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# New Integron-Associated Gene Cassette Encoding a 3-*N*-Aminoglycoside Acetyltransferase

Renee S. Levings,[1,2] Sally R. Partridge,[3] Diane Lightfoot,[4] Ruth M. Hall,[5]* and Steven P. Djordjevic[1]

*Department of Biological Sciences, Macquarie University, Sydney,[3] and School of Molecular and Microbial Biosciences, The University of Sydney,[5] Sydney, New South Wales Department of Primary Industries, Microbiology and Immunology Section, Elizabeth Macarthur Agricultural Institute, Camden,[1] and Department of Biological Sciences, University of Wollongong, Wollongong,[2] New South Wales, and Microbiological Diagnostic Unit, Public Health Laboratory, Department of Microbiology and Immunology, University of Melbourne, Melbourne, Victoria,[4] Australia*

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**A fifth gene cassette containing an *aacC* gene, *aacCA5*, was found in an *aacCA5-aadA7* cassette array in a class 1 integron isolated from a multiply drug resistant *Salmonella enterica* serovar Kentucky strain. The AacC-A5 or AAC(3)-Ie acetyltransferase encoded by *aacCA5* is related to other AAC(3)-I enzymes and confers resistance to gentamicin.**

Acetyltransferases that modify the 3-amino group of aminoglycosides represent one type of enzyme that confers resistance to this important group of antibiotics. The known 3-*N*-aminoglycoside acetyltransferases [AAC(3) enzymes] are classified into several groups based on phenotypic differences in the specific spectra of aminoglycosides they are able to modify (22). However, they fall into only two clearly distinct groups based on the relationships between the proteins. The four *aacC* genes in family A, *aacC1* [here designated *aacCA1* and also sometimes referred to as *aac*(*3*)*-Ia*] (GenBank accession no. X15852) (25), a variant of *aacCA1* (97.6% identical) (9, 15) previously named *aacC4* (15) and here designated *aacCA4*, *aac*(*3*)*-Ib* (here designated *aacCA2*) (L06157) (21), and *aac*(*3*)*Ic* (here designated *aacCA3*) (AJ511268) (18) belong to the *aac*(*3*)*-I* phenotypic group and are found in gene cassettes. The products of these genes are all small proteins, of 154 to 156 amino acids, that are related to one another (Table 1) and confer resistance to gentamicin, sisomicin, and fortimicin but not to tobramycin, amikacin, or kanamycin. The remaining *aacC* genes are not found in gene cassettes and encode longer proteins, of 261 to 300 amino acids, that do not appear to be significantly related (generally less than 25% identical) to members of the AAC(3)-I group. This type B protein family currently includes at least 14 distinct members and variants (<2% difference) of some of the members.

Here, we report the identification of a further *aacCA* gene cassette that was found in a class 1 integron in a multiply drug resistant *Salmonella enterica* serovar Kentucky strain.

**The isolate.** *Salmonella* serovar Kentucky SRC73 was isolated in 2001 from spice imported into Australia from India. The strain was serotyped by using standard procedures according to the Kauffman and White scheme (16). *Salmonella* serovar Kentucky SRC73 was scored as resistant to ampicillin (at 32 μg/ml), gentamicin (2.5 μg/ml), streptomycin (25 μg/ml), spectinomycin (50 μg/ml), sulfathiazole (550 μg/ml), tetracycline (20 μg/ml), and nalidixic acid (50 μg/ml) but susceptible to chloramphenicol (10 μg/ml), trimethoprim (50 μg/ml), kanamycin (10 μg/ml), and ciprofloxacin (2 μg/ml) by using the plate-replicator method as previously described (2, 3). Briefly, antibiotics at the concentrations indicated were in lysed blood Iso Sensitest agar plates (Oxoid, Hampshire, England), and the inoculum was $10^5$ CFU per spot. Plates were incubated overnight at 37°C.

