HAL
open science

Molecular epidemiology and resistance mechanisms involved in reduced susceptibility to amoxicillin/clavulanic acid in isolates from a chronic care centre

Mar Olga Pérez-Moreno, Maria José Centelles-Serrano, Maria Cortell-Ortolá, Isabel Fort-Gallifa, Joaquim Ruiz, Maria Isabel Llovet-Lombarte, Ester Picó-Plana, Anna Maria Jardí-Baiges

To cite this version:

Mar Olga Pérez-Moreno, Maria José Centelles-Serrano, Maria Cortell-Ortolá, Isabel Fort-Gallifa, Joaquim Ruiz, et al.. Molecular epidemiology and resistance mechanisms involved in reduced susceptibility to amoxicillin/clavulanic acid in isolates from a chronic care centre. International Journal of Antimicrobial Agents, 2011, 37 (5), pp.462. 10.1016/j.ijantimicag.2010.12.010 . hal-00685790

HAL Id: hal-00685790

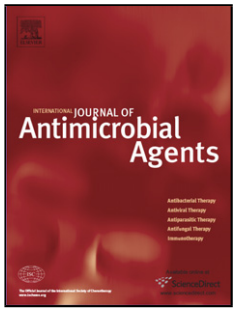
https://hal.science/hal-00685790v1

Submitted on 6 Apr 2012

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Accepted Manuscript



Title: Molecular epidemiology and resistance mechanisms involved in reduced susceptibility to amoxicillin/clavulanic acid in *Klebsiella pneumoniae* isolates from a chronic care centre

Authors: Mar Olga Pérez-Moreno, Maria José Centelles-Serrano, Maria Cortell-Ortolá, Isabel Fort-Gallifa, Joaquim Ruiz, Maria Isabel Llovet-Lombarte, Ester Picó-Plana, Anna Maria Jardí-Baiges

PII: S0924-8579(11)00018-5
DOI: doi:10.1016/j.ijantimicag.2010.12.010
Reference: ANTAGE 3520

To appear in: *International Journal of Antimicrobial Agents*

Received date: 19-8-2010
Revised date: 11-11-2010
Accepted date: 6-12-2010

Please cite this article as: Pérez-Moreno MO, Centelles-Serrano MJ, Cortell-Ortolá M, Fort-Gallifa I, Ruiz J, Llovet-Lombarte MI, Picó-Plana E, Jardí-Baiges AM, Molecular epidemiology and resistance mechanisms involved in reduced susceptibility to amoxicillin/clavulanic acid in *Klebsiella pneumoniae* isolates from a chronic care centre, *International Journal of Antimicrobial Agents* (2010), doi:10.1016/j.ijantimicag.2010.12.010

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Edited manuscript ACCEPTED MANUSCRIPT

Molecular epidemiology and resistance mechanisms involved in reduced susceptibility to amoxicillin/clavulanic acid in *Klebsiella pneumoniae* isolates from a chronic care centre

Mar Olga Pérez-Moreno [a,*], Maria José Centelles-Serrano [a], Maria Cortell-Ortolá [a], Isabel Fort-Gallifa [a], Joaquim Ruiz [b,c], Maria Isabel Llovet-Lombarte [a], Ester Picó-Plana [a], Anna Maria Jardí-Baiges [a]

[a] *Servei Anàlisis Clíniques, Hospital de Tortosa Verge de la Cinta, C/Esplanetes s/n 43500 Tortosa, Spain*

[b] *Centre de Recerca en Salut Internacional de Barcelona (CRESIB), IDIBAPS/Hospital Clinic, Barcelona, Spain*

[c] *CIBER, Epidemiología y Salud Pública (CIBERESP), Spain*

ARTICLE INFO

Article history:

Received 19 August 2010

Accepted 6 December 2010

Keywords:

Klebsiella pneumoniae

Amoxicillin/clavulanic acid

Resistance

IRT-11

OXA-1

Accepted Manuscript

ACCEPTED MANUSCRIPT

Class 1 integrons

* Corresponding author. Tel.: +34 977 519 103.

E-mail address: marolgap@terra.es (M.O. Pérez-Moreno).

