and approved the final manuscript.

Acknowledgements
This study was supported by a grant from the Department of Infectious Diseases, National Institute of Health Dr. Ricardo Jorge (in 2012). V. Manageiro was supported by grant SFRB/BDP/77486/2011 from the Fundação para a Ciência e a Tecnologia, Lisbon, Portugal. This work was funded by “Strategic Plan for Environmental and Natural Sciences, Project UF/211–2011–2012” referenced as “ Pest-OE/AGR/UI0211/2011” from CECA-ICETA. The GenBank accession numbers for the new AmpC-encoding genes are FN556186 for blaCMY-46 and FN645444 for blaCMY-2.

Author details
1Department of Infectious Diseases, National Reference Laboratory of Antibiotic Resistances and Healthcare Associated Infections, National Institute of Health Dr. Ricardo Jorge, Av. Padre Cruz, 1649-016 Lisbon, Portugal.
2Centre for the Study of Animal Sciences (ICETA), University of Oporto, Oporto, Portugal. 3Laboratory of Microbiology, Hospital Garcia de Orta, EPE, Almada, Portugal. 4Laboratory of Clinical Pathology, Hospital de Santa Luzia, Viana do Castelo, Portugal. 5CHU Clermont-Ferrand, Laboratoire de Bactériologie, Clermont-Ferrand, France. 6Present address: Laboratory of Microbiology, Centro Hospitalar de Lisboa Central, EPE, Lisbon, Portugal. 7Present address: Laboratory of Clinical Pathology, Centro Hospitalar de Póvoa de Varzim-Vila do Conde, EPE, Póvoa de Varzim, Portugal.

Received: 1 October 2014 Accepted: 24 February 2015
Published online: 18 March 2015

References
1. Jacoby GA. AmpC-β-lactamases. Clin Microbiol Rev. 2009;22:161–82.
2. Rice LB, Bonomo RA. β-Lactamases: which ones are clinically important? Drug Resist Updat. 2000;3:178–89.
3. Ambler RP, Coulson AF, Frere JM, Ghuysen JM, Joris B, Forsman M, et al. A standard numbering scheme for the class A β-lactamases. Biochem J. 1991;276:269–70.
4. Bush K, Jacoby GA. Updated functional classification of β-lactamases. Antimicrob Agents Chemother. 2010;54:969–76.
5. Pai H, Kang O, Byeon JH, Lee KD, Park WB, Kim HB, et al. Epidemiology and clinical features of bloodstream infections caused by AmpC-type β-lactamase-producing Klebsiella pneumoniae. Antimicrob Agents Chemother. 2004;48:3720–8.
6. Lindquist S, Lindberg F, Normark S. Binding of the Citrobacter freundii AmpR regulator to a single DNA site provides both autoregulation and activation of the inducible ampC-β-lactamase gene. J Bacteriol. 1989;171:3746–53.
7. Mendonça N, Leitão J, Manageiro V, Ferreira E. Antimicrobial Resistance Surveillance Program in Portugal (ARSIP). Caniça M. Spread of extended-spectrum β-lactamase CTX-M-producing Escherichia coli clinical isolates in community and nosocomial environments in Portugal. Antimicrob Agents Chemother. 2007;54:1946–55.
8. Pérez-Pérez FJ, Hansen ND. Detection of plasmid-mediated AmpC-β-lactamases in clinical isolates by using multiplex PCR. J Clin Microbiol. 2002;40:2153–62.
9. Jones-Dias D, Manageiro V, Francisco AP, Martins AP, Domingues G, Louro D, et al. Assessing the molecular basis of transferable quinolone resistance in Escherichia coli and Salmonella spp. from food-producing animals and food products. Vet Microbiol. 2013;167:523–31.
10. Manageiro V, Ferreira E, Caniça M, Manaia CM. CES-5 among the β-lactamases detected in ubiquitous bacteria isolated from aquatic environment samples. FEMS Microbiol Lett. 2014;351:4–9.
11. Leverstein-Van Hall MA, Paauw A, Box AT, Blok HE, Verhoef J, Fluit AC. Presence of integron-associated resistance in the community is widespread and contributes to multidrug resistance in the hospital. J Clin Microbiol. 2002;40:3038–40.
12. Shahada F, Sekizuka T, Kuroda M, Kusumoto M, Chishi D, Matsumoto A, et al. Characterization of Salmonella enterica serovar Typhimurium isolates harboring a chromosomally encoded CMY-2 β-lactamase gene located on a multidrug resistance genomic island. Antimicrob Agents Chemother. 2011;55:4114–21.
13. Zioa A, Whichard JM, Joyce KJ, Tzilepi E, Tsouvelakis LS, Miragou V. Evidence for chromosomal and plasmid location of CMY-2 cephalosporinase gene in Salmonella serotype Typhimurium. J Antimicrob Chemother. 2008;61:1389–90.
14. D’Andrea MM, Literacka E, Zioa A, Giani T, Baranik A, Fett J, et al. Evolution and spread of a multidrug-resistant Proteus mirabilis clone with chromosomal AmpC-type cephalosporinases in Europe. Antimicrob Agents Chemother. 2011;55:2735–42.
15. Nordmann P, Mammari H. Extended-spectrum cephalosporinases: structure, detection and epidemiology. Future Microbiol. 2007;2:297–307.
16. Rodríguez-Martínez JM, Poirel L, Nordmann P. Extended-spectrum cephalosporinases in Pseudomonas aeruginosa. Antimicrob Agents Chemother. 2009;53:766–71.
17. Rodríguez-Martínez JM, Nordmann P, Ronco E, Poirel L. Extended-spectrum cephalosporinases in Acinetobacter baumannii. Antimicrob Agents Chemother. 2010;54:3484–8.
18. Rodríguez-Martínez JM, Fernández-Echarri P, Fernández-Cuenca F, Díaz-de-Alba P, Briales A, Pascual A. Genetic characterization of an extended-spectrum AmpC cephalosporinase with hydrolyzing activity against fourth-generation cephalosporins in a clinical isolate of Enterobacter aerogenes selected in vivo. J Antimicrob Chemother. 2012;67:48–54.
19. Bauernfeind A, Ste mplinger J, Jungwirth R, Giamarelli H. Characterization of the plasmidic β-lactamase CMY-2, which is responsible for cephamycin resistance. Antimicrob Agents Chemother. 1996;40:2241–4.
20. Bartoswky E, Normark S. Interactions of wild-type and mutant AmpR of Citrobacter freundii with target DNA. Mol Microbiol. 1993;10:555–65.
21. Hanson ND, Sanders CC. Regulation of inducible AmpC-β-lactamase expression among Enterobacte riaceae. Curr Pharm Des. 1999;5:881–94.
22. Call DR, Singer RS, Meng O, Broschat SL, Orfe LH, Anderson JM, et al. blaCMY-2-positive IncA/C plasmids from Escherichia coli and Salmonella enterica are a distinct component of a larger lineage of plasmids. Antimicrob Agents Chemother. 2010;54:590–6.
23. Baker-Austin C, Wright MS, Stepanauskas R, Mc Arthur J. Co-selection of antibiotic and metal resistance. Trends Microbiol. 2006;14:176–82.
24. Frost LS, Leplae R, Summers AO, Toussaint A. Mobile genetic elements: the agents of open source evolution. Nat Rev Microbiol. 2005;3:722–32.
25. Norman A, Hansen LH, Sørensen SJ. Conjugal plasmids: vessels of the communal gene pool. Phil Trans R Soc B. 2009;364:2275–89.