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ARTICLE

# Prevalence and molecular epidemiology of acquired AmpC β-lactamases and carbapenemases in *Enterobacteriaceae* isolates from 35 hospitals in Spain

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**Abstract** The purpose of this investigation was to determine the prevalence of plasmid-mediated AmpC (pAmpC) and carbapenemases in *Enterobacteriaceae* collected from 35 hospitals in Spain and to establish their epidemiological relationships. We conducted a prospective multi-centre study on pAmpC- or carbapenemase-producing *Enterobacteriaceae* isolates from clinical samples collected from February to July 2009. The strains suspected to carry pAmpC were resistant or showed intermediate susceptibility to co-amoxiclav and second- or third-generation cephalosporins. Strains suspected to carry a carbapenemase were selected because they showed a minimum inhibitory

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concentration (MIC) to imipenem >1 mg/L. Polymerase chain reaction (PCR) and a sequencing strategy were used to characterise the enzymes. The clonal relationships between isolates was analysed by pulsed field gel electrophoresis (PFGE). Among 100,132 *Enterobacteriaceae* isolates collected, 1,654 were compatible with the production of pAmpC or carbapenemases. We found a prevalence of 0.64 % of pAmpC ($n$=635) and 0.04 % of carbapenemases ($n$=43). The most prevalent pAmpC enzymes were CMY-type (78.3 %), DHA-type (19.5 %), ACC-type (1.6 %) and FOX-type (0.6 %). The CMY-type was the most frequent in *Escherichia coli* and *Proteus mirabilis* species, whereas the DHA-type was mainly found in *Klebsiella* spp. The enzymes involved in carbapenem resistance were VIM-1, IMP-22 and the new IMP-28. Nine new *bla* genes were described: $bla_{\text{CMY-54}}$, $bla_{\text{CMY-55}}$, $bla_{\text{CMY-56}}$, $bla_{\text{CMY-57}}$, $bla_{\text{CMY-96}}$, $bla_{\text{DHA-6}}$, $bla_{\text{DHA-7}}$, $bla_{\text{FOX-8}}$ and $bla_{\text{IMP-28}}$. The prevalence of pAmpC or carbapenemases found is not negligible. The CMY-types were the predominant pAmpC, whereas the VIM or IMP enzymes were the predominant carbapenemases. Furthermore, we observed a great genetic diversity among pAmpC-producing strains and a close clonal relationship between carbapenemase-producing strains.

## Introduction

AmpC β-lactamases (AmpC) are clinically relevant cephalosporinases encoded on the chromosome of many *Enterobacteriaceae*. Some of them are also described on plasmids, usually in species that do not naturally produce AmpC, such as *Klebsiella* spp., *Salmonella enterica* or *Proteus mirabilis*.

Plasmid AmpC (pAmpC) derive from chromosomal *amp*C genes of the families *Enterobacteriaceae* and *Aeromonadaceae*. First characterised in 1988, pAmpC described to date can be grouped into seven families: ACC, CMY, DHA, FOX, MIR, ACT and MOX [1]. These enzymes have been identified throughout the world, with CMY-2 being the most prevalent. A previous study performed in Barcelona (Spain) showed a significant increase of pAmpC in *Enterobacteriaceae* from 0.06 % in 1999 to 1.3 % in 2007 [2], in agreement with other authors (0.13 % to 3 %) [3–6].

Classes A, B and D of acquired carbapenemases are also increasing in *Enterobacteriaceae*. Often involved in nosocomial outbreaks [7–12], these emerging enzymes are worrisome because their broad activity profiles encompass all β-lactam antibiotics. Additionally, carbapenemases are difficult to detect using phenotypical methods because their resistance level to carbapenems is frequently below the established clinical breakpoints [13, 14].

The prevalence of pAmpC and carbapenemases could be underestimated because of the lack of consensus on the methodology to detect them [15]. This study aimed to determine the prevalence of these enzymes in *Enterobacteriaceae* isolates from clinical samples collected in Spain over a six-month period in 2009.

