ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Aug. 2001, p. 2381–2382 Vol. 45, No. 8
0066-4804/01/$04.00+0 DOI: 10.1128/AAC.45.8.2381–2382.2001

Nonenzymatic Chloramphenicol Resistance Mediated by IncC Plasmid R55 Is Encoded by a *floR* Gene Variant

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Received 23 January 2001/Returned for modification 6 April 2001/Accepted 3 May 2001

The IncC plasmid R55, initially described in the 1970s and isolated from *Klebsiella pneumoniae,* confers nonenzymatic chloramphenicol resistance. The gene coding for this resistance was cloned and sequenced and shows 95 to 97% nucleotide identity with the recently reported *floR* gene from *Salmonella enterica* serovar Typhimurium DT104 and from *Escherichia coli* animal isolates, respectively, conferring cross-resistance to florfenicol.

Resistance to chloramphenicol (CHL) has been reported to be mainly due to the production of inactivating enzymes, the CHL acetyl transferases (CATs) (11). Nonenzymatic CHL resistance, however, was described in the late 1970s and early 1980s for plasmids of different incompatibility groups from gram-negative bacteria, such as the IncP-1 plasmid R26 from *Pseudomonas aeruginosa* and the IncC plasmid R55 from *Klebsiella pneumoniae* (10, 11). The *cml* gene of plasmid R26 conferring nonenzymatic CHL resistance was reported in 1986 by Dorman et al. (9) and codes for a putative efflux pump related to the more recently described CmlA protein of the *P. aeruginosa* In*4* integron of transposon Tn*1696* (3). Nonenzymatic CHL resistance has gained importance with the spread of multidrug-resistant *Salmonella enterica* serovar Typhimurium DT104 world-wide epidemic strains, which harbor on their chromosome an antibiotic resistance gene cluster comprising a nonenzymatic CHL resistance gene conferring cross-resistance to florfenicol (FFC) (1, 2, 4, 5).

FFC is a fluorinated analog of CHL approved in Europe for use against pasteurellosis in cattle since January 1995. Previous studies have shown that FFC is active against CHL-resistant strains producing either CATs (6) or nonenzymatic CHL resistance mediated by the CmlA efflux pump (3). The gene conferring cross-resistance to FFC has been named *floR, floSt, flo,* or *cmlA*-like (1, 2, 4, 5, 15) and is closely related (97% identity) to the pp-*flo* gene described in 1996 from a transferable R plasmid of the fish pathogen *Pasteurella piscicida* (13), recently renamed *Photobacterium damselae* subsp. *piscicida*. Their deduced amino acid sequences show about 47% identity to that of the CmlA protein. All of the gene products are assumed to belong to the 12 transmembrane segments family of export proteins of the major facilitator superfamily reviewed by Paulsen et al. (14). FFC resistance conferred by the *floR* gene has also recently been reported in *Escherichia coli* strains isolated from cattle and poultry (7, 12, 15) and in *S. enterica* serovar Agona strains isolated from poultry (8).

In the present study we analyzed nonenzymatic CHL resistance, with particular attention to possible FFC cross-resistance, conferred by the IncC plasmid R55 (150 kb; Tra^+ Ap Cm Gm Su), among the first to have been described as conferring nonenzymatic CHL resistance in the 1970s (10, 11). Interestingly, this plasmid, isolated from *K. pneumoniae*, has been reported to encode enzymatic CHL resistance as well, namely, a type I CAT (11).

R55 FFC resistance and detection of the *floR* gene. *E. coli* strain K-12 BM14 (*pro met azi*) carrying plasmid R55 was verified as FFC resistant. Antibiograms and the MICs of FFC were determined as described previously (1, 2). FFC disks and the drug itself were purchased from Schering-Plough Animal Health (Kenilworth, N.J.). *E. coli* BM14 carrying plasmid R55 showed resistance to FFC (MIC, 32 μg/ml) to the same extent as *S. enterica* serovars Typhimurium DT104 and Agona (8) and previously described *E. coli* strains (7). PCR was performed on the extracted plasmid DNA using internal primers of the *floR* gene, cml01 and cml15, as described previously (1, 2, 8). An amplification fragment of the expected size (496 bp) was obtained (data not shown). Nucleotide sequencing of the fragment revealed 95% identity with the *floR* nucleotide sequence of *S. enterica* serovar Typhimurium DT104 (data not shown), thus indicating that plasmid R55 carries a *floR* gene variant conferring resistance to FFC. Southern blot hybridization of plasmid R55 digested by *Sac*I, *Bam*HI, and *Bgl*I using a *floR* probe produced and labeled as described previously (1, 2, 7, 8) revealed bands of 12, 6, and 3 kb, respectively (not shown), and thus confirmed the presence of a *floR* gene variant on this plasmid.

