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A novel IncQ plasmid type harbouring a class 3 integron from *Escherichia coli*

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Objectives: To determine the genetic structures at the origin of the mobilization of the extended-spectrum β -lactamase (ESBL) *bla*_{GES-1} gene in an *Escherichia coli* clinical isolate.

Methods: ESBL-encoding genes and class 1 or class 3 integron-specific motifs were screened. Conjugation experiments were performed to determine whether the plasmid-carrying *bla*_{GES-1} gene was self-transferable. Plasmid sequencing was achieved by a primer-walking approach.

Results: The *bla*_{GES-1} gene was located in a class 3 integron. That unusual genetic structure was itself located on an \sim 9 kb plasmid, pQ7, which was not self-transferable. Sequence analysis revealed that plasmid pQ7 belonged to a novel subtype of the IncQ group.

Conclusions: This study identified for the first time the *bla*_{GES-1} gene in *E. coli* and in Switzerland. It describes a novel IncQ-type plasmid subgroup that possesses original features, in particular iteron sequences that constitute a hot spot for integration of foreign DNA.

Keywords: IncQ, ESBLs, GES-1

Introduction

Among all the extended-spectrum β -lactamases (ESBLs) identified so far in Gram-negative bacteria, the GES enzymes have been found in Enterobacteriaceae and *Pseudomonas aeruginosa*, and recently in *Acinetobacter baumannii*.^{1–5} Interestingly, several GES variants possess a significant carbapenemase activity.^{1,6–8} The *bla*_{GES}-type genes are part of the class 1 integrons,⁹ with the exception of a single report describing the *bla*_{GES-1} gene as part of a class 3 integron in *Klebsiella pneumoniae*.¹⁰ Class 3 integrons differ from class 1 integrons by the integrase type.^{11,12}

Although the mechanisms leading to the dissemination of class 1 integrons (which are themselves non-self-transferable structures) are well known,^{13–15} including mostly Tn21-like and Tn402-like transposon structures, those that are responsible for the spread of class 3 integrons remain unknown. In our study, the complete sequence of a plasmid bearing a class 3 integron containing two antibiotic resistance genes was characterized.

Materials and methods

Bacterial isolates and susceptibility testing

Escherichia coli isolate TB7 was identified by using the API20E system (bioMérieux, Carponne, France). The antibiotic susceptibility of isolate

TB7 and its corresponding *E. coli* transformant was determined by the disc diffusion technique on Mueller–Hinton agar plates with β -lactam and non- β -lactam antibiotic-containing discs.¹⁶ The MICs of β -lactams, quinolones and fluoroquinolones were determined by an agar dilution technique.¹⁶ Double-disc synergy testing was performed with discs containing cefotaxime or ceftazidime on the one hand, and ticarcillin/clavulanic acid or ceftiofloxacin on the other hand.

Azide-resistant *E. coli* J53 and the electrocompetent *E. coli* TOP10 (Invitrogen, Cergy-Pontoise, France) were used as hosts in conjugation and transformation experiments, respectively. *E. coli* (pRP4) harbouring an IncP-type and mobilizing plasmid was used to perform mobilization experiments.¹⁷

PCR amplification and sequencing

Total DNA of *E. coli* TB7 was extracted as described previously¹⁸ and used as a template in standard PCR conditions with a series of primers designed for the detection of Ambler class A β -lactamase genes (*bla*_{TEM}, *bla*_{SHV}, *bla*_{CARB}, *bla*_{VEB}, *bla*_{PER}, *bla*_{GES} and *bla*_{CTX-M}).¹⁹ PCRs with primers specific for 5'-CS and 3'-CS regions of class 1 integrons combined with *bla*_{GES}-specific primers were performed as described previously.⁸ Amplified DNA fragments were purified with the Qiaquick PCR purification kit (Qiagen, Courtaboeuf, France). Both strands of the amplification products obtained were sequenced with an ABI 3100 sequencer (Applied Biosystems, Foster City, CA, USA). The nucleotide and deduced protein sequences were analysed with software available over the Internet at

the National Center for Biotechnology Information web site (www.ncbi.nlm.nih.gov). The whole nucleotide sequence of plasmid pQ7 was obtained using a primer-walking sequencing approach.