**Resistance genes in *Salmonella* serovar Kentucky SRC73.** Whole cell DNA isolated from the *Salmonella* serovar Kentucky strain by using standard methods (20) was screened by PCR for several known antibiotic resistance genes with primers pairs internal to the genes (Table 2). PCR amplification reactions were carried out with PCR buffer (Roche Molecular Biochemicals, Mannheim, Germany) containing 160 μM of each deoxynucleoside triphosphate, 20 pmol of each primer, approximately 10 to 50 ng of template, and 1 U of Taq DNA polymerase (Roche). Reaction conditions were generally 94 to 96°C for 3 to 5 min; 30 to 40 cycles of 94 to 96°C for 30 s, 53 to 62°C for 30 to 60 s, and 72°C for 30 s to 2 min; and a final incubation at 72°C for 10 to 15 min. A product of the appropriate size for each gene of the *strAB* gene pair that confers resistance to streptomycin and for the $bla_{TEM}$ ampicillin resistance gene was detected. The tetracycline resistance determinant was *tet*(A), but spectinomycin resistance was not due to

TABLE 1. Relationships between members of the AacC-A or AAC(3)-I protein family[a]

| Protein | AacC-A1 | AacC-A2 | AacC-A3 | AacC-A4 | AacC-A5 |
|---|---|---|---|---|---|
| AacC-A1 AAC(3)-Ia | — | 71.6 | 59.4 | 95.5 | 51.0 |
| AacC-A2 AAC(3)-Ib | 87.1 | — | 60.1 | 72.1 | 49.0 |
| AacC-A3 AAC(3)-Ic | 74.2 | 77.1 | — | 60.4 | 55.6 |
| AacC-A4 AAC(3)-Id | 98.7 | 86.4 | 74.0 | — | 51.6 |
| AacC-A5 AAC(3)-Ie | 64.1 | 61.4 | 71.9 | 64.1 | — |

[a] Values represent the percent amino acid identities (top right) and similarities (bottom left) between the different AacC-A proteins. Dashes represent the 100% line.

* Corresponding author. Mailing address: School of Molecular and Microbial Biosciences, Biochemistry and Microbiology Building, G08, The University of Sydney, Sydney, NSW 2006, Australia. Phone: 61-2-9351-6014. Fax: 61-2-9351-4571. E-mail: Ruth.Hall@mmb.usyd.edu.au.

TABLE 2. PCR primer pairs

| Primer name | 5′-to-3′ sequence | Location | Product size | Accession no. | Reference[a] |
|---|---|---|---|---|---|
| L1 | GGCATCCAAGCAGCAAGC | 5′-CS | Variable | M95287.4 | 11 |
| R1 | AAGCAGACTTGACCTGAT | 3′-CS | | U12338.2 | 11 |
| L2 | GACGATGCGTGGAGACC | 5′-CS | 297 | M95287.4 | 19 |
| L3 | CTTGCTGCTTGGATGCC | 5′-CS | | M95287.4 | 12 |
| QS-1 | ATGAAAGGCTGGCTTTTTCTTG | 3′-CS | 722 | U12338.2 | 5 |
| QS-2 | TGAGTGCATAACCACCAGCC | 3′-CS | | U12338.2 | 5 |
| *sulI*-F | GTGACGGTGTTCGGCATTCT | *sul1* | 668 | U12338.2 | 10 |
| *sulI*-R | TTTACAGGAAGGCCAACGGT | *sul1* | | U12338.2 | 10 |
| *sulII*-F | GGCAGATGTGATCGACCTCG | *sul2* | 405 | M28829 | 10 |
| *sulII*-R | ATGCCGGGATCAAGGACAAG | *sul2* | | M28829 | 10 |
| *aadA2*-L | TGTTGGTTACTGTGGCCG | *aadA2* | 538 | X68227 | 14 |
| *aadA2*-R2 | TGCTTAGCTTCAAGTAAGACG | *aadA2* | | X68227 | 4 |
| *strA*-F | CTTGGTGATAACGGCAATTC | *strA* | 548 | M95402 | 8 |
| *strA*-R | CCAATCGCAGATAGAAGGC | *strA* | | M95402 | 8 |
| *strB*-F | ATCGTCAAGGGATTGAAACC | *strB* | 509 | M95402 | 8 |
| *strB*-R | GGATCGTAGAACATATTGGC | *strB* | | M95402 | 8 |
| *tem*-F | TTCTTGAAGACGAAAGGGC | $bla_{TEM}$ | 1,208 | L27758 | 6 |
| *tem*-R | ACGCTCAGTGGAACGAAAAC | $bla_{TEM}$ | | L27768 | 6 |
| *tet*(A)-L | GCTACATCCTGCTTGCCTTC | *tetA*(A) | 210 | X61367 | 14 |
| *tet*(A)-R | CATAGATCGCCGTGAAGAGG | *tetA*(A) | | X61367 | 14 |
| *tet*(B)-L | TTGGTTAGGGGCAAGTTTTG | *tetA*(B) | 659 | AP000342 | 14 |
| *tet*(B)-R | GTAATGGGCCAATAACACCG | *tetA*(B) | | AP000342 | 14 |
| *tet*(G)-L | CAGCTTTCGGATTCTTACGG | *tetA*(G) | 844 | S52437 | 14 |
| *tet*(G)-R | GATTGGTGAGGCTCGTTAGC | *tetA*(G) | | S52437 | 14 |

