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Short Communication

Characterisation of IncA/C_2 plasmids carrying an In*416*-like integron with the *bla*$_{VIM-19}$ gene from *Klebsiella pneumoniae* ST383 of Greek origin

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ABSTRACT

The complete nucleotide sequences of three multidrug resistance (MDR) IncA/C-like plasmids from Enterobacteriaceae isolates carrying the VIM-type carbapenemase-encoding integrons In*4863* (*bla*$_{VIM-19}$–*aacA7*–*dfrA1*–Δ*aadA1*–*smr2*) or In*4873* (*bla*$_{VIM-1}$–*aacA7*–*dfrA1*–Δ*aadA1*–*smr2*) were determined, which are the first In*416*-like elements identified in Greece. Plasmids pKP-Gr642 and pKP-Gr8143 were from *Klebsiella pneumoniae* ST383 isolates, whereas plasmid pEcl-Gr4873 was from an *Enterobacter cloacae* ST88 isolate. Sequencing showed that pKP-Gr642 (162 787 bp) and pKP-Gr8143 (154 395 bp) consisted of the type 1 IncA/C_2 conserved backbone, the *bla*$_{CMY-2}$-like gene-containing region, and the ARI-B (with the *sul2* gene) and ARI-A (with a class 1 integron) resistance islands, like the plasmid pUMNK88_161 from the USA. The third plasmid, pEcl-Gr4873 (153 958 bp), exhibited extensive similarity with the type 2 IncA/C_2 plasmid pR55 from France. pEcl-Gr4873 carried only one resistance island of a hybrid transposon structure inserted in a different location to ARI-A in type 1 A/C_2 plasmids. In all three plasmids, the In*416*-like integrons In*4863* or In*4873* were identified within non-identical class II transposon structures. All three In*416*-like-carrying regions presented significant similarities with the MDR region of the IncA/C_2 plasmid pCC416 from Italy, carrying the prototype In*416* integron (*bla*$_{VIM-4}$–*aacA7*–*dfrA1*–Δ*aadA1*–*smr2*). These findings provided the basis for speculations regarding the evolution of IncA/C_2 plasmids with In*416*-like integrons, and confirmed the rapid evolution of some IncA/C_2 plasmid lineages. Considering the broad host range of IncA/C_2 molecules, it seems that pKP-Gr642, pKP-Gr8143 and pEcl-Gr4873 plasmids might support the diffusion of In*416*-like integrons among Enterobacteriaceae.

1. Introduction

In recent years there has been a growing interest in IncA/C-type multidrug resistance (MDR) plasmids, which are commonly identified among agricultural and clinical bacterial isolates in the USA, Europe and elsewhere. IncA/C plasmids share a conserved backbone into which resistance fragments are integrated at various positions, and they readily circulate in numerous species and

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across taxonomic borders [1,2]. Among many resistance genes observed, the A/C plasmids have often been found to disseminate bla_{CMY-2}-like cephalosporinase genes [1]; more recently, the spread of bla_{NDM} carbapenemase genes has also been in part associated with such molecules [3]. Carattoli et al. [1] reported that bla_{CMY-2}-like-carrying plasmids from the USA represented a new replicon variant, named *repA*/C_2 (IncA/C_2), exhibiting 26 nucleotide substitutions with respect to the IncA/C reference plasmid pRA1 (IncA/C_1) [4]. In addition, a comparison of IncA/C_2 plasmids from different geographic areas showed that those with the bla_{CMY-2}-like region represent a unique phylogenetic lineage [5], increasingly spreading in bacterial populations [1,5]. The DNA segment containing the bla_{CMY-2}-like gene is a part of an IS*Ecp1* transposition unit (TU), which is always found in the same location. In some plasmids, the bla_{CMY-2}-like gene has been duplicated and is associated both with complete and partial copies of IS*Ecp1*. Along with bla_{CMY-2}-like genes, this lineage also carries *sul2*- and class 1 integron-containing segments, the so-called resistance islands ARI-B and ARI-A, respectively [2,4,5]. A recent analysis of backbones of numerous complete A/C_2 plasmid sequences identified two distinct types (types 1 and 2) that diverged a long time ago [2]. The ARI-A island was found only in type 1 A/C_2 plasmids, whereas ARI-B was observed in both types [2]. Overall, the IncA/C_2 type 1 plasmid lineage that also possesses the bla_{CMY-2}-like region is remarkable since it contains three resistance islands serving as hotspots for further plasmid evolution by in situ acquisition of resistance determinants.