## Materials and methods

### Study design, bacterial strains and susceptibility tests

A prospective multi-centre study was undertaken in Spain to collect pAmpC- or carbapenemase-producing *Enterobacteriaceae* isolates from clinical samples from February to July 2009. Thirty-five Spanish hospitals participated, six of which acted as co-ordinating centres. These hospitals were distributed throughout 14 of the 18 Autonomous Communities in Spain.

Only one isolate per patient was considered for analysis. Isolates were identified in each hospital, using standard methods. Disc-diffusion and microdilution susceptibility tests were performed according to Clinical and Laboratory Standards Institute (CLSI) guidelines [16]. As some pAmpC do not confer resistance to cefoxitin, each hospital collected all the strains showing resistance or intermediate susceptibility to both co-amoxiclav (<18 mm; >8/4 mg/L) and any of the following: cefotaxime (<23 mm; >8 mg/L), ceftazidime (<18 mm; >8 mg/L) or aztreonam (<21 mm; >8 mg/L).

All extended-spectrum β-lactamase (ESBL)-producing isolates were excluded, except those that were resistant to or had intermediate susceptibility to cefoxitin (<15 mm; >8 mg/L) or co-amoxiclav. ESBL production was studied using the CLSI confirmatory test [16], the double-disc synergy test [14] and/or Etest ESBL (AB Biodisk, Solna, Sweden). *Enterobacteriaceae* with inducible chromosomal AmpC β-lactamase were also excluded from this part of the study.

Isolates suspected of carrying a carbapenemase were first selected because they showed a minimum inhibitory concentration (MIC) to imipenem >1 mg/L. The modified Hodge test [17] and Etest MBL IP/IPI (AB bioMérieux, Solna, Sweden) were used to confirm carbapenemase activity.

Susceptibility to aminoglycosides, quinolones or co-trimoxazole was determined by broth microdilution (MicroScan, Siemens Healthcare Diagnostics, Deerfield, IL, USA).

### Characterisation of pAmpC and carbapenemases

The detection and characterisation of their genes were performed using the specific primers listed in Table S1 (supplementary data) or as previously described [1, 7, 18–22]. All amplicons obtained were purified and sequenced as previously described [7].

### PFGE

For clonal studies, a group of strains were selected according to the following criteria: (i) all carbapenemase-producing

isolates, (ii) all pAmpC-producing isolates from the coordinating centres and (iii) two pAmpC-producing isolates per species and enzyme (one at the start and one at the end of the study period) from the remaining participant hospitals. The genetic relationship among isolates of the same species was determined by pulsed field gel electrophoresis (PFGE) using a CHEF-DRII device (Bio-Rad, Hercules, CA, USA) after total chromosomal DNA digestion with *Xba*I (*Escherichia coli, Klebsiella pneumoniae, K. oxytoca, Enterobacter cloacae* and *Citrobacter koseri*) [23] or *Not*I (*P. mirabilis*) [24]. DNA relatedness was calculated based on the criteria of Tenover et al. [25].

Nucleotide sequence accession numbers

The new β-lactamase gene sequences were submitted to GenBank under accession numbers HM544039 ($bla_{\text{CMY-54}}$), HM544040 ($bla_{\text{CMY-55}}$), HQ322613 ($bla_{\text{CMY-56}}$), HQ285243 ($bla_{\text{CMY-57}}$), HQ267531 ($bla_{\text{CMY-96}}$), HQ322612 ($bla_{\text{DHA-6}}$), HQ456945 ($bla_{\text{DHA-7}}$), HM565917 ($bla_{\text{FOX-8}}$) and HQ263342 ($bla_{\text{IMP-28}}$).

## Results and discussion

Our study showed that the prevalence of pAmpC and carbapenemases in Spain in 2009 was 0.64 % and 0.04 %, respectively. These data were obtained from 100,132 isolates of *Enterobacteriaceae*, of which 1,654 showed a susceptibility phenotype compatible with the production of pAmpC or carbapenemases. These results were similar to the prevalences found around the world [2, 4–6, 26].