[a] References for primers are shown.

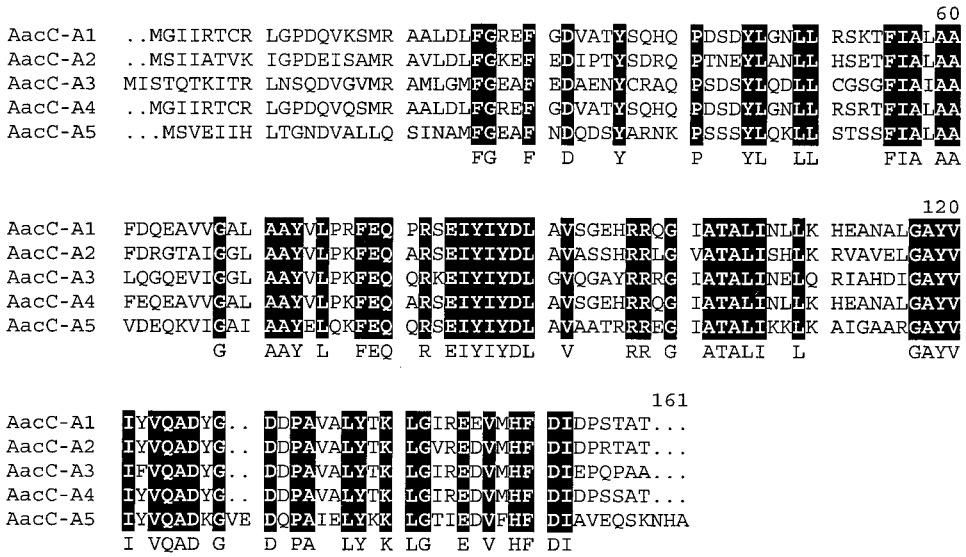


FIG. 1. Alignment of AacC-A proteins in the AAC(3)-I family. Amino acids completely conserved in all sequences are shown as white letters on a black background and are indicated by uppercase letters below the sequence. The sequences of AacC-A1 [AAC(3)-Ia], AacC-A2 [AAC(3)-Ib], AacC-A3 [AAC(3)-Ic], and AacC-A4 were obtained from GenBank and have accession nos. U12338, L06157, AJ511268, and AF318077, respectively. AacC-A5 [AAC(3)-Ie] is from this study.

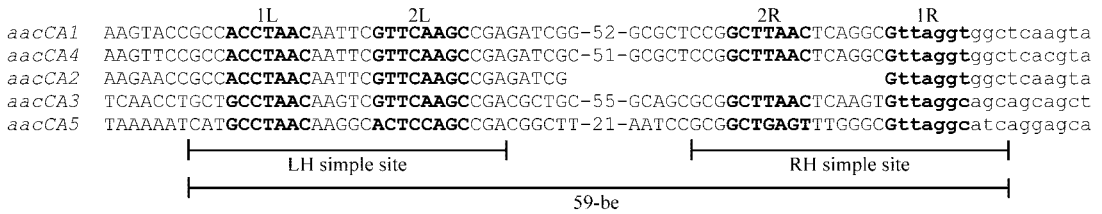


FIG. 2. Alignment of the 59-be of *aacCA* family gene cassettes. Bases in lowercase letters are derived from the beginning of the cassette. The core sites are in boldface type and are designated 1L, 1R, 2L, and 2R according to reference 23. The extents of the simple sites (LH, left-hand; RH, right-hand) and the 59-be are indicated by bars (bottom). Sequences are from sources given in Fig. 1.

the *aadA2* gene. The *sul1* gene, which confers resistance to sulfonamides and is found only in association with class 1 integrons, and the *sul2* gene were both present.