VIM-type metallo-β-lactamase-producing Enterobacteriaceae have been observed in Greece since 2001 [6]. In 2002–2007, a variety of *Klebsiella pneumoniae* sequence types (STs) and other species expressing VIM-1 disseminated into hospitals throughout the country [7]. In most of these isolates, bla_{VIM-1} was part of In-e541 (*bla*$_{VIM-1}$–*aacA7*–*dfrA1*–*aadA1*)-like integrons [6,8] carried by self-transferable IncN plasmids [6,7], similar to the fully sequenced pNL194 [9]. Later, the In-e541-like elements have also been found on molecules with replicons W, R or FII_k, multiple replicons R+FII_K or R+A/C, non-typeable plasmids or in the chromosome [8]. Other bla_{VIM}-type genes found in Enterobacteriaceae of Greek origin encoded the VIM-1 variants VIM-4, VIM-19, VIM-26, VIM-27 and VIM-39 [8,10–13] as well as the variant VIM-12, being a VIM-1/VIM-2 hybrid [14]. The bla_{VIM-26}, bla_{VIM-27} and bla_{VIM-39} variants were identified in In-e541-like integrons [8,12,13], whilst the bla_{VIM-12} gene was part of the class 1 integron In-h12 (*aacA7*–bla_{VIM-12}–*aacA7*) [14]. A recent study showed that bla_{VIM-19}, which is often linked with *K. pneumoniae* ST383 [8], occurred as the first cassette of In*4863* (*bla*$_{VIM-19}$–*aacA7*–*dfrA1*–Δ*aadA1*–*smr2*), being the first In*416*-like element identified in Greece [8]. Interestingly, in the same study, a second In*416*-like element, In*4873* (*bla*$_{VIM-1}$–*aacA7*–*dfrA1*–Δ*aadA1*–*smr2*), encoding VIM-1, was found in an *Enterobacter cloacae* ST88 isolate [8]. The original In*416* element, reported in Italy, Russia and the United Arab Emirates [15–17], comprises bla_{VIM-4}, *aacA7*, *dfrA1*, Δ*aadA1* and *smr2* gene cassettes [18], sharing the first four cassettes with the In-e541-like variants with the *aadA1* cassette truncated by 82 bp at the 5′-end (Δ*aadA1*). VIM-19 differs by a single amino acid substitution from VIM-4 (Asn215Lys) and by two substitutions from VIM-1 (Asn215Lys and Ser228Arg). In Italy, In*416* was associated with transposons Tn*1696* and Tn*8802* [15]. In*416*-like integrons are usually present on IncA/C-type plasmids [8,15,17], like the CMY-4-encoding pCC416 from Italy [15].

In the present study, we describe the complete nucleotide sequences of two IncA/C-type plasmids carrying the In*4863* integron from *K. pneumoniae* ST383. We have also sequenced the IncA/C-type plasmid carrying In*4873* from *E. cloacae* ST88 in order to examine whether the same or different plasmid with In*416*-like integrons are spreading in different species of Enterobacteriaceae in Greece.

2. Materials and methods

2.1. Plasmids

Plasmid pKP-Gr642 was from a *K. pneumoniae* ST383 isolate (Kpn-642) recovered in the Czech Republic in 2011 from a patient who had been previously hospitalised in Northern Greece. Plasmids pKP-Gr8143 and pEcl-Gr4873 were from *K. pneumoniae* ST383 (Kpn-8143) and *E. cloacae* ST88 (Ecl-4873) isolates cultured in 2010 and 2009, respectively, from patients in different Athens hospitals and reported previously [8].

2.2. Plasmid sequencing

Plasmid DNA was extracted from *Escherichia coli* transconjugants or transformants and was sequenced using a 454 Genome Sequencer Junior System (Roche, Prague, Czech Republic) on a standard DNA fragment library. Reads were assembled using the GS De Novo Assembler software (Roche). Sequence gaps were filled by sequencing of PCR amplicons. The BLAST algorithm (http://www.ncbi.nlm.nih.gov/BLAST), IS Finder (http://www-is.biotoul.fr/) and open reading frame (ORF) Finder (http://www.bioinformatics.org/sms/) were used for data analysis.

2.3. Nucleotide sequence accession numbers

The nucleotide sequences of the plasmids pKP-Gr642, pKP-Gr8143 and pEcl-Gr4873 were deposited in GenBank under accession nos. KR559888, KR559889 and KR559890, respectively.

3. Results

Plasmids carrying In*416*-like integrons from three Enterobacteriaceae isolates of Greek origin were analysed. Sequencing of pKP-Gr642, pKP-Gr8143 and pEcl-Gr4873 confirmed that all of these plasmids belonged to the IncA/C group, subgroup IncA/C_2. Furthermore, the backbones of the three plasmids were highly conserved (>99% identity). This finding is in agreement with previous studies documenting that core backbones of IncA/C plasmids are highly syntenic with no genetic rearrangements [4].

3.1. pKP-Gr642

The self-transferable plasmid pKP-Gr642 (10^{-6} transconjugants per donor cell) is a 162 787-bp molecule with a sequence closely related to the type 1 A/C_2 plasmids, like pUMNK88_161 (91% coverage, 99% identity) (Fig. 1) from the USA [5]. The pKP-Gr642 backbone was composed of regions responsible for replication (*repA* gene), conjugative transfer (Tra1 and Tra2 regions) and plasmid maintenance (*higBA* and *parAB* operons, and *xerD* and *kfrA*-like genes). Apart from the backbone, pKP-Gr642 carried the bla_{CMY-2}-like-containing region, and the ARI-B and ARI-A resistance islands, as previously described in other type 1 A/C_2 MDR plasmids [2,4,5]. In pKP-Gr642, the bla_{CMY-2}-like-containing region resembled the type II structure, which includes a single copy of the bla_{CMY-2}-like gene. This region was inserted into the Tra1 region, 99 bp downstream of *traA*, and consisted of an IS*Ecp1* TU with bla_{CMY-4}, *blc*, *sugE* and Δ*ecnR* genes of *Citrobacter freundii* origin. The IS*Ecp1* TU was flanked by direct repeats of 5 bp (ATTTC; DR1) and differed only in the bla_{CMY} sequence (bla_{CMY-4} vs. bla_{CMY-2}) from the respective regions of other type 1 A/C_2 plasmids. The ARI-B with two copies of IS*26*, an IS*CR2* element, and the *floR*, *tetA*(A), *strA*, *strB* and *sul2* genes (resistance to phenicols, tetracyclines, aminoglycosides and sulfonamides) occurred upstream of Tra1 and was identical to the respective part of pUMNK88_161 as well as other type 1 A/C_2