Plasmid analysis

Conjugation assays were performed between an *E. coli* TB7 isolate as the donor and an azide-resistant *E. coli* J53 as the recipient strain, as described previously,²⁰ using a selection based on ceftazidime (30 mg/L) and azide (100 mg/L). Plasmid DNAs were extracted using the Kieser method²¹ and electrotransformation assays were performed as described previously.³

Results and discussion

E. coli TB7 was isolated in 1998 from the urine sample of a Swiss patient who did not have a recent history of travel. It was resistant to most β -lactams, except piperacillin/tazobactam, cephamycins and carbapenems (Table 1). In addition, it was resistant to kanamycin, tobramycin, trimethoprim, sulphonamides, tetracycline and rifampicin. Synergy images between clavulanic acid and broad-spectrum cephalosporins were observed, in addition to a peculiar synergy between ceftaxime and cefuroxime. PCR results followed by sequencing identified the ESBL gene *bla*_{GES-1} together with the β -lactamase gene *bla*_{TEM-1}. PCR mapping using class 1 integron and *bla*_{GES-1}-specific primers failed,²² indicating that the *bla*_{GES-1} gene was located in an unusual genetic context in *E. coli* TB7.

Mating assays using *E. coli* TB7 isolate as a donor remained unsuccessful. Electrotransformation assays resulted in an *E. coli* J53 (pQ7) transformant expressing ESBL GES-1 (Table 1). In addition, the transformant was additionally resistant to amikacin and kanamycin, but susceptible to other compounds, including sulphonamides. Plasmid analysis showed that *E. coli* TB7 harboured two plasmids of ~65 and 9 kb, although the *E. coli* transformant only harboured the 9 kb plasmid, named pQ7.

The entire sequence of plasmid pQ7 was determined and analysis of the DNA sequences flanking the *bla*_{GES-1} gene showed that it corresponded to a gene cassette,²³ as previously observed for the *bla*_{GES}-like genes.⁴ It possessed core and inverse core sites, together with a 59-be element.²⁴ Upstream of the *bla*_{GES-1} gene cassette, a gene encoding the class 3 integrase Int3 was identified, sharing 60% amino acid identity with the

class 1 integrase Int1. The Int3 protein identified in *E. coli* TB7 shared 100% identity with the Int3 associated with the *bla*_{GES-1} gene of *K. pneumoniae* FFUL 22K from Portugal,¹⁰ had a single amino acid substitution compared with the Int3 found in two *Delftia* spp. isolates (a Gram-negative species)²⁵ and had four amino acid substitutions compared with the Int3 identified with the metallo- β -lactamase gene *bla*_{IMP-1} from *Serratia marcescens*.^{12,26} Downstream of the *bla*_{GES-1} gene, the *bla*_{OXA-10}/*aac*(6')-Ib fused gene cassette was identified, as found in *K. pneumoniae* FFUL 22K.¹⁰ The extremities of the class 3 integron were not clearly defined, probably being identical to those identified previously in *Delftia* spp.²⁵ and suggesting that a deletion of those ends had occurred during evolution.

In addition to this *int3-bla*_{GES-1}-*bla*_{OXA-10}/*aac*(6')-Ib structure, the backbone of plasmid pQ7 contained five open reading frames: RepC (308 amino acids); RepA (322 amino acids); a fused relaxase-primase protein Mob/DnaG (813 amino acids); MobC (143 amino acids); and a hypothetical protein (155 amino acids) (Figure 1a). This plasmid has replication genes that are similar to those of other IncQ-type plasmids, including the replicase genes (*repA*, *repB* and *repC*) and *oriV* with the iteron-based incompatibility determinant (Figure 1b). The *oriV* region contains repeated sequences called iterons that serve as specific binding sites for the RepC protein. The IncQ plasmids have been divided into different groups on the basis of the sequence homology of their Rep proteins and their iterons.²⁴ The comparative analysis of the replication proteins of pQ7 showed that this plasmid was homologous to two other plasmids, the sequences of which are available in the GenBank database, being: (i) pGNB2 recovered from a wastewater treatment plant from an unknown host strain and harbouring the quinolone resistance gene *qnrS2*,²⁷ and an identical plasmid named pBRST7.6 identified from *Aeromonas hydrophila* in India (GenBank accession no. EU925817); and (ii) pWES-1 identified from *Salmonella enterica* Westhampton in France and carrying the ESBL-encoding gene *bla*_{CTX-M-53} (Figure 1a).²⁸ However, plasmid pQ7 did not show any significant homology with the IncQ-type plasmid pCHE-A of 7 kb carrying the *bla*_{GES-1} gene that was associated with the original integron mobilization unit (IMU) elements.⁸ Overall, it is interesting to underline that the IncQ-type plasmid plays a significant role in the dissemination of antibiotic resistance genes.

