A type 2 A/C2 plasmid carrying the aacC4 apramycin resistance gene and the erm(42) erythromycin resistance gene recovered from two Salmonella enterica serovars

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Objectives: To determine the relationships between RepA/C2 plasmids carrying several antibiotic resistance genes found in isolates of Salmonella enterica serovars Ohio and Senftenberg from pigs.

Methods: Illumina HiSeq was used to sequence seven S. enterica isolates. BLAST searches identified relevant A/C2 plasmid contigs and contigs were assembled using PCR.

Results: Two serovar Ohio isolates were ST329 and the five Senftenberg isolates were ST210. The A/C2 plasmids recovered from the seven isolates belong to type 2 and contain two resistance islands. Their backbones are closely related, differing by five or fewer SNPs. The sul2-containing resistance island ARI-B is 19.9 kb and also contains the kanamycin and neomycin resistance gene aphA1, the tetracycline resistance gene tetA(D) and an erythromycin resistance gene, erm(42), not previously seen in A/C2 plasmids. A second 30.3 kb resistance island, RI-119, is in a unique location in the A/C2 backbone 8.2 kb downstream of rhs. RI-119 contained genes conferring resistance to apramycin, netilmicin and tobramycin (aacC4), hygromycin (hph), sulphonamides (sul1) and spectinomycin and streptomycin (aadA2). In one of the seven plasmids, this resistance region contained two IS26-mediated deletions. A discrete 5.7 kb segment containing the aacC4 and hph genes and bounded by IS26 on one side and the inverted repeat of Tn5393 on the other was identified.

Conclusions: The presence of almost identical A/C2 plasmids in two serovars indicates a common origin. Type 2 A/C2 plasmids continue to evolve via addition of new resistance regions such as RI-119 and evolution of existing ones.

Keywords: antibiotic resistance, RepA/C2, S. enterica, Ohio, Senftenberg

Introduction

Plasmids of the incompatibility groups A and C (later combined as A/C) were among the earliest plasmids to be associated with antibiotic resistance in Gram-negative bacteria. However, the sequence of the repA gene in the reference plasmid RA1 differs significantly from that of most of the sequenced A/C plasmids and they are now designated A/C1 (RA1) or A/C2 based on the repA gene sequence.1 A/C2 plasmids contribute to multiple antibiotic resistance in Salmonella enterica, a major cause of foodborne illness,2–6 and several A/C2 plasmids from S. enterica serovars Newport, Heidelberg and Typhimurium have been sequenced.3–5,8 A/C2 plasmids also mobilize S. enterica genomic island 1 (SGI1), carrying genes conferring resistance to multiple antibiotics.9,10

Recently, the A/C2 group was subdivided into two types, type 1 and type 2 (Figure 1a), which diverged a long time ago.11 They have accumulated extensive SNPs and differ by two insertions or deletions (i1 and i2 in Figure 1a) within the backbone and two regions where part of a large gene has been replaced (R1 and R2 in Figure 1a).11 The replacements give rise to two versions of the rhs gene (rhs1 and rhs2) and ORFs between traA and traC that predict proteins of 1832 amino acids (orf1832) in type 1 and 1847 amino acids (orf1847) in type 2.

We previously reported an unusual class 1 integron-associated gene cassette configuration in seven S. enterica isolates, two of serovar Ohio and five of serovar Senftenberg sourced in Australia from pigs. These isolates all carried an A/C2 plasmid, and also shared resistance to apramycin, gentamicin, kanamycin, neomycin, streptomycin, spectinomycin, sulfamethoxazole and
Figure 1. Comparison of pSRC119-A/C with other A/C_2 plasmids. (a) A/C_2 backbone structure showing difference between type 1 and type 2 groups as defined previously and (b) backbone structure of type 2 plasmid pSRC119-A/C. Regions containing genes involved in plasmid replication (rep), partitioning (par) and conjugative transfer (tra) and the rhs gene are indicated by arrows below. The remaining genes and ORFs are annotated in GenBank accession number KM670336. The location of insertions i1 and i2 found in type 2 and two regions of replacement R1 (in the ORF) and R2 (in rhs) are shown above. In pSRC119-A/C, vertical arrows indicate the location of the ARI-B and RI-119 resistance islands. Gaps in the horizontal line indicate segments of sequence that are missing relative to the A/C_2 backbone. The figure is drawn to scale with lengths in kb shown above the line in (a).

