2006

New integron-associated gene cassette encoding a trimethoprim-resistant DfrB-type dihydrofolate reductase

Renee Levings
University of Wollongong

D. Lightfoot
University of Melbourne

Liam Elbourne
University of Sydney

R. M. Hall
University of Sydney

Steven P. Djordjevic

New integron-associated gene cassette encoding a trimethoprim-resistant DfrB-type dihydrofolate reductase

Abstract

A sixth gene cassette containing a dfrB-type gene, dfrB6, was found in a dfrB6-aadA1 cassette array in class 1 integrons. This array was isolated from several multiply antibiotic-resistant Salmonella enterica serovar Infantis strains that appear to be clonally related. The DfrB6 dihydrofolate reductase conferred resistance to trimethoprim.

Publication Details

Levings, R., Lightfoot, D., Elbourne, L., Hall, R. & Djordjevic, S. (2006). New integron-associated gene cassette encoding a trimethoprim-resistant DfrB-type dihydrofolate reductase. Antimicrobial Agents and Chemotherapy, 50 (8), 2863-2865.

ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Aug. 2006, p. 2863–2865 Vol. 50, No. 8
0066-4804/06/$08.00+0 doi:10.1128/AAC.00449-06

New Integron-Associated Gene Cassette Encoding a Trimethoprim-Resistant DfrB-Type Dihydrofolate Reductase

Renee S. Levings,[1,2] Diane Lightfoot,[3] Liam D. H. Elbourne,[4] Steven P. Djordjevic,[1] and Ruth M. Hall[4]*

New South Wales Department of Primary Industries, Elizabeth Macarthur Agricultural Institute, Microbiology and Immunology Section, Camden, NSW 2570,[1] Department of Biological Sciences, University of Wollongong, NSW 2522,[2] Microbiological Diagnostic Unit, Public Health Laboratory, Department of Microbiology and Immunology, University of Melbourne, Victoria 3010,[3] and School of Molecular and Microbial Biosciences, The University of Sydney, NSW 2006,[4] Australia

Received 11 April 2006/Returned for modification 6 May 2006/Accepted 23 May 2006

A sixth gene cassette containing a *dfrB*-type gene, *dfrB6*, was found in a *dfrB6-aadA1* cassette array in class 1 integrons. This array was isolated from several multiply antibiotic-resistant *Salmonella enterica* serovar Infantis strains that appear to be clonally related. The DfrB6 dihydrofolate reductase conferred resistance to trimethoprim.

Resistance to trimethoprim, which inhibits the production of the essential cofactor tetrahydrofolate, is generally achieved by a bypass mechanism. Acquired genes that confer resistance to trimethoprim encode dihydrofolate reductases that are inhibitor resistant. These enzymes fall into two quite distinct groups (5), designated DfrA and DfrB, that are encoded by *dfrA* and *dfrB* genes (12). Members of the DfrA group are about 160 amino acids (aa) long and related to the chromosomally encoded dihydrofolate reductases of bacteria. Several of the known *dfrA* genes are found in gene cassettes (3). Members of the second, smaller group, DfrB (encoded by *dfrB* genes), are proteins of 78 aa that form a tetramer that binds both the substrate, dihydrofolate, and the cofactor, NADP, in equivalent positions, thus allowing reduction of the dihydrofolate to occur (1, 5). The five known *dfrB* genes (Table 1), which are all found in gene cassettes, confer resistance to substantially lower levels of trimethoprim than the *dfrA* genes (1).

Here we report the identification of a sixth *dfrB* gene cassette, *dfrB6*, found in class 1 integrons in multiply drug-resistant *Salmonella enterica* serovar Infantis strains, most of which were not recorded as resistant to trimethoprim.

Isolates. Eight multiply antibiotic-resistant *S. enterica* serovar Infantis strains isolated from chickens or chicken meat (six isolates) or infected animals (one isolate from a cat and one from a dog) were identified in a larger collection of 136 *S. enterica* strains of various serovars because they all carried an unusual array of gene cassettes (see below). The strains were serotyped using procedures standard to the Kauffman and White scheme (10), and the resistance profiles were determined as described previously (7). These strains were mostly resistant to streptomycin, spectinomycin, sulfathiazole, and tetracycline but susceptible to ampicillin, gentamicin, chloramphenicol, kanamycin, nalidixic acid, and ciprofloxacin (Table 2). Only one strain was recorded as resistant to trimethoprim.