The gene encoding RepC that was identified in pQ7 is identical to that of plasmid p22K9 from *K. pneumoniae*,¹⁰ suggesting that

Table 1. MICs (mg/L) of β -lactams for the *E. coli* TB7 clinical isolate, the *E. coli* J53 transformant harbouring plasmid pQ7 expressing β -lactamase GES-1 and the *E. coli* J53 reference strain

β -Lactam	<i>E. coli</i> TB7 (GES-1)	<i>E. coli</i> J53 (pQ7) (GES-1)	<i>E. coli</i> J53
Amoxicillin	>512	>512	2
Amoxicillin+CLA	>256	16	2
Ceftazidime	>512	128	0.06
Ceftazidime+CLA	256	16	0.06
Cefoxitin	>512	128	4
Imipenem	>32	1	0.06
Meropenem	32	0.25	0.03
Ertapenem	>32	0.12	0.03

CLA, clavulanic acid.

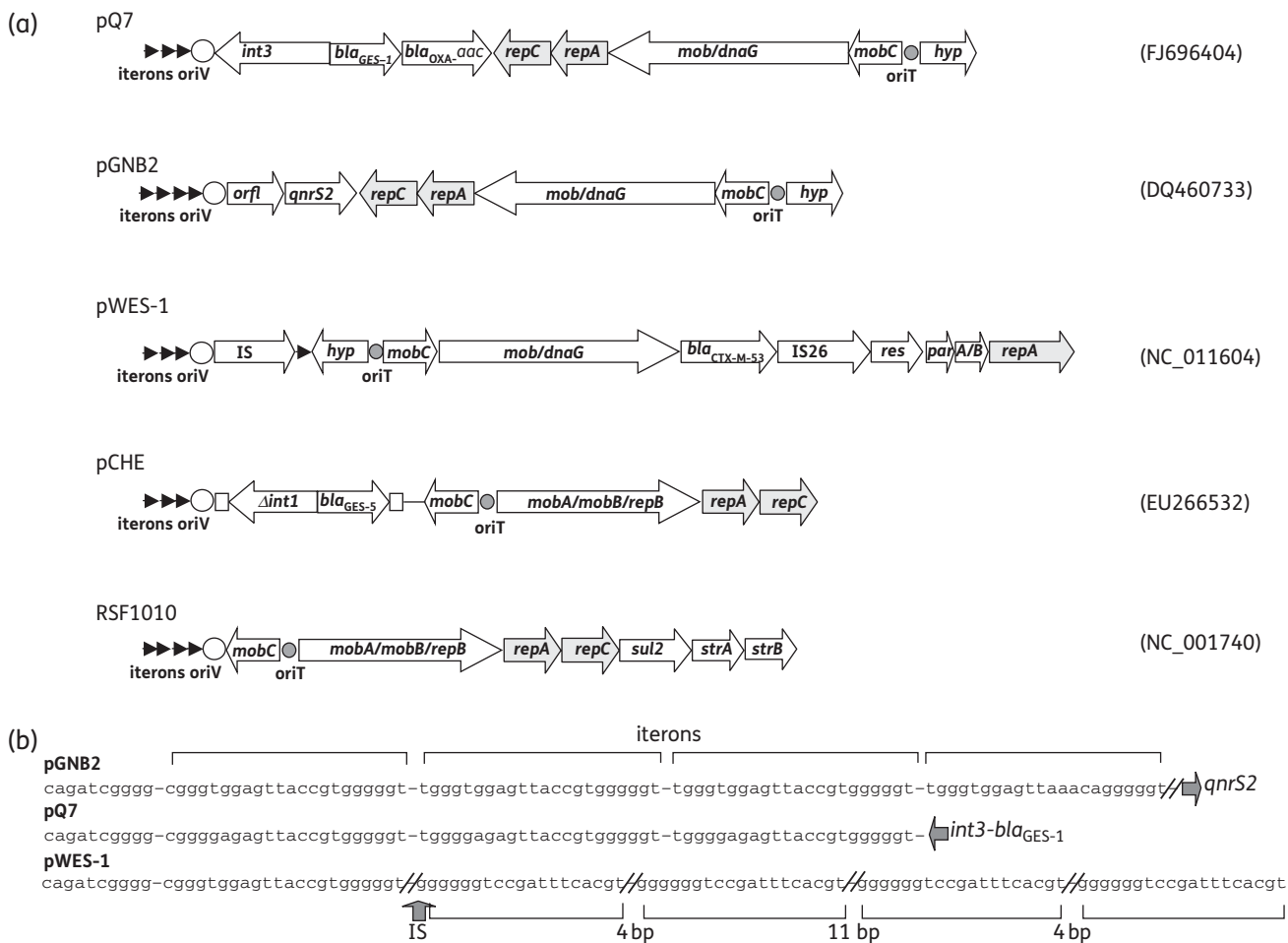


Figure 1. Map of major structural features of IncQ plasmids and iterons of pQ7, pGNB2 and pWES-1 plasmids. (a) Linear maps of the IncQ plasmids. Coding regions are indicated by arrows giving the directions of transcription. The plasmid backbone is made of the replication initiation genes *repA* and *repC* (pale grey arrows), and the putative mobilization module genes *mobA* and *mobC*. The *oriT* region required for mobilization and the *oriV* region required for replication are indicated by circles, and the position of iterons is indicated by short arrows. The plasmid variable region, including resistance, integrase and transposase genes, is indicated by white arrows. IS, insertion sequence; *hyp*, hypothetical protein. (b) Nucleotide comparison of iterons. Integration site and orientation of the resistance genes are indicated by grey arrows.