(c) pSRC119-A/C ARI-B and related ARI-B structures. Regions are drawn to scale from GenBank accession numbers KM670336 (pSRC119-A/C), NC_008612 (pP99-018) and NC_009141 (pIP1202). (d) Regions surrounding the erm(42) gene. Structures are drawn to scale from GenBank accession numbers CP003022 (P. multocida) and AB601890 (P. damselae). In (c) and (d), shared regions are indicated by shading. ISs and the small mobile element CR2 are shown as open boxes with a vertical bar indicating the ori end of CR2. ISs numbers or names are indicated above. Genes and ORFs are shown below the line as named arrows indicating the direction of transcription. Segments derived from the IncN plasmid R46 or GIsul2 are indicated below.
tetracycline. They carry the aacC4 gene, which confers resistance to apramycin, netilmicin and tobramycin. However, conjugative transfer of the shared resistance genes and the A/C2 plasmids could not be detected. Here, we have sequenced the seven isolates and completed the sequence of the A/C2 plasmids.

Materials and methods

DNA sequencing and sequence analysis

The S. enterica isolates examined in this study are described elsewhere. Genomic DNA was isolated as described previously and was sequenced using an Illumina HiSeq platform at the Australian Genome Research Facility. Paired-end reads of 100 bp were assembled using Velvet, yielding 100–200 contigs with an average read depth of 47–60-fold. Contigs carrying parts of the A/C2 backbone defined previously were recovered using standalone BLAST (www.ncbi.nlm.nih.gov/books/NBK52640). Contigs containing resistance genes were identified using ResFinder 2.1. All junctions between contigs were confirmed by PCR using 20 ng of genomic DNA. Amplicons were resolved by electrophoresis and sequenced as described previously. Sequencer 5.2.3 (Gene Codes Corporation, Ann Arbor, MI, USA) was used for the final sequence assembly. Reading frames not annotated previously were predicted using ORF Finder (www.ncbi.nlm.nih.gov/projects/gorf/) and annotated manually. The ST of each strain was determined using the Warwick MLST scheme (http://mlst.warwick.ac.uk/mlst/dbs/Senterica).

Nucleotide sequence accession number

The complete sequence of a representative plasmid, pSRC119-A/C, is deposited in GenBank under accession number KM670336.

Results

pSRC119-A/C

The sequence of pSRC119-A/C, the A/C2 plasmid from isolate SRC119, was assembled from five contigs by using PCR to link across four copies of IS26 (GenBank accession number KM670336). pSRC119-A/C is 174,068 bp and the backbone (Figure 1b) includes the i1 and i2 insertions together with R1-2 (orf1847) and R2-2 (rhs2), the type 2 versions of R1 and R2, making it a type 2 A/C2 plasmid. It contains two resistance regions at the locations shown in Figure 1(b). The first resistance island contains the sul2 gene and islands at this position were recently named ARI-B. In pSRC119-A/C a deletion has removed 4477 bp from the adjacent plasmid backbone (corresponding to bases 25590–30066 in pRMH760, GenBank accession number KF976462). The second resistance island was named RI-119 and is 30,345 bp. We have recently shown that additional resistance islands in type 2 A/C2 plasmids are found in several different positions, mostly clustered within or around the rhs gene. RI-119 is located further away at a new position, 8.2 kb downstream of the rhs stop codon. It replaces an 891 bp segment of the A/C2 backbone (corresponding to bases 157537–158427 in pRMH760) that includes part of the uvrD and kfrA genes.

ARI-B in pSRC119-A/C

ARI-B is 19.9 kb. The boundary at the sul2 (right-hand) end, defined by comparison with pRMH760, which lacks an ARI-B island, is the same as in all other A/C2 plasmids with ARI-B. The left-hand boundary is identical to that found in the plasmids pIP1202 and pP99-018 (GenBank accession numbers NC_009141 and NC_008612, respectively), both of which are A/C2 type 2. These three plasmids have a segment from one end of Gsul2 at one end and a 4.4 kb segment sharing 98.9% nucleotide identity with the IncN plasmid R46 (GenBank accession number AYO46276) at the other end (Figure 1c). In addition to sul2 (sulphonamide resistance), the Gsul2 fragment includes a complete copy of the small mobile element CR2. In pSRC119-A/C, 4286 bp at the right end are identical to the sul2 end of Gsul2 (bases 3902209–3906494 in GenBank accession number CP001918) and in pIP1202 and pP99-018, 4920 bp of Gsul2 are present (Figure 1c). Indeed, it appears that the island was originally formed via the integration of Gsul2. However, the internal composition of ARI-B differs. ARI-B in pSRC119-A/C also carries genes conferring resistance to kanamycin and neomycin (aphA1b) and tetracycline (tetA(D)), and a novel gene, erm(D2), that confers resistance to erythromycin, tilmicosin and clindamycin and to gamithromycin and tildipirosin. The erm(D2) gene was only recently identified in Pasteurella multocida (GenBank accession numbers FR734406 and CP003022), where it is part of an integrative, conjugative element that can transfer across species and genus boundaries. In GenBank there is only one other example of this resistance gene and it is found in Photobacterium damselae (GenBank accession number AB601890). Comparison of the three sequences revealed a shared boundary upstream of the gene but different divergence points downstream (Figure 1d).

