Characterization of a 2.6 kbp variable region within a class 1 integron found in an Acinetobacter baumannii strain isolated from a horse

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Objectives: A complete gene cassette contained in a class 1 integron from a multidrug-resistant (MDR) isolate of Acinetobacter baumannii cultured from a horse was characterized by molecular methods.

Methods: Template genomic DNA purified from the A. baumannii isolate was investigated by PCR. A gene cassette-associated amplicon was detected and completely characterized.

Results: A 2.6 kbp DNA fragment containing four gene cassettes was amplified from the MDR A. baumannii isolate. Sequence analysis showed it was similar to sequences recently reported in Klebsiella pneumoniae, Serratia marcescens and an Escherichia coli plasmid p1658/97 which conferred aminoglycoside resistance. Aminoglycoside resistance-encoding genes aacC1 and aadA1 were located within the 2.6 kbp amplicon, separated by two open reading frames (ORFs) coding for unknown products. This cassette structure (and some variants) was identified in unrelated Acinetobacter spp. from human sources, based on sequence comparisons of the current databases.

Conclusions: Identification of a complete class 1 integron in an equine isolate of A. baumannii suggests that the screening of isolates from animals for these elements should be considered, as this information could influence the selection of chemotherapeutic agents.

Keywords: antimicrobial resistance, A. baumannii, integrons

Introduction

Acinetobacter spp. are regarded as important opportunistic pathogens. Acinetobacter baumannii is a nosocomial pathogen often associated with wound and urinary tract infections and has also been isolated from the respiratory tract of infected animals.1

There are several reports of Acinetobacter species causing infection in pets2 and in horses.3,4 As in human medicine, treatment of animals now includes extensive use of antimicrobial substances, particularly associated with prolonged hospitalization of animals with chronic infections. These developments can create the conditions conducive for increased spread of multidrug-resistant (MDR) Acinetobacter spp. in veterinary hospital settings.

Integron structures have been reported in Acinetobacter isolates suggesting that gene cassettes encoding antimicrobial resistance may act as a possible means for dissemination of resistance among Acinetobacter spp.5,6 Antimicrobial susceptibility patterns among Acinetobacter spp. in Europe were reviewed recently.7 There are no published reports describing class 1 integrons in animal Acinetobacter spp. isolates. This brief report provides molecular data on an isolate of A. baumannii cultured from a horse.

Materials and methods

Bacterial isolate

The isolate of A. baumannii in this study was recovered from a wound swab, taken from a horse hospitalized in a veterinary hospital...
teaching hospital. The organism was cultured using conventional bacteriological methods, and identified by amplified ribosomal DNA restriction analysis (ARDRA) as previously described.8

Antimicrobial susceptibility testing

Susceptibility to a panel of 10 antimicrobial agents was determined by the disc diffusion method in accordance with NCCLS guidelines.9

DNA isolation

Total DNA was prepared using the Wizard Genomic DNA purification kit (Promega, Madison, WI, USA) in accordance with the manufacturer’s recommendations. Purified DNA concentrations were determined spectrophotometrically.

Amplification of integron variable region by PCR

Amplification of the integron variable region by PCR was carried using a modified version of the PCR assay described by White et al.10 Briefly, 50 μL reaction mixtures were assembled, containing 5 μL of purified template DNA and 25 mM MgCl₂, in the 1× PCR buffer. Amplified DNA products were analysed by conventional agarose gel (1.5% (w/v)) electrophoresis.

DNA sequence analysis

The 2.6 kbp DNA amplicon was sequenced commercially (Qiagen Sequencing Services, Hilden, Germany). Sequences were compared to the current GenBank sequence databases using the BLAST suite of programs.11 CLUSTALW amino acid sequence alignments were produced for comparison.12

Nucleotide accession number

The complete 2.6 kbp sequence was submitted to the GenBank sequence database and assigned the accession number AJ784787.

Results

The A. baumannii isolate investigated in this study was cultured from a horse that was hospitalized and treated for cellulitis. This isolate was resistant to the following antimicrobial agents (with concentrations in parentheses): co-amoxiclav (25 mg/L), gentamicin (5 mg/L), cefalothin (30 mg/L), lincomycin (2 mg/L), penicillin G (10 mg/L) and sulfamethoxazole/trimethoprim (25 mg/L).

Template DNA was purified from this MDR isolate and was investigated for class 1 integrons by molecular methods. A large 2.6 kbp amplicon was detected and subsequently characterized.

Complete sequencing revealed the presence of four ORFs of 354, 502, 305 and 791 bp. Additional features included the 59-base element (be) associated with the terminal 30-ends of an ORF required for the recombination of cassettes with corresponding attI sites on the integron. A schematic illustration of the genetic organization of this complete variable gene cassette-encoding region is shown in Figure 1. The remaining conserved structures including the 5-CS located intI along with the 3-CS qacEAl-sul1 coding sequences were identified by PCR, indicating the presence of a complete class 1 integron structure (data not shown) in this A. baumannii isolate.

Analysis of the first ORF (Figure 1) identified perfectly matching sequences with a 3-N-aminoglycoside acetyltransferase (aacC1), in an A. baumannii isolate (accession number AY307113). In addition, this ORF was 99.4% identical at the amino acid level to a recent Serratia marcescens isolate (AF45399).