Accepted Manuscript

ACCEPTED MANUSCRIPT

ABSTRACT

The aim of this work was to investigate the molecular epidemiology and mechanisms responsible for reduced susceptibility to amoxicillin/clavulanic acid (AMC) among cefazolin-susceptible *Klebsiella pneumoniae* isolates from patients admitted to a chronic care institution. In total, 51 (29.8%) of 171 *K. pneumoniae* isolates recovered between 2006 and 2008 were non-susceptible to AMC, of which 45 were susceptible to cefazolin. Nucleotide sequencing analysis revealed that 19 produced IRT-11 and the remaining 26 were OXA-1-producers. All of the OXA-1-producing isolates harboured the *aac(6')-Ib-cr*–bla_{OXA-1} cassette array, which in 23 isolates was located together with *catB3* and *arr3* within a class1 integron and associated with *qnrS2* (in 3 cases the integron lacked the *qacE*Δ*1* and *sul1* or *sul3* genes). Genotyping analysis performed by enterobacterial repetitive intergenic consensus polymerase chain reaction (ERIC-PCR) identified three different patterns among IRT-11-producing isolates (E1 to E3), with E1 being the most prevalent (63.2%), whilst the OXA-1-producing isolates were assigned to patterns E3 and E3a (isolates carrying typical class 1 integrons), E4 (isolates carrying defective integrons) and E5 (isolates without integrons). Genes encoding IRT-11 and OXA-1 were transferred by conjugation, and *aac(6')-Ib-cr* and *qnrS2* were systematically co-transferred with bla_{OXA-1}. These results demonstrate that the high prevalence of decreased susceptibility to AMC among *K. pneumoniae* isolates from a chronic care hospital was mainly due to the simultaneous spread of two different clones, one of which comprised isolates producing IRT-11 and the other one comprised isolates that had acquired either the bla_{OXA-1} gene located in a class 1 integron and linked to *qnrS2* or the bla_{IRT-11} gene.

Accepted Manuscript

ACCEPTED MANUSCRIPT

1. INTRODUCTION

Klebsiella pneumoniae is a Gram-negative bacterium that is present as a saprophyte in the nasopharynx and intestinal tract of humans at rates of 1–6% and 5–38%, respectively. However, these colonisation rates increase dramatically in hospitalised patients in direct proportion to the length of stay and antibiotic use. Moreover, *K. pneumoniae* is very well adapted to the hospital environment since it exhibits higher survivability on hands and environmental surfaces than other Enterobacteriaceae and the wherewithal to spread rapidly and provoke nosocomial outbreaks [1].

Klebsiella pneumoniae is naturally resistant to amino- and carboxy-penicillins owing to the production of a chromosomally encoded SHV-1-like β-lactamase. Most strains of *K. pneumoniae* are susceptible to narrow-spectrum cephalosporins and to associations of β-lactams with β-lactamase inhibitors (BLIs). Hyperproduction of SHV-1 is the main mechanism responsible for resistance to these compounds in *K. pneumoniae*, whilst production of plasmid-borne enzymes conferring resistance to BLIs but not to narrow-spectrum cephalosporins, such as inhibitor-resistant TEM (IRT)-type or SHV-type β-lactamases, is uncommon in this species [2,3]. On the other hand, simultaneous production of OXA-1 β-lactamase (another enzyme poorly inhibited by clavulanic acid, tazobactam or sulbactam and with negligible activity against first-generation cephalosporins) and the cr variant of Acc(6')-Ib has recently been reported among *K. pneumoniae* isolates producing either plasmid-encoded cephamycinases linked to complex class 1 integrons and to *qnr* genes [4] or extended-spectrum β-lactamases (ESBLs) [5].

Accepted Manuscript

ACCEPTED MANUSCRIPT

A retrospective survey of antibiotic resistance carried out in the microbiology laboratory of Hospital Verge de la Cinta (Tortosa, Catalonia, Spain) in 2005 revealed a high rate of decreased susceptibility to amoxicillin/clavulanic acid (AMC) among cefazolin-susceptible *K. pneumoniae* isolates recovered from patients from a chronic care hospital since 2003. The aim of this study was to follow the evolution of *K. pneumoniae* isolates displaying the former β-lactam resistance phenotype and to investigate their molecular epidemiology and the underlying resistance mechanisms.

2. Material and methods

2.1. Bacterial isolates

All *K. pneumoniae* isolates with decreased susceptibility to AMC [minimum inhibitory concentration (MIC) >8/4 mg/L], recovered from clinical samples from patients admitted to a chronic care centre for whom the microbiology laboratory of the Hospital Verge de la Cinta provides its services, were prospectively collected between January 2006 and December 2008. Only one isolate per patient was considered.

2.2. Antimicrobial susceptibility

In vitro susceptibility to amoxicillin, AMC, piperacillin/tazobactam (TZP), cefazolin, cefoxitin, cefuroxime, ceftazidime, cefotaxime, cefepime, imipenem, meropenem, nitrofurantoin, fosfomycin, tetracycline, chloramphenicol, sulfamethoxazole/trimethoprim, nalidixic acid, ciprofloxacin, gentamicin, kanamycin and tobramycin was studied using the Wider commercial microdilution system (Francisco Soria Melguizo, Madrid, Spain) and to kanamycin, streptomycin,

Accepted Manuscript

ACCEPTED MANUSCRIPT

spectinomycin and sulfonamides by disk diffusion following the guidelines and applying the criteria for susceptibility and resistance of the Clinical and Laboratory Standards Institute (CLSI) [6]. AMC was tested at a 2:1 ratio and TZP was assayed at a fixed inhibitor concentration of 4 mg/L.

