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Identification of a cassette-borne *dfrA7*-like gene that shows a 97 bp extension at the 3′-end of the reading frame

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Sir,

Sulphonamides and trimethoprim are among the most frequently used antimicrobial drugs in Nigeria, where the combination of both drugs is used preferentially for the treatment of urinary tract infections. However, sulphonamides and trimethoprim are commonly available, alone or in combination, over the counter in pharmacies in Nigeria without a doctor's prescription. Self-medication and misuse of these drugs favours the development of resistance to sulphonamides and trimethoprim in various bacterial pathogens.

During the course of a study on antimicrobial resistance in uropathogenic *Escherichia coli* from humans in Nigeria, the multi-resistant *E. coli* strain UCH10386 was obtained from a 52-year-old female out-patient suffering from a urinary tract infection at the University College Hospital, Ibadan, Nigeria. This strain showed resistance to ampicillin, tetracycline, streptomycin, sulphonamides and trimethoprim. All five resistance properties were associated with a plasmid of *c.* 58 kb, designated pOJO10386, as confirmed by transformation into the *E. coli* laboratory strain JM107 and conjugation into the rifampicin-resistant *E. coli* HK225.

Extended PCR analyses were conducted using previously described primers to determine the type of the resistance genes.[1–3] For this, plasmid DNA prepared from an *E. coli* JM107:pOJO10386 transformant was used. These analyses revealed the presence of the tetracycline-resistance gene *tet*(A), the two sulphonamide-resistance genes *sulI* and *sul2*, the streptomycin-resistance gene *aadA2* and a bla_{TEM}-type ampicillin-resistance gene.

Furthermore, plasmid pOJO10386 was screened for the carriage of class 1 integrons by PCR using a *Pwo* polymerase (Peqlab, Erlangen, Germany) with an extended proof-reading activity and primers that corresponded to the 5′-conserved segment (5′-GGCATCCAAGCAGCAAG-3′) and the 3′-conserved segment (5′-AAGCAGACTTGACCTGA-3′) of class 1 integrons.[3] A single amplicon of *c.* 0.76 kb was obtained from plasmid pOJO10386, whereas the whole cell DNA of the recipient strain *E. coli* JM107 did not yield an amplicon. This amplicon was cloned into pCR-BluntII-TOPO (Invitrogen, Groningen, The Netherlands) and the recombinant plasmid was transformed into *E. coli* TOP10. *E. coli* TOP10 cells that carried this recombinant plasmid showed resistance to trimethoprim. Sequence analysis of the amplicon conducted on both strands confirmed the presence of a *dfrA7*-like gene that was part of a gene cassette (Figure).[4]

The amplicon comprised 764 bp; the 96 bp at the 5′-end corresponded exactly to part of the *attI1* site of class 1 integrons, whereas the 56 bp at the 3′-end were identical to the *qacE* sequence of the 3′-conserved segment of class 1 integrons.[5,6] The 612 bp between these conserved segments represented a complete *dfrA7* gene cassette that differed from known *dfrA7* cassettes in several aspects. Homology searches (http://www.ncbi.nlm.nih.gov/BLAST/; last accessed 7 November 2001) revealed three database entries of *dfrA7* genes (accession nos AF139109, U31119 and X58425), all of which code for virtually identical proteins of 157 amino acids. In comparison with the other *dfrA7* genes, a single bp exchange (a→c in *dfrA7* from pOJO10386) at position 178 was found to cause a conservative amino acid exchange at codon 21 from isoleucine in all other DfrA7 variants to leucine in DfrA7 from pOJO10386. However, more important was a mutation at positions 558/559. Instead of the 'aa' that was present in all other *dfrA7* genes, *dfrA7* from pOJO10386 had a single 'c' at this position. Although the exchange of 'a' by 'c' at position 558 did not even change the amino acid (isoleucine) at codon 147, the loss of the second 'a' caused a frameshift mutation that extended the *dfrA7* reading frame by 97 bp and, consequently, the length of the DfrA7 protein by 32 amino acids. This extension of the reading frame obviously did not