Genes coding for pAmpC or carbapenemase were detected in 674 isolates (Table 1). Using phenotypic methods, we found that those isolates with negative polymerase chain reaction (PCR) results were ESBL-producers, serine class A-producers (*Klebsiella* spp.) or hyperproducers of chromosomal AmpC (*E. coli*). However, non-enzymatic resistance mechanisms such as altered permeability may have also contributed to resistance in these isolates [14].

The highest prevalence of pAmpC in Spain was found in the Autonomous Community of Catalonia (0.92 %), followed by Asturias (0.85 %). The lowest prevalence was observed in the Balearic Islands (0.35 %). No cases of pAmpC were detected in Castilla y León (Figure S1, supplementary data). This geographical variability was probably due to both the number of centres and the different kind of healthcare institutions included per autonomous community.

The most prevalent pAmpC enzymes found were CMY (78.3 %), followed by DHA (19.5 %), ACC (1.6 %) and FOX (0.6 %) (Table 2). CMY-2 remained the most widely distributed, especially in *E. coli* (87.1 %) and *P. mirabilis* (88.8 %) species, whereas DHA-1 enzyme was the most predominant in *Klebsiella* spp. isolates. Remarkably, both these enzymes were also found in other *Enterobacteriaceae* species, such as *C. koseri*, *P. penneri* and *S. enterica* (Table 2, Figure S1, supplementary data). FOX-type enzymes were found exclusively in *E. coli*. Nucleotide sequence analysis of all *bla* genes identified eight new pAmpC ($bla_{\text{CMY-54}}$, $bla_{\text{CMY-55}}$, $bla_{\text{CMY-56}}$, $bla_{\text{CMY-57}}$, $bla_{\text{CMY-96}}$, $bla_{\text{DHA-6}}$, $bla_{\text{DHA-7}}$ and $bla_{\text{FOX-8}}$).

Table 3 shows the correlation between resistance to non-β-lactam antibiotics and the most frequent species/pAmpC

**Table 1** Prevalence of pAmpC and carbapenemases in *Enterobacteriaceae* isolated in Spain in 2009

| Micro-organism | No. of isolates | No. of pAmpC-producing isolates | No. of carbapenemase-producing isolates | No. of pAmpC- and carbapenemase-producing isolates |
|---|---|---|---|---|
| *E. coli* | 66,935 | 465 (0.69 %) | 1 (0.001 %) | – |
| *K. pneumoniae* | 8,964 | 92 (1.02 %) | 18 (0.20 %) | 1 (0.01 %) |
| *P. mirabilis* | 7,901 | 62 (0.78 %) | – | 1 (0.01 %) |
| *E. cloacae* | 3,809 | – | 9 (0.23 %) | 2 (0.05 %) |
| *K. oxytoca* | 2,657 | 7 (0.26 %) | 11 (0.41 %) | – |
| *S. enterica* | 1,678 | 1 (0.06 %) | – | – |
| *C. koseri* | 890 | 3 (0.33 %) | – | – |
| *M. morganii* | 2,520 | – | – | – |
| *S. marcescens* | 1,490 | – | – | – |
| *E. aerogenes* | 1,190 | – | – | – |
| *C. freundii* | 1,100 | – | – | – |
| *P. stuartii* | 531 | – | – | – |
| *H. alvei* | 169 | – | – | – |
| Others | 297 | 1[a] | – | – |
| Total | 100,132 | 631 (0.63 %) | 39 (0.04 %) | 4 (0.004 %) |