TABLE 1. *dfrB* gene cassettes

Gene name[a]	Old or other name(s)	Cassette length (bp)	59-be length (bp)	GenBank accession no.	Reference or source
dfrB1	*dhfrIIa*, *dfr2a*	411[b]	57	AY139601	16
dfrB2	*dhfrIIb*, *dfr2b*	384	57	J01773	15
dfrB3	*dhfrIIc*, *dfr2c*	408	57	X72585	11
dfrB4	*dfr2d*	408	57	AJ429132	4
dfrB5		411	57	AY943084	8
dfrB6		410	57	DQ274503	This study

[a] Cassettes are named after the gene.
[b] An earlier sequence for this cassette is 485 bp long and contains a duplication of 72 of 73 bp (GenBank accession no. U36276).

Gene cassettes in class 1 integrons. Whole-cell DNA isolated from the *S. enterica* serovar Infantis strains by using

TABLE 2. Multiply antibiotic-resistant *S. enterica* serovar Infantis strains

Strain	Source	Yr isolated	Antibiotic resistance phenotype[a]	Cassette array	*tet*(B)[b]
SRC70	Feline	2001	Sm Sp Su Tc	*dfrB6-aadA1*	+
SRC71	Chicken	2001	Sm Sp Su Tc	*dfrB6-aadA1*	+
SRC72	Chicken	2001	Sm Sp Su Tc	*dfrB6-aadA1*	+
SRC92	Canine	2000	Sm Sp Su Tc Tp	*dfrB6-aadA1*	+
SRC93	Chicken	2000	Sp Su Tc	*dfrB6-aadA1*	+
SRC94	Chicken	2000	Sm Sp Su Tc	*dfrB6-aadA1*	+
SRC95	Chicken mince	2000	Sm Sp Su Tc	*dfrB6-aadA1*	+
SRC96	Chicken carcass	2000	Cm Sm Sp Su Tc	*dfrB6-aadA1*	+

[a] Cm, chloramphenicol; Sm, streptomycin; Sp, spectinomycin; Su, sulfonamides; Tc, tetracycline; Tp, trimethoprim.
[b] +, gene present.

* Corresponding author. Mailing address: School of Molecular and Microbial Biosciences, Biochemistry and Microbiology Building G08, The University of Sydney, NSW 2006, Australia. Phone: 61-2-9351-3465. Fax: 61-2-9351-4571. E-mail: Ruth.Hall@mmb.usyd.edu.au.

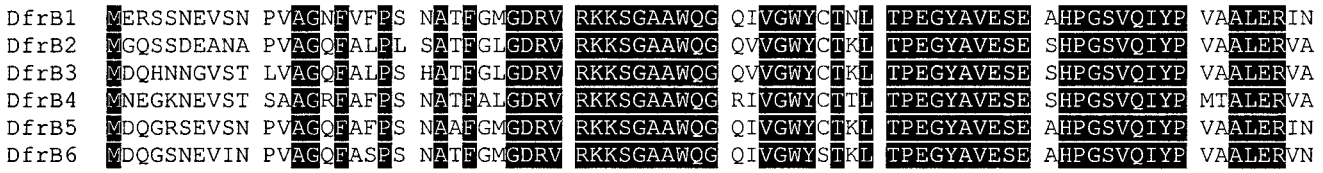

FIG. 1. Alignment of DfrB proteins. Amino acids completely conserved in all sequences are shown as white on black. The sequences were obtained from GenBank accession numbers listed in Table 1.