2.3. Detection of resistance genes and class 1 integrons

Polymerase chain reaction (PCR) amplification followed by nucleotide sequencing was performed under conditions and with primers previously described (Table 1) to screen for genes encoding resistance to β-lactams (bla_{TEM}, bla_{SHV-1}, bla_{OXA-1} and bla_{PSE-1}), aminoglycosides [*aac(6')-Ib*] and sulfonamides (*sul1* and *sul3*) and for the *qnrA*, *qnrB* and *qnrS* (plasmid-mediated quinolone resistance), *qacEΔ1* and *orf513* (element associated with insertion sequence IS*CR1*) as well as to detect mutations in the quinolone-resistance determining regions (QRDRs) of *gyrA* and *parC* genes. The presence of class 1 integrons was investigated by PCR amplification using specific primers aimed to detect the *intI1* gene (Table 1). Gene integron content within the variable region of class 1 integrons was assessed by PCR using 5'-conserved segment (5'-CS) and 3'-CS set of primers or by PCR mapping assays employing 5'-CS plus bla_{OXA-1} reverse primers and 3'-CS plus bla_{OXA-1} forward primers and subsequent nucleotide sequencing (Table 1). In isolates lacking the *qacEΔ1* gene, the *arr3* reverse specific primer (Table 1) was used instead of 3'-CS.

2.4. Molecular epidemiological analysis

DNA fingerprinting was achieved by means of enterobacterial repetitive intergenic consensus (ERIC)-PCR using the ERIC-2 primer (5'-

ACCEPTED MANUSCRIPT

AAGTAAGTGACTGGGGTGAGCG-3') and repeating reactions at least twice in each isolate. Isolates were considered epidemiologically unrelated if their patterns differed by more than one band [7].

2.5. Conjugation assays

Conjugation experiments were carried out in Luria–Bertani broth with azide-resistant *Escherichia coli* J53 as the recipient strain. Transconjugants were selected on trypticase soy agar plates containing 150 mg/L sodium azide and 100 mg/L amoxicillin.

3. Results

3.1. β-Lactamase characterisation and antibiotic susceptibility

In total, 51 (29.8%) of 171 *K. pneumoniae* isolates recovered from clinical samples of different patients admitted to the chronic care hospital over the 3-year study period showed decreased susceptibility to AMC, 6 of which were resistant to cefazolin and displayed a resistant phenotype consistent with the production of AmpC (four isolates) or ESBLs (two isolates), whilst the remaining 45 isolates, all intermediately resistant to AMC and recovered from urine (88.9%), blood (4.4%), wounds (4.4%) and ascitic fluid (2.2%), remained susceptible to first-generation cephalosporins and underwent further studies. PCR and nucleotide sequencing analysis revealed that 19 isolates produced the β-lactamase IRT-11 (TEM-40) and the other 26 were OXA-1-producers, whereas all harboured the bla_{SHV-1} gene. The percentage of AMC-non-susceptible isolates dropped from 46.6% in 2006 to 14.3% in 2008.

Accepted Manuscript

ACCEPTED MANUSCRIPT

The 19 IRT-11-producing isolates were susceptible to TZP, extended-spectrum cephalosporins, carbapenems, gentamicin, tobramycin and amikacin, whilst resistance to other antibiotics assayed was variable, with fosfomycin (26.3%), ciprofloxacin (26.3%) and nalidixic acid (57.9%) showing the highest rates of resistance. All OXA-1-producing isolates were susceptible to cefotaxime, ceftazidime, cefepime (MIC between 1 mg/L and 4 mg/L in 61.5% of isolates), carbapenems and gentamicin and were resistant to tobramycin, kanamycin and ciprofloxacin. Resistance rates to sulfonamides, nitrofurantoin, chloramphenicol, nalidixic acid and TZP were 76.9%, 80.8%, 84.6%, 92.3% and 92.3% and were <25% for the remaining antimicrobials studied.

3.2. Molecular epidemiology and detection of integrons, qnr genes and mutations in the QRDRs of gyrA and parC genes

The characteristics (ERIC-PCR pattern, resistance phenotype, presence and content of class 1 integrons, presence of *qnr*, amino acid changes in GyrA and ParC, and year of isolation) of the 19 IRT-11- and 26 OXA-1-producing isolates are shown in Table 2.

Three ERIC-PCR patterns were observed among the IRT-11-producing isolates, with E1 being the most prevalent (12; 63.2%), followed by E3 (5; 26.3%) and E2 (2; 10.5%). None of the IRT-11-producing isolates possessed *qnr* genes and only 1 presented a class 1 integron with a 2 kb/*dfrA12–orf–aadA2* variable region.

