

## Correspondence

### Identification of class 1 integrons-associated gene cassettes in *Escherichia coli* isolated from *Varanus* spp. in Indonesia

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*Escherichia coli* isolates play a major role in the horizontal gene transfer of resistance genes located on mobile genetic elements between intestinal bacteria of different species and genera.<sup>1</sup> While data on antimicrobial resistance in *E. coli* are commonly obtained from clinical isolates of humans, food-producing animals or pets, little is known about the antimicrobial resistance of *E. coli* from free-living animals in remote geographical areas. In this study, we investigated 28 *E. coli* isolates obtained from faecal samples of monitor lizards (*Varanus* spp.) for the presence of antimicrobial resistance-mediating gene cassettes located in class 1 integrons.

The 28 *E. coli* isolates were obtained from free-living individual animals of different monitor lizard species captured alive in various regions in Indonesia. Antimicrobial resistance testing by disc diffusion was performed and evaluated according to the NCCLS document M31-A.<sup>2</sup> Of the 28 *E. coli* isolates, 21 proved to be resistant to tetracycline (75.0%), 20 to streptomycin (71.4%), 19 to sulfamethoxazole (67.9%), 18 to ampicillin (64.3%), seven to kanamycin or chloramphenicol (25.0%) and five to gentamicin or trimethoprim (17.9%). Many of the *E. coli* isolates showed multiresistance to the antimicrobial agents tested. In this regard, *E. coli* from the intestinal flora of lizards did not differ distinctly from the *E. coli* isolates commonly found in mammals where they

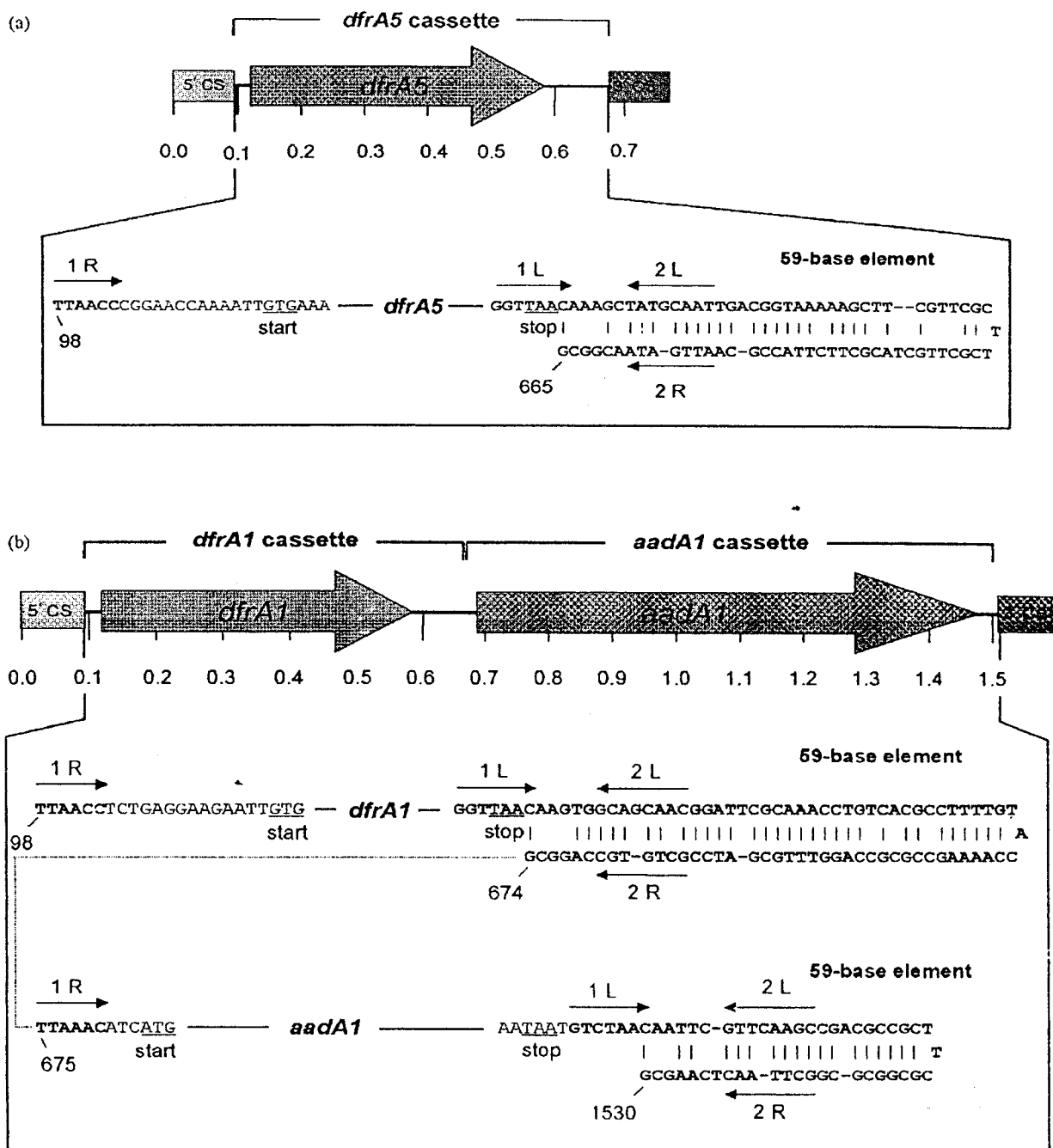
represented the main carrier of antimicrobial resistance in the intestinal flora.<sup>3</sup>