the two plasmids harbouring the integron-borne *bla_{GES-1}* gene were identical. The RepC, RepA and Mob/DnaG proteins of pQ7 shared 92%–98% amino acid identity with those of plasmid pGNB2 harbouring the *qnrS2* gene. Furthermore, pQ7 and pGNB2 possessed identical iterons, whereas iteron sequences of pWES-1 differ. These three plasmids exhibit replicase genes and iteron sequences that differ from those of all the previously reported IncQ plasmids. The molecular basis of the compatibility among IncQ-type plasmids relies on sequence divergence of iterons and on the inability of RepC to bind to iterons of divergent IncQ plasmids. For instance, the iterons of IncQ2-type plasmids pTF-FC2 and pTC-F14 have sufficiently diverged (together with their associated RepC proteins) to allow each other to be compatible.²⁹ In contrast, plasmids pTC-F14 and RSF1010 that belong to the IncQ2 and IncQ1 groups, respectively, are non-compatible, despite possessing different iteron sequences. The fact that distantly related replicons are non-compatible while closely related replicons may be compatible represents

an unexpected but interesting finding among the IncQ plasmid types.³⁰

The fact that iteron sequences of this plasmid cluster including pQ7 correspond to an integration site for mobile elements or resistance genes (the *qnrS2* gene in pGNB2, the *int3-bla_{GES-1}* locus in pQ7 and an insertion sequence element in pWES-1) may have a significant impact on the incompatibility properties of this novel IncQ plasmid.