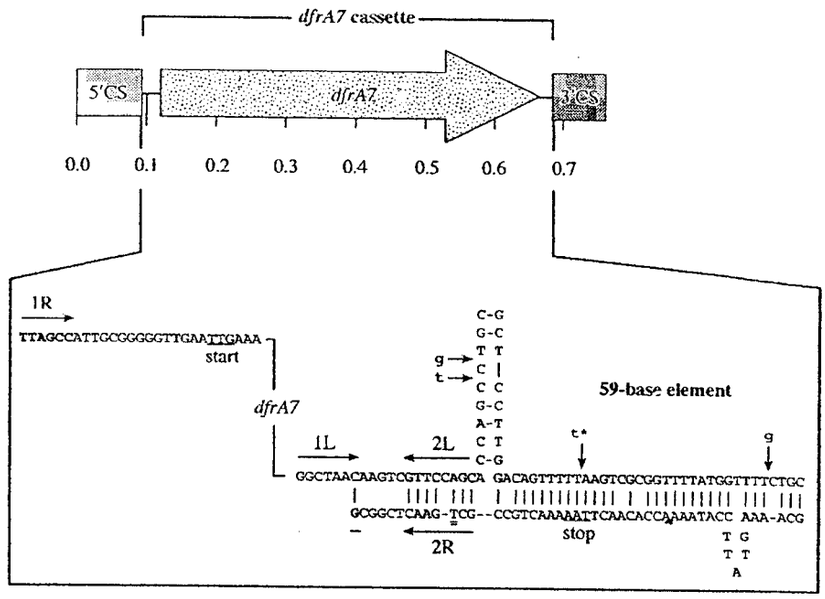

Figure. Schematic presentation of the *dfrA7* gene cassette from plasmid pOJO10386. The *dfrA7* reading frame is shown as an arrow, whereas the 5′- and 3′-conserved segments (5′ CS and 3′ CS) of the class 1 integron are shown as boxes. The beginning and the end of the cassette are shown in detail below. The translational start (TTG) and stop (TAA) codons are underlined. In the 59-base element, the putative IntI1 integrase binding domains 1L, 2L, 2R and 1R are indicated by arrows. The 7 bp core site of the cassette, which also represents the 1R sequence, is shown in bold type. The positions of the bases absent in the 59-base elements of the *dfrA7* cassette from pOJO10386, but present in those of the other *dfrA7* cassettes, are shown as lower-case letters marked by arrows. The 't' marked by an asterisk is only present in the sequences deposited under accession nos U31119 and X58425. These two sequences also show the exchange of the double underlined 'T' by 'C'. The sequence of the 764 bp amplicon from pOJO10386 has been submitted to the EMBL database and assigned accession no. AJ419170.

reduce the level of trimethoprim resistance mediated by this DfrA7 protein. The original *E. coli* strain UCH10386, its *E. coli* JM107:pOJO10386 transformant, its *E. coli* HK225:pOJO10386 transconjugant and the *E. coli* TOP10 that carried the *dfrA7* amplicon cloned into pCR-BluntII-TOPO exhibited trimethoprim MICs of >256 mg/L, whereas those of the *E. coli* strains HK225 and TOP10 were <2 mg/L.

Analysis of the 59-base element of the *dfrA7* cassette from pOJO10386 (Figure) showed that this element consisted of 130 bp, whereas the corresponding elements of the other known *dfrA7* genes were 134 bp (accession nos U31119 and X58425) or 133 bp (accession no. AF139109).[5] The loss of 4 or 3 bp, respectively, had no influence on the overall symmetry of this element and also did not affect the putative IntI1 integrase binding domains 1L, 2L, 2R and 1R.[6] Although in all other *dfrA* genes the coding sequence ended in the region of the inverse core site of the 59-base element, the *dfrA7* reading frame from pOJO10386 ended at a translational stop codon in the final part of the 59-base element (Figure). Whether this extension of the reading frame into the 59-base element might influence the mobility of this gene cassette is unknown.

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