OXA-1-producing isolates could be classified into three different groups. The first group comprised 20 isolates that carried a typical class 1 integron with the following

ACCEPTED MANUSCRIPT

variable region [3.6 kb/*aac(6')-Ib-cr*, bla_{OXA-1}, *catB3*, *arr3*] as well as *qnrS2* and were ascribed to two closely related ERIC patterns (E3 and E3a) that differed from each other by only one band. The second group comprised three sulfonamide-susceptible isolates that shared the same ERIC-PCR pattern (E4) and harboured *qnrS2* and a class 1 integron whose variable region was identical to that described above, although they were negative for *qacE*Δ*1* and *sul1* (quaternary ammonium compounds and sulfonamide resistance genes associated with 3'-conserved region of class 1 integrons) as well as for *sul3*, a sulfonamide resistance gene encountered in the 3'-end of some atypical class 1 integrons [8]. Finally, the third group was made up of another three sulfonamide-susceptible isolates that contained the *aac(6')-Ib-cr*–bla_{OXA-1} array but were negative for *intI1*, *qnrA*, *qnrB* and *qnr*S and were assigned to the E5 ERIC-PCR pattern. None of the OXA-1-producing isolates possessed *orf513*.

All nalidixic acid-resistant isolates analysed presented at least one point mutation in the *gyrA* or *parC* genes leading to amino acid substitutions.

3.3. Transfer of amoxicillin/clavulanic acid resistance and characteristics and antimicrobial resistance of transconjugants

Conjugation assays were carried out in selected IRT-11- and OXA-1-producing isolates displaying the different ERIC-PCR patterns identified in this work. Transfer by conjugation of genes involved in AMC resistance (bla_{IRT-11} or bla_{OXA-1}) was achieved in all the isolates studied. Moreover, bla_{OXA-1} was systematically co-transferred with *aac(6')-Ib-cr*, and transconjugants from integron-carrying OXA-1-producing isolates were positive for *qnrS2* and bore class 1 integrons that were

ACCEPTED MANUSCRIPT

identical to those presented by donor strains. The MIC and other characteristics of the donor strains and their transconjugants are shown in Table 3.

4. Discussion

The results of this work demonstrate that the high prevalence of decreased susceptibility to AMC among *K. pneumoniae* isolates recovered over a 3-year period from patients from a chronic care hospital was mediated by the production of IRT-11 or OXA-1, two penicillinases weakly inhibited by clavulanic acid, and was mainly due to the simultaneous spread of two different clones, one of which comprised isolates that produced IRT-11 (E1) and the other one comprising isolates that had acquired either the $bla_{\text{OXA-1}}$ gene placed within a class 1 integron and linked to *qnrS2* or the $bla_{\text{IRT-11}}$ gene (E3).

In this series, isolates harbouring the $bla_{\text{IRT-11}}$ gene located in a conjugative plasmid accounted for 37.3% of AMC-resistant *K. pneumoniae* isolates and for up to 11.1% of total isolates. IRT-11-producing isolates were distributed in three different clones, a finding which indicates that, in addition to clonal dissemination, horizontal transfer or independent emergence of $bla_{\text{IRT-11}}$ may have occurred. Although IRT β-lactamases have been described in sporadic clinical isolates of *K. pneumoniae* [2], these enzymes, unlike TEM-derived ESBLs, are still infrequent and occur chiefly in community-acquired *E. coli* isolates [9–11]. The high diversity of mutant enzymes and the lack of clonal relatedness observed in some studies suggest the independent emergence and selection of these TEM variants under antibiotic selective pressure, posed by increasing use of inhibitor-containing combinations in clinical practice, rather than an epidemic dissemination [10,11]. Although IRT enzymes are usually

ACCEPTED MANUSCRIPT

encoded by genes harboured by conjugative plasmids, they appear to be less prone to be transmitted or selected than ESBLs or TEM-1 β-lactamases, perhaps because their production constitutes a less efficient resistance mechanism, protecting bacteria specifically against the action of inhibitors but not against the effects of other β-lactams. In fact, to our knowledge there is only one previous description reporting an outbreak involving IRT β-lactamases. This outbreak was caused by the dissemination of clonally related *K. pneumoniae* isolates recovered in a geriatric facility in France that produced IRT-2 [12]. It is appropriate to point out that the isolates in the current study were also obtained from patients from a chronic care centre, a setting where bacteria are submitted to heavy antibiotic selection pressure and where strict adherence to infection control precautions may be difficult to achieve.

With regard to isolates bearing the bla_{OXA-1} gene, which represented 51% of AMC-non-susceptible isolates and 15.2% of total isolates, all of them also carried *aac(6')-Ib-cr*, a gene encoding a new variant of the acetyltransferase Acc(6')-Ib that exhibits the ability to acetylate not only tobramycin, kanamycin and amikacin but also quinolones with a piperazinyl amine such as ciprofloxacin. In most of these isolates the *aac(6')-Ib-cr*–bla_{OXA-1} cassette array was located within the variable region of a class 1 integron together with *catB3* and *arr3*, which confer resistance to chloramphenicol and rifampicin, respectively, and was associated with a *qnrS2* gene, probably in the same plasmid, since this determinant was consistently co-transferred by conjugation with the former integron. The aforementioned variable region was identical to that described in various enterobacterial clinical isolates from a different geographical origin [4,13] and to that recently identified in the class 1 integron of an aquatic isolate of *Aeromonas allosaccharophila* [14]. However, when specified, the