The pQ7 features revealed that it lacked the machinery for conjugation and therefore cannot be self-conjugative, which agrees with our experimental observation. However, the *nic* site required for mobilization was identified between the *mobC* gene and that encoding the hypothetical protein; thus, suggesting that plasmid pQ7, similarly to other IncQ plasmids, might be mobilized in the presence of a helper plasmid and might possess a mobilization system based on relaxase proteins. The IncQ plasmids have been successfully mobilized to a large number of Gram-negative hosts, but also to *Arthrobacter* spp.,

	Motif I	Motif II
pWES-1	MKAKVSRGGGFRGALNYVFDVGKEATHTKNAE RVGGNMAGNDPRELSREFSAVRQLRPDI GKPVVHCSLSLP PGERLSAEKW EAVAADF	
pQ7	MKAKVSRGGGFRGALNYVFDVGKEATHTKNAE RVGGNMAGNDPRELSREFSAVRQLRPe IGKPVVHCSLSLP PGERLSAEKW EAVtADF	
pGNB2	MKAKVSRGGGFRGALNYVFDVGKEATHTKNAE RVGGNMAGNDPRE LSREFSAVRQLRPDI GKPVVHCSLSLP PGERLSAEKW EAVttDF	
pCHE	maiyhltak tgsr ngGqsakak adYiqreGrysr drdevl htqsg hlpk waer padywdvadlyeranGr l fkevefaLP veltLdqqrelvde. . F	
RSF1010	maiyhltak tgsr SgGqsaRak adYiqreGKyAr dmdevl haesghmpe fver padywdAadlyeranGr l fkevefaLP veltLd qqKalAse.F	
Consensus	G Y G	G LP L F

	Motif III	
pWES-1	MQRMGFDQTNTPWVAVRHQD TDK DHIHIVASRVGLDGK VVLGQWEARRAI EATQELEHHTHGLT	152
pQ7	MQRMGFDQTNTPWVAVRHQD TDK DHIHIVASRVGLDGK VVLGQWEARRAI EATQELEHHTHGLT	152
pGNB2	MQRMGFDQTNTPWVAVRHQD TDK DHIHIVASRVGLDGK VVLGQWEARRAI EATQELEHHTHGLT	152
pCHE	arhlt dger lpyt lAihagDgen p HcHlmiSer knDGie	134
RSF1010	aQhlt gaer lpyt lAihaggen p HcHlmiSer inDGie	134
Consensus	A H H S DG	

Figure 2. Amino acid sequence alignment of the 152 N-terminal residues of MOB relaxases from IncQ plasmids. The three putative relaxase domains (motif I, II and III) are indicated.

Streptomyces lividans, *Mycobacterium smegmatis*, cyanobacteria, and even plant and animal cells.^{31,32}

In order to experimentally assess that hypothesis, we conducted a conjugation experiment by using as donor an *E. coli* strain harbouring both pQ7 and the IncP-type RP4 plasmid, known to mobilize IncQ plasmids. Despite repeated attempts, no transconjugant was obtained, which would suggest that pQ7 has lost its ability to be mobilized. This could be the result of some rearrangements that could have modified its potential for spread.

Interestingly, plasmid pQ7 and its related plasmids possess a novel type of relaxase protein, forming a distinct clade with respect to all other relaxases previously identified in fully sequenced bacterial genomes and plasmids (Figure 2). This protein is a peculiar relaxase/primase fusion protein (Mob/DnaG), possessing at its N-terminal extremity a relaxase/mobilization nuclease domain (pfam03432) and at its C-terminal extremity a DNA primase domain (PRK05667 DnaG domain) which are different from the RepB proteins previously described in fusion with MobA in IncQ-1 and IncQ-2 plasmids.³⁰ The comparative analysis of the relaxase amino acid sequences has been proposed as a method for the classification of plasmids³¹ and, therefore, pQ7-like plasmids must be classified into a novel subfamily of IncQ plasmids, defined here as IncQ-3 type, since they possess new relaxases, in accordance with the nomenclature proposed by Rawlings and Tietze.³⁰

Interestingly, the entire GC content of plasmid pQ7 (60.2%) increased to 63% if the two gene cassettes were excluded. Considering that the GC content of the *int3* gene is 61.3%, it could be hypothesized that this gene originates from those species from which those IncQ-type plasmids originate also.