[a]Corresponding to a *P. penneri* strain

**Table 2** Prevalence of pAmpC and carbapenemases in the 674 *Enterobacteriaceae* strains studied

| Micro-organism (*n*) | pAmpC | No. of strains (%) | Carbapenemases | No. of strains (%) | pAmpC + carbapenemases | No. of strains (%) |
|---|---|---|---|---|---|---|
| *E. coli* (466)[a] | **CMY-2** | **406 (87.1)** | VIM-1 | 1 (0.2) | | |
| | **DHA-1** | **37 (8.1)** | | | | |
| | ACC-1 | 3 (0.6) | | | | |
| | ACC-1+FOX | 1 (0.2) | | | | |
| | CMY-2t[a] | 3 (0.8) | | | | |
| | CMY-4 | 3 (0.8) | | | | |
| | CMY-7 | 1 (0.2) | | | | |
| | CMY-27 | 2 (0.4) | | | | |
| | CMY-43 | 1 (0.2) | | | | |
| | CMY-48 | 1 (0.2) | | | | |
| | CMY-54 | 1 (0.2) | | | | |
| | CMY-55 | 1 (0.2) | | | | |
| | CMY-57 | 1 (0.2) | | | | |
| | DHA-6 | 1 (0.2) | | | | |
| | FOX-3 | 1 (0.2) | | | | |
| | FOX-8 | 2 (0.4) | | | | |
| *K. pneumoniae* (111) | **DHA-1** | **68 (60.7)** | VIM-1 | 9 (8.0) | DHA-1+VIM-1 | 1 (0.9) |
| | CMY-2 | 18 (16) | IMP-22 | 9 (8.0) | | |
| | ACC-1 | 3 (2.6) | | | | |
| | CMY-56 | 1 (0.9) | | | | |
| | CMY-96 | 1 (0.9) | | | | |
| | CMY-2+DHA-1 | 1 (0.9) | | | | |
| *P. mirabilis* (63) | **CMY-2** | **56 (88.8)** | | | CMY-2+VIM-1 | 1 (1.6) |
| | ACC-1 | 3 (4.7) | | | | |
| | DHA-1 | 3 (4.7) | | | | |
| *K. oxytoca* (18) | DHA-1 | 5 (38.8) | **VIM-1** | **10 (55.5)** | | |
| | CMY-2 | 2 (11.1) | IMP-28 | 1 (5.5) | | |
| *E. cloacae* (11) | | | **VIM-1** | **9 (75)** | DHA-7+VIM-1 | 2 (16.6) |
| *C. koseri* (3) | CMY-2 | 1 | | | | |
| | DHA-1 | 2 | | | | |
| *S. enterica* (1) | DHA-1 | 1 | | | | |
| *P. penneri* (1) | CMY-2 | 1 | | | | |

[a]CMY-2t: CMY-2 related enzyme. Three isolates yielded positive PCR results for the internal fragment of CIT enzymes and sequences of the fragments were identical to CMY-2, despite several attempts to amplify the full gene consistently failing

enzymes. The resistance of pAmpC-producers to non-β-lactam antibiotics was high: 70.2 % to ciprofloxacin, 65.9 % to co-trimoxazole, 44.8 % to tobramycin, 35.8 % to gentamicin and 7.4 % to amikacin. Co-resistance to ciprofloxacin, co-trimoxazole, gentamicin and tobramycin was detected in 23.4 % of the isolates. Regarding resistance

**Table 3** Resistance to non-β-lactam antibiotics in the most frequent species/pAmpC associations

| Species/resistance mechanism (*n*) | Ciprofloxacin (%) | Gentamicin (%) | Tobramycin (%) | Amikacin (%) | Co-trimoxazole (%) |
|---|---|---|---|---|---|
| *E. coli*/CMY-2 (131) | 98 (74.8) | 48 (36.6) | 45 (34.4) | 7(5.3) | 81 (61.8) |
| *E. coli*/DHA-1 (22) | 18 (81.8) | 7 (31.8) | 6 (27.3) | 0 | 15 (68.2) |
| *K. pneumoniae*/CMY-2 (14) | 9 (64.3) | 10 (71.4) | 12 (85.7) | 2 (14.3) | 8 (57.1) |
| *K. pneumoniae*/DHA-1 (24) | 13 (54.2) | 6 (25) | 9 (37.5) | 3 (12.5) | 13 (54.2) |
| *P. mirabilis*/CMY-2 (40) | 26 (65) | 15 (37.5) | 13 (32.5) | 6 (15) | 28 (70) |

to non-β-lactams, we did not find any statistically significant differences between CMY-2- and DHA-1-producing isolates.