**Gene cassettes in a class 1 integron.** The presence of a class 1 integron was confirmed using primers within the *intI1* gene (L2 and L3) and in the 3′-conserved segment (3′-CS) (QS-1 and QS-2) (Table 2). Amplification of the gene cassettes with standard primers in the 5′-CS and 3′-CS (L1 and R1) yielded a product of 1.6 kb, indicating the presence of gene cassettes totaling 1.45 kb. The sequence of this amplicon (GenBank accession no. AY463797) revealed two gene cassettes. The first is 564 bp long and contains an open reading frame with a GTG start codon at positions 22 to 24 relative to the beginning of the cassette that is preceded by a GAGG ribosome binding site at positions 11 to 14. Translation from this GTG gives a protein of 158 amino acids that is related to the known AacC-A [AAC(3)-I] proteins (Table 1), and alignment of the sequences (Fig. 1) revealed 65 completely conserved amino acids. Using the next available number, the gene and cassette were named *aacCA5* and the protein AacC-A5 or AAC(3)-Ie.

The *aacCA5* cassette has a 59-be (59-base element) of 78 bp made up of two simple sites (Fig. 2) and a central region, as is characteristic for 59-be (23). This 59-be is not closely related to those of other cassettes in the *aacCA* group nor to those of any other known cassettes. The 59-be of the *aacCA1* and *aacCA4* cassettes, which are closely related, differ at only three positions, as expected if these two cassettes had diverged by accumulation of mutations over their full lengths (17). However, the more distantly related *aacCA2* [*aac*(*3*)-*Ib*] cassette (73% identical to *aacCA1*), for which a complete sequence is not available, appears to have the same 59-be (Fig. 2), as the 34 bp of the *aacCA2* 59-be sequence present in GenBank accession no. L06157 is identical to the corresponding part of the *aacCA1* 59-be. In this case, the 59-be may have been acquired recently from the *aacCA1* cassette. The 59-be in the *aacCA3* [*aac*(*3*)-*Ic*] cassette is most closely related to that of the $bla_{GES/IBC}$ cassette, as noted previously (18). Thus, the *aacCA5* and *aacCA3* cassettes appear to have separate origins.

The second cassette in the integron is identical to the *aadA7* cassette (13) (GenBank accession no. AF224733), found in an *Escherichia coli* strain from the ECOR collection that was isolated from a leopard in Washington Zoo in 1973 and in a Shiga toxin-producing *E. coli* O157:H7 strain (AF234167) (26). In both of these strains, *aadA7* is the only cassette in a class 1 integron. The *aadA7* gene confers resistance to streptomycin and spectinomycin and accounts for the spectinomycin resistance of SRC73. Since our work was completed, the *aacCA5-aadA7* cassette array (AB114632) has been found in *Vibrio fluvialis* (1), but the *aacCA5* gene was incorrectly designated *aac*(*3*)-*Id*. The same cassette array is also found in *Vibrio cholerae* (AY605683) and *Salmonella enterica* serovar Newport (AY458224) (7). The *aacCA5* cassettes are all identical, but there are minor differences in the *aadA7* cassettes (T65G C837Δ in AB114631 and AY605683; T669C A786G in AY458224).