RI-119

The 30,345 bp RI-119 (Figure 2a) is a mosaic that contains adjacent genes conferring resistance to apramycin, netilmicin and tobramycin (aacC4) and to hygromycin (hph), as well as the unusual class 1 integron configuration previously shown to carry an adaA2 cassette conferring resistance to streptomycin and spectinomycin and the sul1 gene conferring resistance to sulphonamides. The irr, the inverted repeat (IR) at the trnI end of the 6988 bp integron is at the right-hand boundary of the resistance island and IR; the IR at the intI1 end, is separated from an IS26 by 20 bp of sequence derived from Tn21.

The remainder of RI-119 is 23,357 bp and at the left-hand boundary of the island there are 364 bp from the trnPA end of Tn1721, with the IR adjacent to the A/C2 backbone sequence. The aacC4 and hph genes are in a 5693 bp structure (bases 138374–144066 in GenBank accession number KM670336) bounded by IS26 at one end and IRmp of Tn5393 at the other end (Figure 2b). This configuration is present in three other plasmids, p9134, pPWd4_103 and pkHV (GenBank accession numbers KF705205, HQ114284 and HF545434, respectively; Figure 2b). Although the structure is within a resistance island in each of these plasmids, the surrounding sequence is different, suggesting that this may be a mobile unit that can spread between plasmids. The IS26-aacC4-hph-ΔtnpA5393 structure in pSRC119-A/C separates two parts of a 13.5 kb segment sharing 99.8% nucleotide identity with a segment found in the draft genome of Klebsiella pneumoniae strain KPN1IH18 (AKA10100038). The genes in this segment encode proteins predicted to be associated with plasmid replication or conjugative transfer. In RI-119, part of this segment has been inverted, probably due to inversion of the segment between the two oppositely
An IS26-mediated deletion has also removed 864 bp belonging to the \textit{trbC} and \textit{trbD} genes found between \textit{trbB} and \textit{trbE} in KPNIH18.

Closely related A/C2 plasmids in different serovars

Closure of the A/C2 plasmids from four additional Senftenberg isolates, SRC69, SRC91, SRC102 and SRC103, and two Ohio isolates, SRC22 and SRC74, were identical to the A/C2 plasmids that they carried. The resistance islands were in the same locations in all seven plasmids and the backbones differed from pSRC119-A/C by only two to five SNPs. Three SNPs were shared by the two Ohio isolates and a single SNP was shared by two of the four remaining Senftenberg isolates. However, the plasmid from SRC102 had two IS26-mediated deletions relative to RI-119, one of which has truncated the \textit{hph} gene. These deletions have removed 5453 bp (bases 132921–138373 in GenBank accession number KM670336) and 10608 bp (bases 141081–151688 in GenBank accession number KM670336).

Discussion

The type 2 A/C2 plasmids recovered in this study have acquired three resistance genes \(\text{erm}(42), \text{aacC4}\) and \text{hph} not previously
seen in A/C₂ plasmids. These genes complement genes conferring resistance to kanamycin, neomycin, tetracycline, tobramycin, sulphonamides, spectinomycin and streptomycin. The aacC4 and hph genes are located in a discrete structure that appears to have moved as a unit into three different plasmids.

The ARI-B island in pSRC119-A/C belongs to a specific subgroup of type 2 plasmids that include a fragment from the IncN plasmid R46 in ARI-B, and the erm(42) gene has been incorporated into ARI-B. One member of this group (pP99-018) includes only an ARI-B island whereas pSRC119-A/C and pJIP1202 have each acquired an additional resistance island.

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Transparency declarations

None to declare.

References