VIM-1, VIM-2 and IMP-1 are the most frequent carbapenemases reported in *Enterobacteriaceae* around the world [27]. In Spain, the first VIM-1 enzyme was described in 2003 in *K. pneumoniae* and *E. coli* [7, 28]. Since then, several outbreaks involving *K. pneumoniae* and *E. cloacae* strains have been published [7, 28, 29]. In the present study, we found that the VIM-1 enzyme was the most frequent carbapenemase. We also detected IMP-22-producers and the new IMP-28-producing strain that was recently published [30]. As Gaibani et al. [31], we did not find any KPC, NDM or OXA carbepenemases. Our study, therefore, preceded the emergence of carbapenemases such as OXA-48 and NDM-1 that were recently described in Spain [32, 33]. The resistance of carbapenemase-producers to non-β-lactam antibiotics was high and similar to the pAmpC-producers: 60 % to ciprofloxacin, 80 % to co-trimoxazole, 33 % to tobramycin, 27.5 % to gentamicin and 7.5 % to amikacin. Co-resistance to ciprofloxacin, co-trimoxazole, gentamicin and tobramycin was detected in 17.5 % of the isolates.

A total of 324 non-duplicate isolates were selected to determine their molecular typing. PFGE analysis showed a great genetic diversity: 172 different PFGE patterns in 179 *E. coli* isolates, 51 in 67 *K. pneumoniae* isolates, 45 in 48 *P. mirabilis* isolates, 10 in 15 *K. oxytoca* isolates, 11 in 11 *E. cloacae* isolates and 3 in 3 *C. koseri* isolates. The PFGE results revealed four well-defined clusters: (i) all nine IMP-22-producing *K. pneumoniae* isolates from Hospital Puerta del Mar (Cadiz), (ii) five VIM-1-producing *K. oxytoca* from Hospital Ramón y Cajal (Madrid), (iii) three CMY-2-producing *E. coli* from Hospital Virgen del Rocío (Seville) and (iv) three CMY-2-producing *P. mirabilis* from Hospital Sant Pau (Barcelona). We also detected 15 pairs of identical isolates: seven pairs of CMY-2-producing isolates (four of *E. coli* and three of *K. pneumoniae*), three of VIM-1-producing isolates (two *K. pneumoniae* and one *K. oxytoca*), two of DHA-1-producing *K. pneumoniae*, one of AAC-1-producing *K. pneumoniae*, one of FOX-8-producing *E. coli* and one of DHA-7- and VIM-1-producing *E. cloacae*. All but one of these strain pairs consisted of isolates from the same hospital. The highest number of strain pairs was detected in Hospital Vall d'Hebron (Barcelona) and corresponded to three CMY-2-producing isolates (two of *E. coli* and one of *K. pneumoniae*) and one pair of *E. cloacae* isolates co-producing DHA-7 and VIM-1. Finally, one pair of VIM-1-producing *K. oxytoca* strains comprised of isolates from Hospital Vall d'Hebron and from Laboratori de Referència de Catalunya (both in Barcelona), but no epidemiologic relationship between the patients carrying these isolates could be demonstrated.

pAmpC-producing strains cause nosocomial, healthcare-associated and community infections mainly in predisposed patients. The clinical data of the patients included in our study have already been published, showing that invasive infections were associated with high mortality, which might be partly related to inappropriate empirical therapy [22].

