High frequency transfer and horizontal spread of apramycin resistance in calf faecal Escherichia coli

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Objectives: The aminoglycoside apramycin has been used extensively in animal husbandry in the UK since 1978. This study aimed to determine both whether calves that had never been treated with aminoglycoside antibiotics harboured apramycin-resistant (aprR) commensal Escherichia coli, and the mode of spread of the resistance gene.

Methods: AprR E. coli from weekly calf faecal samples were typed by pulsed-field gel electrophoresis, antibiotic resistance phenotype, plasmid restriction profiles and plasmid transfer frequencies.

Results: During 4 months of weekly sampling, six of 11 calves were found to harbour apr R E. coli. All aprR E. coli (45) were cross-resistant to gentamicin and tobramycin, which are both used in human medicine. Resistance was conferred by the aac(3)IV gene, present on three different conjugative plasmids. Two of these plasmids also mediated tetracycline and streptomycin resistance. One plasmid demonstrated very high transfer frequencies and was found in three different genotypes.

Conclusions: We report the presence of apr R commensal E. coli in cattle that have never been treated with aminoglycosides. The presence of one conjugative plasmid in three different genotypes is evidence of horizontal spread of this plasmid. This is the first report of a very high transfer frequency of aprR plasmid, demonstrating horizontal spread in the commensal flora of food animals.

Keywords: conjugation, commensals, AAC(3)IV, food animals, plasmids

Introduction

The study of antibiotic-resistant bacteria in animals has focused on organisms pathogenic to humans and animals, such as salmonellae and campylobacter.1 Resistance genes commonly reside on transmissible plasmids, transposons, gene cassettes or other mobile genetic elements, allowing the horizontal spread of resistance genes between strains, species and even genera. Because of this genetic mobility, the commensal flora of animals may act as a reservoir of resistance genes.

Apramycin has been used extensively in animal husbandry since 1978. Although it has not been used in human medicine, apramycin resistance has been detected in human isolates of Klebsiella pneumoniae and Escherichia coli.2 These findings, in contrast to a recent report by Phillips et al.,1 indicate that the gene conferring resistance appears to have transferred between animal and human bacteria. Apramycin resistance is conferred by the aminoglycoside-modifying enzyme 3-N-aminoglycoside acetyltransferase type IV [AAC(3)IV],1 which also acetylates tobramycin and gentamicin, used to treat serious infections in humans.

This study aimed to determine whether calves that had not been treated with aminoglycosides harbour aprR commensal E. coli, and whether apramycin resistance was spread clonally or horizontally.

Materials and methods

Bacterial isolates

Rectal faecal samples from 11 beef-suckler calves on a Scottish farm, born between 14–26 September 2001, were taken within 48 h of birth, then weekly until 14 January 2002 (except for 2 weeks at Christmas and New Year). Enrofloxacin, florfenicol, oxytetracycline, penicillin, streptomycin and tylosin were used on the farm during the sampling period, but no tetracyclines or aminoglycosides were used on the sampled calves. Samples were stored at 4°C and processed within 24 h of collection. Samples were diluted 1:10 in maximum recovery diluent (Oxoid, Basingstoke, UK). Ten millilitres of

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Apramycin resistance plasmids in commensal E. coli

Table 1. Calves from which aprR E. coli were isolated, calf antibiotic treatments, dates of aprR E. coli detection, and number and genotype of aprR isolates