These findings add to the diversity of genetic structures at the origin of the dissemination of the *bla*_{GES}-like genes. A variety of class 1 integron structures harbouring *bla*_{GES}-like genes has already been reported.⁴ In addition, the novel IMU genetic structures were recently shown to be at the origin of acquisition of a *bla*_{GES-5}-borne class 1 integron remnant.⁸

We report here the second identification of a *bla*_{GES}-type gene into a class 3 integron structure. This represents one of the very few reports of acquisition of an antibiotic resistance gene mediated by a class 3 integron. Our study identified the ESBL GES-1 for the first time in Switzerland, further indicating the dissemination of GES-type ESBLs after their identification in France, Greece, Portugal, South Africa, Korea, Canada, French Guiana, Argentina and Brazil.^{2,3,6-8,10,33,34}

Nucleotide sequence accession numbers

The nucleotide sequence reported in this work and corresponding to the entire sequence of plasmid pQ7 has been deposited in the GenBank nucleotide database under accession no. FJ696404.

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Transparency declarations

None to declare.

References

- Naas T, Poirel L, Nordmann P. Minor extended-spectrum β-lactamases. *Clin Microbiol Infect* 2008; **14** Suppl 1: 42–52.
- Dubois V, Poirel L, Marie C *et al.* Molecular characterization of a novel class 1 integron containing *bla*_{GES-1} and a fused product of *aac3-Ib/aac6'-Ib'* gene cassettes in *Pseudomonas aeruginosa*. *Antimicrob Agents Chemother* 2002; **46**: 638–45.
- Poirel L, Le Thomas I, Naas T *et al.* Biochemical sequence analyses of GES-1, a novel class A extended-spectrum β-lactamase, and the