**The *aacCA5* cassette confers resistance to aminoglycosides.** The *aacCA5-aadA7* cassette array was amplified by PCR and eluted from a 1% (wt/vol) agarose gel using an Amicon Bioseparations Ultrafree-DNA kit (Millipore Corp., Bedford, Massachusetts), and ligated into pCR-Script (PCR-Script Amp cloning kit; Stratagene, La Jolla, California) using the manufacturer's protocols. The cloned fragment was recovered by transformation with selection on LB agar containing ampicillin (50 μg/ml) and gentamicin (8 μg/ml). Susceptibilities to gentamicin, tobramycin, amikacin, netilmicin, and kanamycin for *E. coli* strain 294 (24) containing either pCR-Script or pCR-Script with the cassette array were determined by using the CDS method for antibiotic disks (Oxoid, Basingstoke, Hampshire, United Kingdom). The cloned fragment conferred resistance to gentamicin (5- versus 11-mm zone size), but not to tobramycin, amikacin, netilmicin, or kanamycin, consistent with the presence of an AAC(3)-I-type aminoglycoside acetyltransferase. Resistance to sisomicin has also been demonstrated (1). The cassette array also conferred resistance to streptomycin and spectinomycin, consistent with the presence of the *aadA7* cassette. SRC73 was resistant to the same set of aminoglycosides, indicating that the *aacCA5* and *aadA7* cassettes are sufficient to account for the observed aminoglycoside resistance.

**Nucleotide sequence accession number.** The nucleotide sequence reported in this paper has been submitted to GenBank under accession no. AY463797.

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## REFERENCES

1. **Ahmed, A. M., T. Nakagawa, E. Arakawa, T. Ramamurthy, S. Shinoda, and T. Shimamoto.** 2004. New aminoglycoside acetyltransferase gene, *aac*(*3*)-*Id*, in a class 1 integron from a multiresistant strain of *Vibrio fluvialis* isolated from an infant aged 6 months. J. Antimicrob. Chemother. **53:**947–951.
2. **Amavisit, P., P. F. Markham, D. Lightfoot, K. G. Whithear, and G. F. Browning.** 2001. Molecular epidemiology of *Salmonella* Heidelberg in an equine hospital. Vet. Microbiol. **80:**85–98.
3. **Bettelheim, K. A., M. A. Hornitzky, S. P. Djordjevic, and A. Kuzevski.** 2003. Antibiotic resistance among verocytotoxigenic *Escherichia coli* (VTEC) and non-VTEC isolated from domestic animals and humans. J. Med. Microbiol. **52:**155–162.
4. **Boyd, D., A. Cloeckaert, E. Chaslus-Dancla, and M. R. Mulvey.** 2002. Characterization of variant *Salmonella* genomic island 1 multidrug resistance

