linG, a new integron-associated gene cassette encoding a lincosamide nucleotidyltransferase

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**Publication Details**
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Abstract
Lincosamide antibiotics include lincomycin, a compound produced by several actinomycetes, and its semisynthetic chlorinated derivative clindamycin. These antibiotics block the peptidyltransferase activity of the 50S subunit of the bacterial ribosome, inhibiting protein synthesis, and are active against most gram-positive cocci and anaerobes. However, they are not generally effective against gram-negative bacilli due to intrinsic resistance.

Keywords
lincosamide, encoding, cassette, gene, associated, integron, nucleotidyltransferase, ling

Disciplines
Medicine and Health Sciences | Social and Behavioral Sciences

Publication Details

This journal article is available at Research Online: http://ro.uow.edu.au/smhpapers/1699
LinG, a New Integron-Associated Gene Cassette Encoding a Lincosamide Nucleotidyltransferase

Lincosamide antibiotics include lincomycin, a compound produced by several actinomycetes, and its semisynthetic chlorinated derivative clindamycin. These antibiotics block the peptidyltransferase activity of the 50S subunit of the bacterial ribosome, inhibiting protein synthesis, and are active against most gram-positive cocci and anaerobes. However, they are not generally effective against gram-negative bacilli due to intrinsic resistance (3, 5).

Resistance to lincosamides is most commonly due to N⁶ dimethylation of an adenine residue in the 23S rRNA, which usually confers broad-spectrum cross-resistance to macrolides, lincosamides, and streptogramin B antibiotics or to efflux (5). However, antibiotic inactivation by nucleotidylation (1, 2) has also been described as a mechanism of resistance. Despite the fact that lincosamides are not used to treat enterobacterial infections, a gene, linF, that confers low levels of resistance to both lincomycin (fourfold) and clindamycin (twofold) was recently found in a gene cassette in a class 1 integron recovered from an Escherichia coli blood isolate (4). The linF gene encodes a 273-amino-acid lincosamide nucleotidyltransferase. The LinF protein shares approximately 35% identity with the nucleotidyltransferases encoded by the linG gene from Enterococcus faecium (1) (GenBank accession no. AF110130) and linB from Enterococcus faecalis (GenBank accession no. AF040159).

We have identified a second lin gene in a gene cassette. This cassette was recovered from a multiply antibiotic-resistant Salmonella enterica serovar Stanley strain (SRC54) isolated in

![FIG. 1. Analysis of LinG sequences. (A) Alignment of Lin proteins. Amino acids that are completely conserved across all sequences are shown as white letters on a black background. The protein sequences of LinB and LinF were from GenBank accession numbers AF110130 and AJ561187, respectively. The LinG protein sequence is from this study. The boundaries of the LH (left-hand) and RH (right-hand) simple sites and the 59-be are indicated by bars. The bases in lowercase are those derived from the beginning of the cassette. The stop codons of the LinG and LinF proteins are indicated by the asterisk. The sequence of each 59-be came from the sources mentioned for panel A.](#)
2001 from a traveler who had recently returned from Thailand. This strain was resistant to chloramphenicol, gentamicin, kanamycin, spectinomycin, streptomycin, sulfathiazole, and tetracycline but susceptible to ampicillin and nalidixic acid at levels described previously (6). It displayed intermediate resistance to ciprofloxacin. The gene cassette array was amplified by using standard primers (L2 and R1) in the 5' conserved sequence and 3' conserved sequence of class 1 integrons, and the 2.25-kb amplicon was cloned into pHPCRscript and sequenced as previously described (see reference 6 for primer details). E. coli strain DH5α containing pHPCRScript with the cassette array was at least 10-fold more resistant to lincomycin (MIC, ≥2,000 μg/ml) than DH5α containing only pHPCR-Script (MIC, 180 μg/ml).

The first cassette in the array was identical to the aadA2 cassette in GenBank accession no. L06822. The second cassette was 937 bp long and 93.4% identical to the linF gene cassette. It encoded a 273-amino-acid protein that is 93.1% identical to LinF (17 amino acid differences). An alignment of these proteins with LinB is shown in Fig. 1A. The sequence of the aadA2-linG cassette array was identical to a region found in GenBank accession no. AY522431. The 59-base elements (59-be; attC sites) of the linG and linF cassettes are 58 bp long (Fig. 1B) and not closely related to any other known 59-be. They retain the critical features of 59-be, namely, complementary sites 1L-1R and 2L-2R (7).

Nucleotide sequence accession numbers. The sequence of the aadA2-linG cassette array has been deposited in GenBank under accession no. DQ836009.

REFERENCES

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