- class 1 integron In52 from *Klebsiella pneumoniae*. *Antimicrob Agents Chemother* 2000; **44**: 622–32.
- 4** Poirel L, Brinas L, Fortineau N et al. Integron-encoded GES-type extended-spectrum β -lactamase with increased activity toward aztreonam in *Pseudomonas aeruginosa*. *Antimicrob Agents Chemother* 2005; **49**: 3593–7.
- 5** Moubareck C, Brémont S, Conroy MC et al. GES-11, a novel integron-associated GES variant in *Acinetobacter baumannii*. *Antimicrob Agents Chemother* 2009; **53**: 3579–81.
- 6** Poirel L, Weldhagen GF, Naas T et al. GES-2, a class A β -lactamase from *Pseudomonas aeruginosa* with increased hydrolysis of imipenem. *Antimicrob Agents Chemother* 2001; **45**: 2598–603.
- 7** Jeong SH, Bae IK, Kim D et al. First outbreak of *Klebsiella pneumoniae* clinical isolates producing GES-5 and SHV-12 extended-spectrum β -lactamases in Korea. *Antimicrob Agents Chemother* 2005; **49**: 4809–10.
- 8** Poirel L, Carrère A, Pitout JD et al. Integron mobilization unit as a source of mobility of antibiotic resistance genes. *Antimicrob Agents Chemother* 2009; **53**: 2492–8.
- 9** Poirel L, Naas T, Nordmann P. Genetic support of extended-spectrum β -lactamases. *Clin Microbiol Infect* 2008; **14** Suppl 1: 75–81.
- 10** Correia M, Boavida F, Grosso F et al. Molecular characterization of a new class 3 integron in *Klebsiella pneumoniae*. *Antimicrob Agents Chemother* 2003; **47**: 2838–43.
- 11** Collis CM, Hall RM. Expression of antibiotic resistance genes in the integrated cassettes of integrons. *Antimicrob Agents Chemother* 1995; **39**: 155–62.
- 12** Collis CM, Kim MJ, Partridge SR et al. Characterization of the class 3 integron and the site-specific recombination system it determines. *J Bacteriol* 2002; **184**: 3017–26.
- 13** Carattoli A. Importance of integrons in the diffusion of resistance. *Vet Res* 2001; **32**: 243–59.
- 14** Partridge SR, Brown W, Hall RM. Transposons Tn1696 and Tn21 and their integrons In4 and In2 have independent origins. *Antimicrob Agents Chemother* 2001; **45**: 1263–70.
- 15** Liebert CA, Hall ARM, Summers AO. Transposon Tn21, flagship of the floating genome. *Microbiol Mol Biol Rev* 1999; **63**: 507–22.
- 16** Clinical and Laboratory Standards Institute. *Performance Standards for Antimicrobial Susceptibility Testing: Twentieth Informational Supplement M100-S20*. CLSI, Wayne, PA, USA, 2010.
- 17** Couturier M, Bex F, Bergquist PL et al. Identification and classification of bacterial plasmids. *Microbiol Rev* 1988; **52**: 375–95.
- 18** Poirel L, Guibert M, Girlich D et al. Cloning, sequence analyses, expression and distribution of *ampC-ampR* from *Morganella morganii* clinical isolates. *Antimicrob Agents Chemother* 1999; **43**: 769–76.
- 19** Poirel L, Brinas L, Verlinde A et al. BEL-1, a novel clavulanic acid-inhibited extended-spectrum β -lactamase, and the class 1 integron In120 in *Pseudomonas aeruginosa*. *Antimicrob Agents Chemother* 2005; **49**: 3743–8.
- 20** Poirel L, Héritier C, Tolün V et al. Emergence of oxacillinase-mediated resistance to imipenem in *Klebsiella pneumoniae*. *Antimicrob Agents Chemother* 2004; **48**: 15–22.
- 21** Kieser T. Factors affecting the isolation of CCC DNA from *Streptomyces lividans* and *Escherichia coli*. *Plasmid* 1984; **12**: 19–36.
- 22** Lévesque C, Brassard S, Lapointe J et al. Diversity and relative strength of tandem promoters for the antibiotic-resistance genes of several integrons. *Gene* 1994; **142**: 49–54.
- 23** Hall RM, Collis CM. Mobile gene cassettes and integrons: capture and spread of genes by site-specific recombination. *Mol Microbiol* 1995; **15**: 593–600.
- 24** Stokes HW, O’Gorman DB, Recchia GD et al. Structure and function of 59-base element recombination sites associated with mobile gene cassettes. *Mol Microbiol* 1997; **26**: 731–45.
- 25** Xu H, Davies J, Miao V. Molecular characterization of class 3 integrons from *Delftia* spp. *J Bacteriol* 2007; **189**: 6276–83.
- 26** Arakawa Y, Murakami M, Suzuki K et al. A novel integron-like element carrying the metallo- β -lactamase gene *bla*_{IMP}. *Antimicrob Agents Chemother* 1995; **39**: 1612–5.
- 27** Bonemann G, Stiens M, Puhler A et al. Mobilizable IncQ-related plasmid carrying a new quinolone resistance gene, *qnrS2*, isolated from the bacterial community of a wastewater treatment plant. *Antimicrob Agents Chemother* 2006; **50**: 3075–80.
- 28** Doublet B, Granier SA, Robin F et al. Novel plasmid-encoded ceftazidime-hydrolyzing CTX-M-53 extended-spectrum β -lactamase from *Salmonella enterica* serotypes Westhampton and Senftenberg. *Antimicrob Agents Chemother* 2009; **53**: 1944–51.
- 29** Rawlings DE. The evolution of pTF-FC2 and pTC-F14, two related plasmids of the IncQ-family. *Plasmid* 2005; **53**: 137–47.
- 30** Rawlings DE, Tietze E. Comparative biology of IncQ and IncQ-like plasmids. *Microbiol Mol Biol Rev* 2001; **65**: 481–96.
- 31** Francia MV, Varsaki A, Garcillan-Barcia MP et al. A classification scheme for mobilization regions of bacterial plasmids. *FEMS Microbiol Rev* 2004; **28**: 79–100.
- 32** Lawley TD, Wilkins BM, Frost LS. Bacterial conjugation in Gram-negative bacteria. In: Funnell B, Philips G, eds. *Plasmid Biology*. Washington, DC: ASM Press, 2004; 203–26.
- 33** Castanheira M, Mendes RE, Walsh TR et al. Emergence of the extended-spectrum β -lactamase GES-1 in a *Pseudomonas aeruginosa* strain from Brazil: report from the SENTRY antimicrobial surveillance program. *Antimicrob Agents Chemother* 2004; **48**: 2344–5.
- 34** Giakkoupi P, Tzouveleki LS, Tsakris A et al. IBC-1, a novel integron-associated class A β -lactamase with extended-spectrum properties produced by an *Enterobacter cloacae* clinical strain. *Antimicrob Agents Chemother* 2000; **44**: 2247–53.