regions from serovars Typhimurium DT104 and Agona. Antimicrob. Agents Chemother. **46:**1714–1722.
5. **Boyd, D., G. A. Peters, A. Cloeckaert, K. S. Boumedine, E. Chaslus-Dancla, H. Imberechts, and M. R. Mulvey.** 2001. Complete nucleotide sequence of a 43-kilobase genomic island associated with the multidrug resistance region of *Salmonella enterica* serovar Typhimurium DT104 and its identification in phage type DT120 and serovar Agona. J. Bacteriol. **183:**5725–5732.
6. **Briñas, L., M. Zarazaga, Y. Sáenz, F. Ruiz-Larrea, and C. Torres.** 2002. β-Lactamases in ampicillin-resistant *Escherichia coli* isolates from foods, humans, and healthy animals. Antimicrob. Agents Chemother. **46:**3156–3163.
7. **Doublet, B., F.-X. Weill, L. Fabre, E. Chaslus-Dancla, and A. Cloeckaert.** 2004. Variant *Salmonella* genomic island 1 antibiotic resistance gene cluster containing a novel 3′-*N*-aminoglycoside acetyltransferase gene cassette, *aac*(3)-*Id*, in *Salmonella enterica* serovar Newport. Antimicrob. Agents Chemother. **48:**3806–3812.
8. **Gebreyes, W. A., and C. Altier.** 2002. Molecular characterization of multidrug-resistant *Salmonella enterica* subsp. *enterica* serovar Typhimurium isolates from swine. J. Clin. Microbiol. **40:**2813–2822.
9. **Gibb, A. P., C. Tribuddharat, R. A. Moore, T. J. Louie, W. Krulicki, D. M. Livermore, M.-F. I. Palepou, and N. Woodford.** 2002. Nosocomial outbreak of carbapenem-resistant *Pseudomonas aeruginosa* with a new $bla_{IMP}$ allele, $bla_{IMP-7}$. Antimicrob. Agents Chemother. **46:**255–258.
10. **Leverstein-van Hall, M. A., A. Paauw, A. T. A. Box, H. E. M. Blok, J. Verhoef, and A. C. Fluit.** 2002. Presence of integron-associated resistance in the community is widespread and contributes to multidrug resistance in the hospital. J. Clin. Microbiol. **40:**3038–3040.
11. **Lévesque, C., and P. H. Roy.** 1993. PCR analysis of integrons., p. 590–594. *In* D. H. Persing, T. F. Smith, F. C. Tenover, and T. J. White (ed.), Diagnostic molecular microbiology: principles and applications. Mayo Foundation, Rochester, Minn.
12. **Maguire, A. J., D. F. Brown, J. J. Gray, and U. Desselberger.** 2001. Rapid screening technique for class 1 integrons in *Enterobacteriaceae* and nonfermenting gram-negative bacteria and its use in molecular epidemiology. Antimicrob. Agents Chemother. **45:**1022–1029.
13. **Mazel, D., B. Dychinco, V. A. Webb, and J. Davies.** 2000. Antibiotic resistance in the ECOR collection: integrons and identification of a novel *aad* gene. Antimicrob. Agents Chemother. **44:**1568–1574.
14. **Ng, L. K., M. R. Mulvey, I. Martin, G. A. Peters, and W. Johnson.** 1999. Genetic characterization of antimicrobial resistance in Canadian isolates of *Salmonella* serovar Typhimurium DT104. Antimicrob. Agents Chemother. **43:**3018–3021.
15. **Poirel, L., G. F. Weldhagen, C. De Champs, and P. Nordmann.** 2002. A nosocomial outbreak of *Pseudomonas aeruginosa* isolates expressing the extended-spectrum β-lactamase GES-2 in South Africa. J. Antimicrob. Chemother. **49:**561–565.
16. **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*, Institute Pasteur, Paris, France.
17. **Recchia, G. D., and R. M. Hall.** 1997. Origins of the mobile gene cassettes found in integrons. Trends Microbiol. **5:**389–394.
18. **Riccio, M. L., J.-D. Docquier, E. Dell'Amico, F. Luzzaro, G. Amicosante, and G. M. Rossolini.** 2003. Novel 3-*N*-aminoglycoside acetyltransferase gene, *aac*(*3*)-*Ic*, from a *Pseudomonas aeruginosa* integron. Antimicrob. Agents Chemother. **47:**1746–1748.
19. **Sallen, B., A. Rajoharison, S. Desvarenne, and C. Mabilat.** 1995. Molecular epidemiology of integron-associated antibiotic resistance genes in clinical isolates of *Enterobacteriaceae*. Microb. Drug Resist. **1:**195–202.
20. **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.
21. **Schwocho, L. R., C. P. Schaffner, G. H. Miller, R. S. Hare, and K. J. Shaw.** 1995. Cloning and characterization of a 3-*N*-aminoglycoside acetyltransferase gene, *aac*(*3*)-*Ib*, from *Pseudomonas aeruginosa*. Antimicrob. Agents Chemother. **39:**1790–1796.
22. **Shaw, K. J., P. N. Rather, R. S. Hare, and G. H. Miller.** 1993. Molecular genetics of aminoglycoside resistance genes and familial relationships of the aminoglycoside-modifying enzymes. Microbiol. Rev. **57:**138–163.
23. **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.
24. **Talmadge, K., and W. Gilbert.** 1980. Construction of plasmid vectors with unique PstI cloning sites in a signal sequence coding region. Gene **12:**235–241.
25. **Wohlleben, W., W. Arnold, L. Bissonnette, A. Pelletier, A. Tanguay, P. H. Roy, G. C. Gamboa, G. F. Barry, E. Aubert, J. Davies, and S. A. Kagan.** 1989. On the evolution of Tn*21*-like multiresistance transposons: sequence analysis of the gene (*aacC1*) for gentamicin acetyltransferase-3-I (AAC(3)-I), another member of the Tn*21*-based expression cassette. Mol. Gen. Genet. **217:**202–208.
26. **Zhao, S., D. G. White, B. Ge, S. Ayers, S. Friedman, L. English, D. Wagner, S. Gaines, and J. Meng.** 2001. Identification and characterization of integron-mediated antibiotic resistance among Shiga toxin-producing *Escherichia coli* isolates. Appl. Envron. Microbiol. **67:**1558–1564.