standard methods (13) was screened for the presence of class 1 integrons by using primers within the *intI1* gene (L2 and L3) and in the 3′-conserved segment (3′-CS) (QS-1 and QS-2) and primers L1 and R1 to amplify the cassette array (see reference 7 for primer details). PCR amplification was carried out in PCR buffer (Roche Molecular Biochemicals, Mannheim, Germany) containing 160 μM deoxynucleoside triphosphates, 20 pmol of each primer, approximately 10 to 50 ng template, and 1 U of *Taq* DNA polymerase (Roche). Reaction conditions were 94 to 96°C for 5 min, followed by 30 cycles of 96°C for 30 s, 54°C, 60°C, or 57°C, respectively, for 30 to 60 s, and 72°C for 90 s, and a final incubation at 72°C for 15 min. The gene cassettes, amplified using standard primers in the 5′-CS and 3′-CS (L1 and R1), yielded a product of 1.4 kb from all strains, indicating the presence of gene cassettes with a total length of approximately 1.25 kb. Digestion of the L1-R1 amplicon with restriction enzymes RsaI and Tsp5091, as described previously (6), revealed that the 1.4-kb amplicons were all the same.

The sequence of the amplicon from a single strain (SRC70) revealed two gene cassettes (GenBank accession no. DQ274503). The first contains an open reading frame with an ATG start codon at positions 71 to 73 relative to the start of the cassette that is preceded by a potential ribosome binding site AGG at positions 61 to 63. Translation from this ATG predicts a protein of 78 aa that is quite closely related to the known DfrB proteins (77 to 92% identical), and alignment of the sequences (Fig. 1) revealed only 7 and 10 amino acid differences from the closest relatives DfrB5 and DfrB1, respectively. The gene and cassette were named, using the next available number, as *dfrB6*, and the protein was named DfrB6. The second cassette in the integron is identical to the *aadA1* cassette in GenBank accession no. AF313471 (9).

The *dfrB6* cassette is 410 bp long, with 70 bp preceding the initiation codon and 53 bp between the termination codon and the 59-be (59-base element). The closest relatives of this cassette are the *dfrB1* cassette and the *dfrB5* cassette, both of which are 90% identical over the full length of the cassette. The 59-be is made up of two simple sites and a central region, as is characteristic for a 59-be (14). The *dfrB6* 59-be is identical to that in the *dfrB1* cassette and very closely related to those of other *dfrB* cassettes, which form a distinct group that are the shortest known, at 57 bp (12).

The *dfrB6* gene confers resistance to trimethoprim. As most of the original *S. enterica* serovar Infantis strains were not recorded as resistant to trimethoprim, the promoter in the integron 5′-CS was also amplified from SRC70 and sequenced. The *dfrB6* cassette is preceded by the strong promoter of class 1 integrons (2). The *dfrB6-aadA1* cassette array from SRC70 was amplified by PCR and cloned into pPCR-Script as described previously (7). The cloned fragment was recovered by transformation with selection on LB agar plates containing ampicillin (50 μg/ml) and trimethoprim (25 μg/ml). Susceptibility to trimethoprim for the *Escherichia coli* DH5α strain containing either pPCR-Script or pPCR-Script with the cassette array was determined using the gradient plate method. The cloned fragment conferred resistance to 550 μg/ml of trimethoprim (control was <1 μg/ml). The plasmid also conferred resistance to streptomycin and spectinomycin, as expected from the presence of the *aadA1* cassette.

The *S. enterica* serovar Infantis strains are clonally related. The strains were also screened by PCR for the presence of several additional antibiotic resistance genes by use of primer pairs internal to the genes (7). The tetracycline resistance determinant was identified as *tet*(B) [not *tet*(A) or *tet*(G)], and the *strAB* spectinomycin resistance determinant and *sul2* sulfonamide resistance gene were not present. IS*200* profiles, determined as described by Weill et al. (17), were identical for all of the eight strains, indicating that the strains are clonally related. This raises the possibility that the presence of rare gene cassettes may be an indicator for closely related strains. The infections of companion animals, a cat and a dog, may have arisen from the consumption of chicken meat.

Nucleotide sequence accession number. The nucleotide sequence reported in this paper has been submitted to GenBank under accession no. DQ274503.

R.S.L. was supported by a postgraduate award from the University of Wollongong. This work was supported by grants from the Department of Primary Industries, the McGarvie Smith Trust, and NHMRC (grant no. 402585).

We thank Linda Falconer and Jenny Liu for competent technical assistance.