ACCEPTED MANUSCRIPT

In*37*-like integron of clinical isolates was associated with *qnrA or qnrB* and with an IS*CR1* element, whilst the integron of *A. allosaccharophila* was not linked to IS*CR1* and was placed in a plasmid carrying *qnrS2*, similar to what occurred with the present *K. pneumoniae* isolates. Interestingly, three of the OXA-1-producing isolates obtained in 2008 carried a class 1 integron lacking both the *qacE*Δ*1* and *sul1* genes typically present in the 3'-CS of class 1 integrons and that was also not associated with *sul3*. This circumstance also appears to be common among aquatic environmental bacteria, perhaps because natural bacterial isolates are less likely to have been exposed to synthetic antimicrobials such as sulfonamides and hence may not yet have found a need to integrate a *sul* resistance gene cassette into their integron [15]. Although genotyping analysis demonstrated that the current spread of OXA-1-producing *K. pneumoniae* isolates in the chronic care centre under discussion was the result of clonal dissemination, the fact that we have recently identified two *qnrS2*-positive *E. coli* isolates, likewise carrying *aac*(6')-*Ib-cr*, bla_{OXA-1}, *catB3 and arr3* genes inserted in the variable region of a defective class 1 integron (unpublished data), in patients admitted to the former institution is of concern and evidences the role of integrons in the dissemination and co-selection of resistance in Gram-negative microorganisms.

Finally, we would like to highlight what in our opinion is the most striking finding of this work, namely the simultaneous emergence and scatter in a healthcare institution of *K. pneumoniae* isolates displaying two different resistance mechanisms, quite uncommon in this species, which confer specific resistance to BLIs. We speculate that the elevated consumption of AMC in the centre, where it was the most frequently prescribed antibiotic (data not shown), could have favoured the emergence and

ACCEPTED MANUSCRIPT

selection of IRT-11- and OXA-1-producing isolates. Additionally, in the latter case, the widespread use of fluoroquinolones could have also contributed, as is suggested by the association of bla_{OXA-1} with the plasmid-mediated quinolone resistance determinants *aac(6')-Ib-cr* and *qnrS2* and the coexistence of chromosomal mutations in the QRDR. Moreover, deficiencies in infection control measures probably led to their propagation among patients, resulting in the consequent epidemic outbreak.

Acknowledgments

The authors thank M. Pilar Cid Ventura and Carmen López Escorihuela for excellent technical support in the conduct of this study as well as Donna Pringle for editorial assistance. They also thank J. Calvo Montes (Hospital Marqués de Valdecilla, Santander, Spain) for kindly providing *E. coli* J53-AzR.

Funding

This work was partially supported by Fundació Doctor Ferran (2006 Research on Health Sciences Grant). The research of JR is supported by project CP05/0130 of the Fondo de Investigaciones Sanitarias (Spain).

Competing interests

None declared.

Ethical approval

Not required.

Accepted Manuscript

ACCEPTED MANUSCRIPT

References

[1] Podschun R, Ullmann U. *Klebsiella* spp. as nosocomial pathogens: epidemiology, taxonomy, typing methods, and pathogenicity factors. Clin Microbiol Rev 1998;11:589–603.

[2] Lemozy J, Sirot D, Chanal C, Huc C, Labia R, Dabernat H, et al. First characterization of inhibitor-resistant TEM (IRT) β-lactamases in *Klebsiella pneumoniae* strains. Antimicrob Agents Chemother 1995;39:2580–2.

[3] Dubois V, Poirel L, Demarthe F, Arpin C, Coulange L, Minarini LA, et al. Molecular and biochemical characterization of SHV-56, a novel inhibitor-resistant β-lactamase from *Klebsiella pneumoniae*. Antimicrob Agents Chemother 2008;52:3792–4.

[4] Quiroga MP, Andres P, Petroni A, Soler Bistué AJ, Guerriero L, Vargas LJ, et al. Complex class 1 integrons with diverse variable regions, including *aac(6')-Ib-cr*, and a novel allele, *qnrB10*, associated with IS*CR1* in clinical enterobacterial isolates from Argentina. Antimicrob Agents Chemother 2007;51:4466–70.

[5] Machado E, Coque TM, Cantón R, Baquero F, Sousa JC, Peixe L; Portuguese Resistance Study Group. Dissemination in Portugal of CTX-M-15-, OXA-1-, and TEM-1-producing Enterobacteriaceae strains containing the *aac(6')-Ib-cr* gene, which encodes an aminoglycoside- and fluoroquinolone-modifying enzyme. Antimicrob Agents Chemother 2006;50:3220–1.

[6] Clinical and Laboratory Standards Institute. *Performance standards for antimicrobial susceptibility testing*; *nineteenth informational supplement*. Document M100-S19. Wayne, PA: CLSI; 2009.