Whole-cell DNA of the 28 *E. coli* isolates was screened for the presence of class 1 integrons by PCR using a high fidelity *Pwo* polymerase (Peqlab, Erlangen, Germany) and primers corresponding to the conserved 5' (5'-GGCATCCAAG-CAGCAAG-3') and 3' (5'-AAGCAGACTTGACCTGA-3') regions of class 1 integrons.<sup>4</sup> Only three of the 28 *E. coli* isolates yielded amplicons with these PCR primers. The amplicons of two isolates were ~0.7 kb in size, whereas that of the third *E. coli* isolate was ~1.6 kb. These amplicons were cloned into pCR-Blunt II-TOPO (Invitrogen, Groningen, The Netherlands). Sequence analysis showed that the two similar-sized amplicons carried a *dfrA5* gene cassette for trimethoprim resistance, whereas the larger amplicon contained two gene cassettes, one harbouring the trimethoprim resistance gene *dfrA1*, the other the streptomycin/spectinomycin resistance gene *aadA1*.<sup>5</sup>

The two *dfrA5* gene cassettes of 721 bp each, detected in unrelated *E. coli* isolates from different *Varanus* spp., were indistinguishable by their nucleotide sequences. The predicted product of the *dfrA5* reading frame comprised 157 amino acids and proved to be identical to the DfrA5 protein of plasmid pLM020 (accession no. X12868). The 59 base element (59 be) of the *dfrA5* gene cassette consisted of 87 bp. The core site for the site-specific insertion, GTTAACC, was found at position 97–103. The recombination site is between the G and the first T, indicating the beginning of the gene cassette at position 98 bp. The inverse core site, GGTTAAC, was located at position 585–591 and included the translational stop codon (Figure 1).

The larger amplicon of 1586 bp contained a *dfrA1* gene cassette followed by an *aadA1* gene cassette. Homology searches revealed three database entries of *dfrA1* genes (accession nos X17478, AF203818 and AF382145). The only amino acid difference was Ser-124 in DfrA1 from *E. coli* of *Varanus* versus Pro-124 in the other DfrA1 variants. The recombination core site was found at position 97–103, while the inverse core site was at position 586–592. The 59 be of the *dfrA1* gene cassette comprised 95 bp and ended with a G at position 674 (Figure 1). Immediately downstream, the recombination core site of the *aadA1* gene cassette was identified at position 675–680. The corresponding AadA1 protein was indistinguishable from the AadA1 proteins encoded by other known *aadA1* genes (accession nos AY007807, AJ278514, AF327727 and M95287). The inverse core site, GTCTAAC, was located at position 1477–1483. The 59 be comprised 60 bp and ended with a G at position 1530 (Figure 1).

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**Figure 1.** Schematic representation of (a) the *dfrA5* gene cassette (accession no. AJ419169) as well as (b) the *dfrA1* and *aadA1* gene cassettes (accession no. AJ419168) detected in this study. The *dfrA5*, *dfrA1* and *aadA1* reading frames are shown as arrows, and the 5' and 3' conserved segments (5' CS, 3' CS) of the class 1 integron are shown as boxes. The beginning and end of the gene cassettes are shown in detail below. The translational start (GTG, ATG) and stop (TAA) codons are underlined. In the 59 be, the putative IndI integrase binding domains 1L, 2L, 2R and 1R are indicated by arrows. The 59 be of the gene cassettes is shown in bold type. Numbers indicating important positions of bases in the 59 be refer to the corresponding database entries. The dotted line in (b) indicates the connection between the two gene cassettes.

In conclusion, this study showed that antimicrobial resistance is rather common in intestinal *E. coli* isolates of free-living lizards captured in remote areas of Indonesia. Since these animals had no known contact with antimicrobials, it is

questionable from where these *E. coli* isolates have acquired their resistance genes. Attempts to answer this question require detailed analysis of the resistance genes and the genetic elements on which they are located. This analysis of

gene cassettes in class 1 integrons was a first step. Although the events in the acquisition of these gene cassettes could not be answered retrospectively, the finding that *E. coli* from *Varanus* spp. carried virtually the same gene cassettes as those previously described in clinical isolates of other Gram-negative bacteria of human or veterinary importance<sup>6</sup> was an interesting observation. This finding underlines the presence of links between bacteria of humans, animals and the environment that might be reflected by the interchange of gene cassettes carrying antimicrobial resistance genes.

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