REFERENCES

1. **Alonso, H., and J. E. Gready.** 2006. Integron-sequestered dihydrofolate reductase: a recently redeployed enzyme. Trends Microbiol. **14:**236–242.
2. **Collis, C. M., and R. M. Hall.** 1995. Expression of antibiotic resistance genes in the integrated cassettes of integrons. Antimicrob. Agents Chemother. **39:**155–162.
3. **Grape, M., A. Farra, G. Kronvall, and L. Sundström.** 2005. Integrons and gene cassettes in clinical isolates of co-trimoxazole-resistant Gram-negative bacteria. Clin. Microbiol. Infect. **11:**185–192.
4. **Grape, M., L. Sundström, and G. Kronvall.** 2003. New *dfr2* gene as a single-gene cassette in a class 1 integron from a trimethoprim-resistant *Escherichia coli* isolate. Microb. Drug Resist. **9:**317–322.
5. **Howell, E. E.** 2005. Searching sequence space: two different approaches to dihydrofolate reductase catalysis. ChemBioChem **6:**590–600.
6. **Levings, R. S., D. Lightfoot, S. R. Partridge, R. M. Hall, and S. P. Djordjevic.** 2005. The genomic island SGI1, containing the multiple antibiotic resistance region of *Salmonella enterica* serovar Typhimurium DT104 or variants of it, is widely distributed in other *S. enterica* serovars. J. Bacteriol. **187:**4401–4409.
7. **Levings, R. S., S. R. Partridge, D. Lightfoot, R. M. Hall, and S. P. Djordjevic.** 2005. New integron-associated gene cassette encoding a 3-*N*-aminoglycoside acetyltransferase. Antimicrob. Agents Chemother. **49:**1238–1241.
8. **Lolans, K., A. M. Queenan, K. Bush, A. Sahud, and J. P. Quinn.** 2005. First nosocomial outbreak of *Pseudomonas aeruginosa* producing an integron-borne metallo-β-lactamase (VIM-2) in the United States. Antimicrob. Agents Chemother. **49:**3538–3540.

9. **Partridge, S. R., H. J. Brown, and R. M. Hall.** 2002. Characterization and movement of the class 1 integron known as Tn*2521* and Tn*1405*. Antimicrob. Agents Chemother. **46:**1288–1294.
10. **Popoff, M. Y., and L. Le Minor.** 2001. Antigenic formulas of the *Salmonella* serovars, 8th ed. WHO Collaborating Centre for Reference and Research on *Salmonella*, Institut Pasteur, Paris, France.
11. **Rådström, P., O. Sköld, G. Swedberg, J. Flensburg, P. H. Roy, and L. Sundström.** 1994. Transposon Tn*5090* of plasmid R751, which carries an integron, is related to Tn*7*, Mu, and the retroelements. J. Bacteriol. **176:** 3257–3268.
12. **Recchia, G. D., and R. M. Hall.** 1997. Origins of the mobile gene cassettes found in integrons. Trends Microbiol. **5:**389–394.
13. **Sambrook, J., E. F. Fritsch, and T. Maniatis.** 1989. Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
14. **Stokes, H. W., D. B. O'Gorman, G. D. Recchia, M. Parsekhian, and R. M. Hall.** 1997. Structure and function of 59-base element recombination sites associated with mobile gene cassettes. Mol. Microbiol. **26:**731–745.
15. **Swift, G., B. J. McCarthy, and F. Heffron.** 1981. DNA sequence of a plasmid-encoded dihydrofolate reductase. Mol. Gen. Genet. **181:**441–447.
16. **Tennstedt, T., R. Szczepanowski, S. Braun, A. Pühler, and A. Schlüter.** 2003. Occurrence of integron-associated resistance gene cassettes located on antibiotic resistance plasmids isolated from a wastewater treatment plant. FEMS Microbiol. Ecol. **45:**239–252.
17. **Weill, F.-X., L. Fabre, B. Grandry, P. A. D. Grimont, and I. Casin.** 2005. Multiple-antibiotic resistance in *Salmonella enterica* serotype Paratyphi B isolates collected in France between 2000 and 2003 is due mainly to strains harboring *Salmonella* genomic islands 1, 1-B, and 1-C. Antimicrob. Agents Chemother. **49:**2793–2801.