Accepted Manuscript

ACCEPTED MANUSCRIPT

[7] Deplano A, Vaneechoutte M, Verschraegen G, Struelens M. Typing of *Staphylococcus aureus* and *Staphylococcus epidermidis* strains by PCR analysis of inter-IS*256* spacer length polymorphisms. J Clin Microbiol 1997;35:2580–7.

[8] Pérez-Moreno MO, Centelles-Serrano MJ, Cortell-Ortolá M, Ruiz J, Llovet-Lomabarte MI, Jardí-Baiges AM, et al. Multidrug resistance related to class 1 integrons in human *Salmonella enterica* serotype Typhimurium isolates and emergence of atypical *sul3*-associated integrons. Int J Antimicrob Agents 2009;34:381–3.

[9] Pérez-Moreno MO, Pérez-Moreno M, Carulla M, Rubio C, Jardí AM, Zaragoza J. Mechanisms of reduced susceptibility to amoxycillin–clavulanic acid in *Escherichia coli* strains from the health region of Tortosa (Catalonia, Spain). Clin Microbiol Infect 2004;10:234–41.

[10] Miró E, Navarro F, Mirelis B, Sabaté M, Rivera A, Coll P, et al. Prevalence of clinical isolates of *Escherichia coli* producing inhibitor-resistant β-lactamases at a university hospital in Barcelona, Spain, over a 3-year period. Antimicrob Agents Chemother 2002;46:3991–4.

[11] Martín O, Valverde A, Morosini MI, Rodríguez-Domínguez M, Rodríguez-Baños M, Coque TM, et al. Population analysis and epidemiological features of inhibitor-resistant-TEM-β-lactamase-producing *Escherichia coli* isolates from both community and hospital settings in Madrid, Spain. J Clin Microbiol 2010;48:2368–72.

[12] Girlich D, Karim A, Poirel L, Cavin MH, Verny C, Nordmann P. Molecular epidemiology of an outbreak due to IRT-2 β-lactamase-producing strains of *Klebsiella pneumoniae* in a geriatric department. J Antimicrob Chemother 2000;45:467–73.

Accepted Manuscript

ACCEPTED MANUSCRIPT

[13] Chang GY, Fang YT, Tsai SM, Chamg LL, Yu WL. Characterization of class 1 integrons and gene cassettes in clinical isolates of *Klebsiella pneumoniae* from Taiwan. Diagn Microbiol Infect Dis 2009;65:214–6.

[14] Picão RC, Poirel L, Demarta A, Ferreira Silva CS, Corvaglia AR, Petrini O, et al. Plasmid-mediated quinolone resistance in *Aeromonas allosaccharophila* recovered from a Swiss lake. J Antimicrob Chemother 2008;62:948–50.

[15] Rosser SJ, Young HK. Identification and characterization of class 1 integrons in bacteria from an aquatic environment. J Antimicrob Chemother 1999;44:11–8.

Accepted Manuscript

ACCEPTED MANUSCRIPT

Table 1

Primers used in this study for polymerase chain reaction (PCR)

Target	Primer	Sequence (5'→3')
bla$_{TEM}$	TEM-1	ATA AAATTCTTGAAGACGAAA
	TEM-2	GACAGTTACCAATGCTTAATCA
bla$_{SHV}$	SHV-F	CACTCAAGGATGTATTGTG
	SHV-R	TTAGCGTTGCCAGTGCTCG
bla$_{OXA}$	OXA-F	AGCAGCGCCAGTGCATCA
	OXA-R	ATTCGACCCCAAGTTTCC
bla$_{PSE}$	OC-1	AATGGCAATCAGCGCTTC
	OC-2	GGGGCTTGATGCTCACTC
aac(6')-Ib	ACC(6')-B-F	TTGCGATGCTCTATGAGTGGCTA
	ACC(6')-B-R	CTCGAATGCCTGGCGTGTTT
sul1	Sul-F	TGGTGACGGTGTTCGGCATTC
	Sul-R	GCGAGGGTTTCCGAGAAGGTG
sul3	Sul3-F	CATTCTAGAAAACAGTCGTAGTTCG
	Sul3-R	CATCTGCAGCTAACCTAGGGCTTTGGA
qnrA	QnrA-F	ATTTCTCACGCCAGGATTTG
	QnrA-R	GATCGGCAAAGGTTAGGTCA
qnrB	QnrB-F	GATCGTGAAAGCCAGAAAGG
	QnrB-R	ACGATGCCTGGTAGTTGTCC
qnrS	QnrS-F	ACGACATTCGTCAACTGCAA
	QnrS-R	TAAATTGGCACCCTGTAGGC
qacEΔ1	Qac-F	GGCTGGCTTTTTCTTGTTATCG
	Qac-R	TGAGCCCCATACCTACAAAGC
orf513	orf513-F	ATGGTTTCATGCGGGTT
	orf513-R	CTGAGGGTGTGAGCGAG
intI1	IntI1-F	GGGTCAAGGATCTGGATTTCG
	IntI1-R	ACATGGGTGTAAATCATCGTC
Int 1 VR [a]	5'-CS	GGCATCCAAGCAGCAAG
	3'-CS	AAGCAGACTTGACCTGA
arr3	arr-3-R	CCCTGTGGTTAGCAAGTC

Accepted Manuscript

ACCEPTED MANUSCRIPT

gyrA	GyrA-F	AAATCTGCCCGT GTCGTTGGT
	GyrA-R	GCCATACCTACGGCGATACC
parC	ParC-F	CCTTGCGCTACATGAATTTA
	ParC-R	CAGGTTATGCGGTGGAATAT

[a] Class 1 integron variable region.