R55 *floR* gene variant and flanking regions. The *floR*-carrying 6-kb *Bam*HI fragment of plasmid R55 was cloned in plasmid pGEM-7Zf (Amp^r) (Promega, Charbonnieres, France) and sequenced. Briefly, *Bam*HI-digested fragments of plasmid R55 were ligated into plasmid pGEM-7Zf. Competent *E. coli* JM109 cells were transformed with the recombinant plasmids. Selection of transformants was done using Luria-Bertani agar plates supplemented with ampicillin (100 μg/ml) and FFC (10 μg/ml). Positive clones were confirmed by *floR* PCR on the extracted plasmids. One pGEM-7Zf plasmid clone carrying the 6-kb *Bam*HI insert was kept and named plasmid pSR511. The FFC and CHL MICs for *E. coli* JM109 carrying plasmid pSR511 were 32 and 128 μg/ml, respectively. Those for *E. coli* JM109 without the plasmid were 4 μg/ml for both antibiotics. DNA sequencing of the insert was performed by Génome Express (Grenoble, France).

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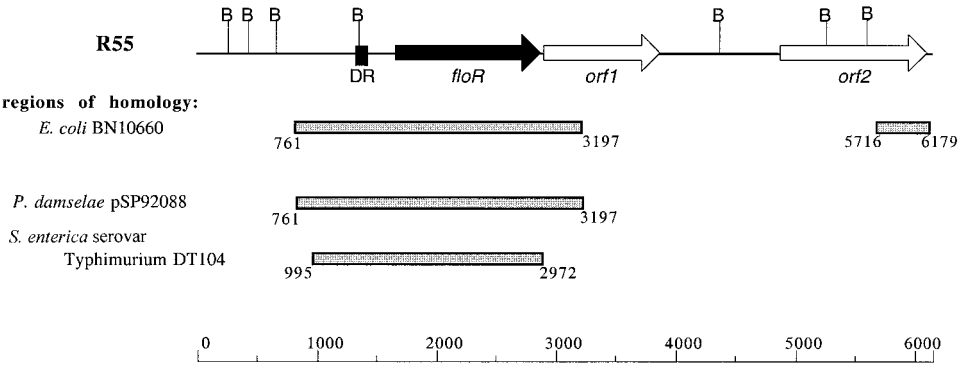

FIG. 1. Structural organization of the 6,179-bp *floR* locus of plasmid R55. Regions which exhibit homology to the *floR*-carrying *E. coli* BN10660 plasmid (GenBank accession no. AF231986), to the pp-*flo*-carrying *P. damselae* plasmid pSP92088 (GenBank accession no. D37826), and to the *S. enterica* serovar Typhimurium DT104 antibiotic resistance gene cluster (GenBank accession no. AF071555) are indicated. The numbers of the homologous segments refer to their positions within the sequence of the plasmid R55 *floR* locus. The extent and the direction of transcription of the *floR, orf1,* and *orf2* reading frames are marked by arrows. The solid box upstream of *floR* (DR) indicates the 99-bp direct repeat. The *Bgl*I restriction sites are abbreviated as B. The distance scale below the map of the *floR* locus is given in basepairs.

Comparative sequence analysis showed that the R55 *floR* gene variant was 95 and 97% identical to previously reported *floR* genes of *S. enterica* serovar Typhimurium DT104 and *E. coli* animal isolates, respectively (data not shown). The deduced amino acid sequence of the R55 *floR* gene variant showed 97 and 98% identity to that of *floR* of *S. enterica* serovar Typhimurium DT104 and *E. coli*, respectively. Amino acid changes occured principally in the sixth transmembrane segment of the protein (data not shown).

A database search for homologies revealed that the flanking regions of the R55 *floR* gene partly matched those of previously described *E. coli* and *S. enterica* serovar Typhimurium DT104 *floR* genes and also those of the pp-*flo* gene of *P. damselae* subsp. *piscicida* (Fig. 1). In all cases, the upstream region of *floR* with its putative promoter region, and a stretch of 99 bp which is repeated in *S. enterica* serovar Typhimurium DT104 downstream of the *floR* gene, appears conserved. Also, two open reading frames were detected downstream of *floR* of plasmid R55 (Fig. 1). The deduced amino acid sequence of *orf1* shows homology with those of transcriptional regulators of the LysR family, the closest homolog being that of *S. enterica* serovar Typhimurium (accession number AAK02052) with 67% amino acid identity (data not shown). The C-terminal end of the deduced amino acid sequence of *orf2* is identical to that of the putative transposases found up- and downstream of the *floR* gene of *E. coli* (Fig. 1) (7).

In conclusion, this study showed that the nonenzymatic CHL resistance described in the late 1970s and mediated by the IncC plasmid R55, initially isolated from *K. pneumoniae*, is conferred by a *floR* gene variant which confers FFC cross-resistance to the same extent as previously described *floR* genes. From a historical point of view, this is thus the first example of FFC resistance, long before its description in *P. damselae* subsp. *piscicida* (13), *S. enterica* serovars Typhimurium DT104 and Agona (1, 2, 4, 5, 8), or *E. coli* of animal origin (7, 12, 15).

Nucleotide sequence accession number. The sequence of the *floR*-containing *Bam*HI fragment from plasmid R55 has been deposited in GenBank under accession no. AF332662.

We thank P. Courvalin for providing *E. coli* strain BM14 and plasmid R55. We also thank M. A. Arcangioli for preliminary studies and C. Mouline for expert technical assistance.

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