Similarly, BLAST searches with the second ORF identified a putative protein of 263 residues designated orfX. This ORF closely matched a similar sequence in Klebsiella pneumoniae (accession no AF282595) and was also similar to a protein encoded on a 12.5 kbp plasmid (p1658/97) in E. coli (AF550679). CLUSTALW multiple sequence alignment12 of the orfX deduced amino acid sequences from several organisms, showed that these were almost identical (with amino acid identities ranging from 98% to 99%). In one of the alignments, a single amino acid substitution was identified wherein an arginine residue (in the equine sequence) was replaced by an alanine residue in a human clinical A. baumannii isolate (AF310480) (data not shown).

A third ORF (designated orfX'0) was located distal to both aacC1 and orfX and was identical to a previously reported orfX' in an A. baumannii isolate (AY307113, Figure 1). Although

![Figure 1](https://academic.oup.com/microbiology/article-abstract/55/3/367/758322/1)
the function of this ORF is unknown, it is present in several bacterial species including *Klebsiella oxytoca*, *K. pneumoniae*, *S. marcescens* and *Salmonella enterica* serovar Typhimurium (data not shown). A CLUSTALW alignment of this deduced sequence showed that it was almost identical (96%) to a putative protein of 103 residues designated *orfF* in a human clinical *A. baumannii* isolate (AJ310480).

The remaining ORF encoding an *aadA1* sequence was located at the 3'-end of the 2.6 kbp sequence. This ORF showed significant sequence similarity to other *aadA1* genes in *K. pneumoniae* (AF282595) and *A. baumannii* (AJ310480 and AY307113).

Finally, the deduced amino acid sequences of the two annotated ORFs, *aacC1* and *aadA1* from the 2.6 kbp sequence in *A. baumannii* were compared to other similar sequences using the CLUSTALW program. Alignment of the *aacC1*-encoding gene product (Figure 2a) showed that the MDR *A. baumannii* isolate in this study contained a similar AacC1 protein to that of a human *A. baumannii* isolate and a *S. marcescens* (AF435999) isolate, with a high level of amino acid similarity (ranging from 97% to 100%) between the sequences. Two amino acid substitutions were identified, as indicated in Figure 2a, and these were associated with one of the isolates, *S. marcescens* (indicated in bold type, in Figure 2a) while the remaining sequences were indistinguishable from each other based on these alignments. A second alignment showing the AadA1 deduced sequence from this study (AJ784787, Figure 2b) showed significant similarity.

**Figure 2.** (a) Amino acid alignment of AacC1 from the 2.6 kbp amplicon (AJ784787, this study) with an unrelated *Acinetobacter* spp. isolate (AY307113) and a *S. marcescens* isolate (AF453999). (b) Amino acid alignment of AadA1 from the 2.6 kbp amplicon (AJ784787, this study) with *Acinetobacter* spp. isolate (AY307113), *K. pneumoniae* (AF282595), and two *A. baumannii* isolates (AJ310480) and (AY307113). Amino acid substitutions are indicated in bold-face type.
with unrelated species, and in this alignment two amino acid substitutions (indicated by the bold-faced residues in Figure 2b) were identified in a clinical A. baumannii isolate (AJ310480).

Discussion

Horizontal gene transfer is a significant mechanism for disseminating antimicrobial resistance in bacterial populations. Integrons contribute to this process and have been identified in several Gram-negative bacterial species. Until recently, these structures were not identified in Acinetobacter spp. from animals and the potential for disseminating resistance by this means, was not established. However, studies are now reporting the existence of these structures in Acinetobacter spp. and therefore their role and contribution to antimicrobial resistance must be assessed.6,13 Several aad genes, encoding resistance to streptomycin/spectinomycin, have been located within integrons as gene cassettes in human and animal isolates. In fact, these gene cassettes are common among class 1 integrons.14

In this study, we report the molecular characterization of a class 1 integron identified in an equine isolate of A. baumannii. The 2.6 kbp amplicon carried two resistance genes, aadA1 and aacC1. In addition, two smaller ORFs of unknown function were also identified. These were noted previously by other authors in a human A. baumannii isolate (AY307113) and were designated orfX and orfX’. Interestingly, the MDR animal isolate of A. baumannii in this study and the human isolate previously referred to, contained a gene cassette arrangement which was similar to that in an A. baumannii (AJ310480) isolate and a K. pneumoniae (AF282595, originating in Poland) isolate. An E. coli and S. marcescens isolate each contained gene cassettes that matched some of those found within the variable region of the class 1 integron in our isolate. When compared with S. Typhimurium (AF203818), only the aadA1 sequence appeared to be conserved.

Our study extends previous observations made by others concerning class 1 integrons in A. baumannii.5,11 The numbers of isolates carrying class 1 integrons with similar cassette assortments appear to be increasing among human isolates and a similar pattern may emerge in animals. It is possible that similar selective pressures are being exerted on both human and animal clinical isolates which may contribute to a human-to-animal or animal-to-human transmission of MDR Acinetobacter spp. This is the first report of a class 1 integron in an animal (equine) isolate of A. baumannii. Accordingly, screening of further animal isolates for these elements should be considered. This investigative approach, relating clinical, epidemiological and molecular information could provide a greater understanding of the mechanism(s) of resistance when selecting chemotherapeutic agents for the treatment of infected animals.

References