In spite of the predominance of CMY-2- and DHA-1-producing *E. coli*, *K. pneumoniae* and *P. mirabilis*, we found a great genetic diversity among these isolates, higher in *E. coli* and *P. mirabilis* than in *K. pneumoniae*. This finding supports the theory that the spread of these antibiotic resistance mechanisms is mainly due to the dissemination of mobile genetic elements among a polyclonal population. Previous studies have demonstrated the polyclonal dissemination of CMY-2-producing *E. coli* isolates [23, 34, 35]. However, nosocomial outbreaks due to DHA-1- or ACC-1-producing *K. pneumoniae* or CMY-2-producing *P. mirabilis* have been only sporadically described [36, 37]. In this study, we describe two small nosocomial outbreaks due to CMY-2-producing *E. coli* and *P. mirabilis*, respectively.

MBL-producing clinical *Enterobacteriaceae* isolates have been reported all over the world [7, 38, 39]. In this study, we describe two nosocomial outbreaks in two hospitals in Spain, one due to VIM-1-producing *K. oxytoca* in Hospital del Mar (Barcelona) and the other due to IMP-22-producing *K. pneumoniae* in Hospital Puerta del Mar (Cadiz). Conejo et al. [9] described a nosocomial outbreak of IMP-8-producing *K. oxytoca* strain in the same period and in the same area. In contrast to the wide spread of the KPC-producing ST258 *K. pneumoniae* strain [10], the dissemination of VIM and IMP carbapenemases seems to be associated to both clonal intra-hospital spread of local strains [7, 8, 31] and to non-clonal dissemination [7, 29, 38].

The detection and surveillance of pAmpC- and carbapenemase-producing strains is needed in order to implement appropriate therapeutic options and appropriate infection control measures [13]. However, the lack of standardised phenotypic methods has become evident in this study, as only 63 % of all evaluated isolates ($n$=1,654) were pAmpC- or carbapenemase-producers.

In conclusion, the prevalence of pAmpC and carbapenemases found is not negligible. The CMY-type enzymes were the predominant pAmpC found, whereas the VIM or IMP enzymes were the most frequent carbapenemases. A great genetic diversity among pAmpC-producing strains and a closer clonal relationship between carbapenemase-producing strains was found.