Accepted Manuscript

ACCEPTED MANUSCRIPT

Table 2

Features of the 19 IRT-11- and 26 OXA-1-producing *Klebsiella pneumoniae* isolates

β-Lactamase	ERIC-PCR pattern	Resistance phenotype [a]	Class 1 integron [b]	*qnr*	Amino acid changes [c]		Year of isolation (*n*)
					GyrA	ParC	
IRT-11 [d]	E1	Am, Amc (Nal, Fos)	No	No	Phe83	–	2006 (8); 2007 (3); 2008 (1)
	E2	Am, Amc (Fos, C)	No	No	–	–	2006 (2)
	E3	Am, Amc, Nal, Cip, Nf, C (K, Fos, Sxt, T, St/Sp, Sul)	2 kb/*dfrA12*–orf–*aadA2* [e]	No	Ile83	Ile80	2006 (2); 2007 (1); 2008 (2)
OXA-1 [d]	E3	Am, Amc, Nal, Cip, Tob, K, Nf, C, Sul (Crx, Fos, Sxt, T, St)	3.6 kb/*aac(6')-Ib-cr*, bla_{OXA-1}, *catB3*, *arr3*	*qnrS2*	Ile83	Ile80	2006 (15); 2007 (1); 2008 (1)
	E3a	Am, Amc, Nal, Cip, Tob, K, Nf, C, Sul, St/Sp	3.6 kb/*aac(6')-Ib-cr*, bla_{OXA-1}, *catB3*, *arr3*	*qnrS2*	Ile83	Ile80	2006 (1); 2007 (2)
	E4	Am, Amc, Cip, Tob, K (Nal, Nf, C)	3.6 kb/*aac(6')-Ib-cr*, bla_{OXA-1}, *catB3*, *arr3* [f]	*qnrS2*	Phe83	–	2008 (3)
	E5	Am, Amc, Nal, Cip, Tob, K (Ak, Nf, C, Fos)	No	No	Phe83/Asn87	Ile80	2008 (3)

ERIC-PCR, enterobacterial repetitive intergenic consensus polymerase chain reaction.

1

ACCEPTED MANUSCRIPT

[a] Resistance phenotypes: Am, amoxicillin; Amc, amoxicillin/clavulanic acid; Nal, nalidixic acid; Fos, fosfomycin; C, chloramphenicol; Cip, ciprofloxacin; Nf, nitrofurantoin; K, kanamycin; Sxt, sulfamethoxazole/trimethoprim; T, tetracycline; St, streptomycin; Sp, spectinomycin; Sul, sulfonamides; Tob, tobramycin; Crx, cefuroxime; Ak, amikacin. Additional antibiotic resistances found in some but not all of the isolates are indicated by parentheses.

[b] Size and resistance genes inserted within the variable region of class 1 integrons identified by PCR amplification and nucleotide sequencing using 5'-CS plus 3'-CS set of primers or 5'-CS plus bla_{OXA-1} reverse primers and 3'-CS or *arr3* reverse primers plus bla_{OXA-1} forward primers:*dfrA12* (trimethoprim resistance); *aadA2* (streptomycin and spectinomycin resistance); ***aac(6')-Ib-cr*** (kanamycin, tobramycin and amikacin resistance and ciprofloxacin decreased susceptibility); bla_{OXA-1} (β-lactam resistance); *catB3* (chloramphenicol resistance), *arr3* (rifampicin resistance).

[c] Amino acid substitutions in GyrA and ParC deduced from mutations detected by PCR amplification and nucleotide sequencing of the quinolone-resistance determining regions of *gyrA* and *parC* genes from nalidixic acid-resistant isolates.

[d] All isolates additionally produced SHV-1.

[e] A single isolate.

[f] Isolates with atypical 3'-end class 1 integron [negative for *qacEΔ1*, *sul1* and *sul3* (quaternary ammonium compounds and sulfonamide resistance genes associated with class 1 integrons)].