<table>
<thead>
<tr>
<th>Calf</th>
<th>Treatment (date)</th>
<th>Sampling dates of aprR E. coli detection</th>
<th>aprR E. coli isolates</th>
<th>PFGE types (number of each type)</th>
</tr>
</thead>
<tbody>
<tr>
<td>690</td>
<td>none</td>
<td>01/10/2001, 12/11/2001</td>
<td>2</td>
<td>A (2)</td>
</tr>
<tr>
<td>687</td>
<td>none</td>
<td>17/10/2001, 24/10/2001</td>
<td>10</td>
<td>A (10)</td>
</tr>
<tr>
<td></td>
<td>florfenicol (23/11/2001)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>florfenicol (05/12/2001)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>696</td>
<td>none</td>
<td>12/11/2001</td>
<td>6</td>
<td>A (1), B(4), D (1)</td>
</tr>
<tr>
<td>698</td>
<td>enrofloxacin (01/10/2001)</td>
<td></td>
<td>1</td>
<td>A (1)</td>
</tr>
</tbody>
</table>
We were unable to restrict pUK2003, but the different sizes and transfer frequencies (Table 2) indicate that pUK2003 and pUK2002 are different plasmids. pUK2001 transferred at the highest frequency (1.15 × 10⁻² h⁻¹) (Table 2), which was substantially greater than that of RP4 and R46 (1.4 × 10⁻³ h⁻¹ and 3.1 × 10⁻⁵ h⁻¹, respectively). MICs for the donor strains, recipient and transconjugants revealed that pUK2002 and pUK2003 also carried tetracycline and streptomycin resistance (Table 2). This was confirmed by the observation of similar transfer frequencies when pUK2002 or pUK2003 were selected on tetracycline, streptomycin or each of these antibiotics in combination with apramycin (data not shown). In addition to aprR (MIC >128 mg/L) all three plasmids conferred resistance to tobramycin and gentamicin (MICs of 16 and 8 mg/L, respectively). All three plasmids harboured the aac(3)IV gene. The sequences were identical to that of the published nucleotide sequence (X01385).

**Discussion**

Previous reports on apramycin resistance have concentrated on human or animal pathogens. Little is known about the epidemiology of resistant commensal bacteria. In this study, PFGE and plasmid analysis were combined to give a better understanding of the spread of apramycin resistance.

A cohort of calves that had not been treated with any aminoglycosides was selected for this work, to avoid any direct selection of aprR E. coli during the study. During 4 months, six of 11 calves were found to carry aprR E. coli.

The farm from which the isolates used in this study came had not used apramycin since July 2000 (when treatment records commenced). The carriage of streptomycin and tetracycline resistance by pUK2002 and pUK2003 suggests either cross-contamination of these antibiotics between calves, or the spread of resistance plasmids or strains between calves. The presence of aprR plasmids in the calves sampled in this study demonstrates the persistence of apramycin resistance without direct selective pressure by the use of apramycin. A similar result has been reported in E. coli from disease outbreaks in pigs, but because antibiotic usage in cattle is dramatically less than that used in pigs, the detection of aprR commensals in calves is of significance.

Restriction analysis of the aprR plasmids showed that the aac(3)IV gene was carried on three different conjugative plasmids. Similar findings have been reported in pathogenic bacteria. The frequency at which the transfer of aprR plasmids occurs is rarely measured, but one study has measured transfer frequencies of aprR plasmids isolated from E. coli implicated in disease outbreaks of cattle, pigs and poultry. Two aprR E. coli isolates from diseased cattle were found to transfer apramycin resistance at frequencies of <10⁻⁸ and <10⁻¹⁰ transconjugants per donor overnight. In addition to the high transfer frequencies of pUK2001 and pUK2002 observed in this study, the use of PFGE to genotype each isolate allowed the detection of pUK2001 in three different genotypes. To the best of our knowledge, this is the first report of horizontal spread of apramycin resistance in commensal organisms.

The selective pressures maintaining these large conjugative resistance plasmids in the commensal flora are unclear. The calves sampled were not treated with aminoglycosides or tetracyclines, but the presence of pUK2002 and pUK2003 suggests that the spread of plasmids or resistant strains resulted from co-selection by tetracycline or streptomycin usage. pUK2001 only carried apramycin resistance, and we hypothesize that some factor, other than antibiotic use, is responsible for the selection and spread of this plasmid. The selective force may be the use of disinfectants or feed supplements on the farm, and work is currently underway to investigate these hypotheses. The presence and spread of aprR plasmids in commensal E. coli, under unknown selective pressures, poses severe implications for the transmission of this resistance determinant into clinical bacteria.

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**References**

Apramycin resistance plasmids in commensal *E. coli*