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## References

1. Pérez-Pérez FJ, Hanson ND (2002) Detection of plasmid-mediated AmpC β-lactamase genes in clinical isolates by using multiplex PCR. J Clin Microbiol 40:2153–2162
2. Mata C, Miró E, Rivera A et al (2010) Prevalence of acquired AmpC β-lactamases in *Enterobacteriaceae* lacking inducible chromosomal *ampC* genes at a Spanish hospital from 1999 to 2007. Clin Microbiol Infect 16:472–476
3. Navarro F, Pérez-Trallero E, Marimón JM et al (2001) CMY-2-producing *Salmonella enterica*, *Klebsiella pneumoniae*, *Klebsiella oxytoca*, *Proteus mirabilis* and *Escherichia coli* strains isolated in Spain (October 1999–December 2000). J Antimicrob Chemother 48:383–389
4. Yamasaki K, Komatsu M, Abe N et al (2010) Laboratory surveillance for prospective plasmid-mediated AmpC β-lactamases in the Kinki region of Japan. J Clin Microbiol 48:3267–3273
5. Song W, Kim JS, Kim HS et al (2006) Increasing trend in the prevalence of plasmid-mediated AmpC β-lactamases in *Enterobacteriaceae* lacking chromosomal ampC gene at a Korean university hospital from 2002 to 2004. Diagn Microbiol Infect Dis 55:219–224
6. Empel J, Baraniak A, Literacka E et al (2008) Molecular survey of β-lactamases conferring resistance to newer β-lactams in *Enterobacteriaceae* isolates from Polish hospitals. Antimicrob Agents Chemother 52:2449–2454
7. Miró E, Segura C, Navarro F et al (2010) Spread of plasmids containing the $bla_{\text{VIM-1}}$ and $bla_{\text{CTX-M}}$ genes and the *qnr* determinant in *Enterobacter cloacae*, *Klebsiella pneumoniae* and *Klebsiella oxytoca* isolates. J Antimicrob Chemother 65:661–665
8. Oteo J, Hernández-Almaraz JL, Gil-Antón J et al (2010) Outbreak of VIM-1-carbapenemase-producing *Enterobacter cloacae* in a pediatric intensive care unit. Pediatr Infect Dis J 29:1144–1146
9. Conejo MC, Domínguez MC, López-Cerero L et al (2010) Isolation of multidrug-resistant *Klebsiella oxytoca* carrying $bla_{\text{IMP-8}}$, associated with OXY hyperproduction, in the intensive care unit of a community hospital in Spain. J Antimicrob Chemother 65:1071–1073
10. Giakoupi P, Maltezou H, Polemis M et al; Greek System for the Surveillance of Antimicrobial Resistance (2009) KPC-2-producing *Klebsiella pneumoniae* infections in Greek hospitals are mainly due to a hyperepidemic clone. Euro Surveill 14. pii: 19218
11. Cuzon G, Ouanich J, Gondret R et al (2011) Outbreak of OXA-48-positive carbapenem-resistant *Klebsiella pneumoniae* isolates in France. Antimicrob Agents Chemother 55:2420–2423
12. Struelens MJ, Monnet DL, Magiorakos AP et al; European NDM-1 Survey Participants (2010) New Delhi metallo-β-lactamase 1-producing *Enterobacteriaceae*: emergence and response in Europe. Euro Surveill 15. pii: 19716
13. Miriagou V, Cornaglia G, Edelstein M et al (2010) Acquired carbapenemases in Gram-negative bacterial pathogens: detection and surveillance issues. Clin Microbiol Infect 16:112–22
14. Navarro F, Calvo J, Cantón R et al (2011) Detection of resistance phenotypes in gram-negative bacteria. Enferm Infecc Microbiol Clin 29:524–534
15. Walther-Rasmussen J, Høiby N (2007) Class A carbapenemases. J Antimicrob Chemother 60:470–482
16. Clinical and Laboratory Standards Institute (CLSI) (2005) Performance standards for antimicrobial susceptibility testing; fourteenth informational supplement, M100-S14. CLSI, Wayne, PA, USA
17. Pasteran F, Mendez T, Guerriero L et al (2009) Sensitive screening tests for suspected class A carbapenemase production in species of *Enterobacteriaceae*. J Clin Microbiol 47:1631–1639
18. Dubois V, Poirel L, Marie C et al (2002) Molecular characterization of a novel class 1 integron containing $bla_{\text{GES-1}}$ and a fused product of *aac(3)-Ib/aac(6")-Ib*" gene cassettes in *Pseudomonas aeruginosa*. Antimicrob Agents Chemother 46:638–645
19. Yigit H, Queenan AM, Anderson GJ et al (2001) Novel carbapenem-hydrolyzing β-lactamase, KPC-1, from a carbapenem-resistant strain of *Klebsiella pneumoniae*. Antimicrob Agents Chemother 45:1151–1161
20. Poirel L, Magalhaes M, Lopes M et al (2004) Molecular analysis of metallo-β-lactamase gene $bla_{\text{SPM-1}}$-surrounding sequences from disseminated *Pseudomonas aeruginosa* isolates in Recife, Brazil. Antimicrob Agents Chemother 48:1406–1409
21. Poirel L, Lambert T, Türkoglü S et al (2001) Characterization of Class 1 integrons from *Pseudomonas aeruginosa* that contain the $bla_{\text{VIM-2}}$ carbapenem-hydrolyzing β-lactamase gene and of two novel aminoglycoside resistance gene cassettes. Antimicrob Agents Chemother 45:546–552
22. Rodríguez-Baño J, Miró E, Villar M et al (2012) Colonisation and infection due to *Enterobacteriaceae* producing plasmid-mediated AmpC β-lactamases. J Infect 64:176–183
23. Oteo J, Navarro C, Cercenado E et al (2006) Spread of *Escherichia coli* strains with high-level cefotaxime and ceftazidime resistance between the community, long-term care facilities, and hospital institutions. J Clin Microbiol 44:2359–2366
24. Sabbuba NA, Mahenthiralingam E, Stickler DJ (2003) Molecular epidemiology of *Proteus mirabilis* infections of the catheterized urinary tract. J Clin Microbiol 41:4961–4965