ACCEPTED MANUSCRIPT

Table 3

Characteristics and resistance profiles of donor strains and transconjugants

Isolate (β-lactamase; ERIC-PCR pattern)	*IntI1/qnrS2* [a]	MIC (mg/L)															
		Am	Amc	Tzp	Cfz	Cx	Crx	Ctx	Cfp	Tob	Ak	Nal	Cip	C	Nf	Fos	Sxt
EC J53 Az-R [b]	No/No	≤4	≤4/2	≤16/4	≤2	≤4	≤1	≤0.12	≤0.5	≤2	≤4	≤4	≤0.12	≤4	≤64	≤16	≤2/38
Kp9 (IRT-11; E2) [c]	No/No	>16	16/8	≤16/4	≤2	≤4	≤1	≤0.12	≤0.5	≤2	≤4	≤4	≤0.12	>8	≤64	>32	≤2/38
TC Kp9 [d]	No/No	>16	16/8	≤16/4	≤2	≤4	≤1	≤0.12	≤0.5	≤2	≤4	≤4	≤0.12	≤4	≤64	≤16	≤2/38
Kp12 (IRT-11; E3) [c]	No/No	>16	16/8	≤16/4	8	16	>16	≤0.12	≤0.5	≤2	≤4	>16	>4	>8	>64	>32	≤2/38
TC Kp12 [d]	No/No	>16	16/8	≤16/4	≤2	≤4	≤1	≤0.12	≤0.5	≤2	≤4	≤4	≤0.12	≤4	≤64	≤16	≤2/38
Kp13 (IRT-11; E1) [c]	No/No	>16	16/8	≤16/4	≤2	≤4	≤1	≤0.12	≤0.5	≤2	≤4	≤4	≤0.12	≤4	≤64	≤16	≤2/38
TC Kp13 [d]	No/No	>16	16/8	≤16/4	≤2	≤4	≤1	≤0.12	≤0.5	≤2	≤4	≤4	≤0.12	≤4	≤64	≤16	≤2/38
Kp15 (IRT-11; E3) [c]	+/No	>16	16/8	≤16/4	≤2	≤4	2	≤0.12	≤0.5	≤2	≤4	>16	>4	>8	>64	16	>4/76
TC Kp15 [d]	+/No	>16	16/8	≤16/4	≤2	≤4	≤1	≤0.12	≤0.5	≤2	≤4	≤4	≤0.12	≤4	≤64	≤16	>4/76

1

Accepted Manuscript

ACCEPTED MANUSCRIPT

Kp18 (OXA-1; E3) [c]	+/+	>16	16/8	32/4	4	4	16	≤0.12	1	>8	8	>16	>4	>8	>64	>32	≤2/38
TC Kp18 [d]	+/+	>16	16/8	32/4	≤2	≤4	16	≤0.12	1	8	8	16	2	8	≤64	≤16	≤2/38
Kp19 (OXA-1; E3) [c]	+/+	>16	16/8	64/4	4	≤4	8	0.25	2	>8	8	>16	>4	>8	>64	32	>4/76
TC Kp19 [d]	+/+	>16	16/8	32/4	≤2	≤4	8	0.25	2	>8	8	16	1	8	≤64	≤16	≤2/38
Kp21 (OXA-1; E3a) [c]	+/+	>16	16/8	64/4	4	≤4	16	0.5	2	>8	≤4	>16	>4	>8	>64	≤16	≤2/38
TC Kp21 [d]	+/+	>16	16/8	32/4	≤2	≤4	4	0.25	1	8	≤4	16	2	8	≤64	≤16	≤2/38
Kp22 (OXA-1; E4) [c]	+/+	>16	16/8	64/4	≤2	≤4	8	0.25	2	>8	8	16	4	≤4	≤64	≤16	≤2/38
TC Kp22 [d]	+/+	>16	16/8	64/4	≤2	≤4	8	0.25	2	>8	16	16	2	8	≤64	≤16	≤2/38
Kp25 (OXA-1; E5) [c]	No/No	>16	16/8	32/4	4	≤4	8	≤0.12	2	>8	16	>16	>4	≤4	≤64	32	≤2/38
TC Kp25 [d]	No/No	>16	8/4	≤16/4	≤2	≤4	8	≤0.12	1	>8	8	≤4	≤0.12	≤4	≤64	32	≤2/38

ERIC-PCR, enterobacterial repetitive intergenic consensus polymerase chain reaction; MIC, minimum inhibitory concentration; Am, amoxicillin; Amc, amoxicillin/clavulanic acid; Tzp, piperacillin/tazobactam; Cfz, cefazolin; Cx, cefoxitin; Crx, cefuroxime; Ctx, cefotaxime; Cfp, cefepime; Tob, tobramycin; Ak, amikacin; Nal, nalidixic acid; Cip, ciprofloxacin; C, chloramphenicol; Nf, nitrofurantoin; Fos, fosfomycin; Sxt, sulfamethoxazole/trimethoprim.

ACCEPTED MANUSCRIPT

[a] Presence of *intI1* (encoding integrase 1) or *qnrs2* (encoding QnrS2).

[b] Recipient strain (azide-resistant *Escherichia coli* J53).

[c] *Klebsiella pneumoniae* donor strains.

[d] Transconjugants.

Accepted Manuscript