25. Tenover FC, Arbeit RD, Goering RV et al (1995) Interpreting chromosomal DNA restriction patterns produced by pulsed-field gel electrophoresis: criteria for bacterial strain typing. J Clin Microbiol 33:2233–2239
26. Ding H, Yang Y, Lu Q et al (2008) The prevalence of plasmid-mediated AmpC β-lactamases among clinical isolates of *Escherichia coli* and *Klebsiella pneumoniae* from five children's hospitals in China. Eur J Clin Microbiol Infect Dis 27:915–921
27. Queenan AM, Bush K (2007) Carbapenemases: the versatile β-lactamases. Clin Microbiol Rev 20:440–458
28. Tórtola MT, Lavilla S, Miró E et al (2005) First detection of a carbapenem-hydrolyzing metalloenzyme in two *Enterobacteriaceae* isolates in Spain. Antimicrob Agents Chemother 49:3492–3494
29. Tato M, Coque TM, Ruíz-Garbajosa P et al (2007) Complex clonal and plasmid epidemiology in the first outbreak of *Enterobacteriaceae* infection involving VIM-1 metallo-β-lactamase in Spain: toward endemicity? Clin Infect Dis 45:1171–1178
30. Pérez-Llarena FJ, Fernández A, Zamorano L et al (2012) Characterization of a novel IMP-28 metallo-β-lactamase from a Spanish *Klebsiella oxytoca* clinical isolate. Antimicrob Agents Chemother 56:4540–4543
31. Gaibani P, Ambretti S, Berlingeri A et al (2011) Rapid increase of carbapenemase-producing *Klebsiella pneumoniae* strains in a large italian hospital: surveillance period 1 March–30 September 2010. Euro Surveill 16. pii: 19800
32. Solé M, Pitart C, Roca I et al (2011) First description of an *Escherichia coli* strain producing NDM-1 carbapenemase in Spain. Antimicrob Agents Chemother 55:4402–4404
33. Pitart C, Solé M, Roca I et al (2011) First outbreak of a plasmid-mediated carbapenem-hydrolyzing OXA-48 β-lactamase in *Klebsiella pneumoniae* in Spain. Antimicrob Agents Chemother 55:4398–4401
34. Oteo J, Cercenado E, Cuevas O et al (2010) AmpC β-lactamases in *Escherichia coli*: emergence of CMY-2-producing virulent phylogroup D isolates belonging mainly to STs 57, 115, 354, 393, and 420, and phylogroup B2 isolates belonging to the international clone O25b-ST131. Diagn Microbiol Infect Dis 67:270–276
35. Naseer U, Haldorsen B, Simonsen GS et al (2010) Sporadic occurrence of CMY-2-producing multidrug-resistant *Escherichia coli* of ST-complexes 38 and 448, and ST131 in Norway. Clin Microbiol Infect 16:171–178
36. Empel J, Hrabák J, Kozińska A et al (2010) DHA-1-producing *Klebsiella pneumoniae* in a teaching hospital in the Czech Republic. Microb Drug Resist 16:291–295
37. Ohana S, Leflon V, Ronco E et al (2005) Spread of a *Klebsiella pneumoniae* strain producing a plasmid-mediated ACC-1 AmpC β-lactamase in a teaching hospital admitting disabled patients. Antimicrob Agents Chemother 49:2095–2097
38. Psichogiou M, Tassios PT, Avlamis A et al (2008) Ongoing epidemic of $bla_{\text{VIM-1}}$-positive *Klebsiella pneumoniae* in Athens, Greece: a prospective survey. J Antimicrob Chemother 61:59–63
39. Cagnacci S, Gualco L, Roveta S et al (2008) Bloodstream infections caused by multidrug-resistant *Klebsiella pneumoniae* producing the carbapenem-hydrolysing VIM-1 metallo-β-lactamase: first Italian outbreak. J Antimicrob Chemother 61:296–300