Plasmid-Mediated High-Level Resistance to Erythromycin in *Escherichia coli*

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_Escherichia coli_ BM2195 was found to be resistant to high levels of erythromycin. This new resistance phenotype was due to the constitutive synthesis of an erythromycin esterase which inactivates the antibiotic. The gene conferring resistance to erythromycin in this strain is carried on a 61-kilobase self-transferable plasmid, pIP1100, belonging to incompatibility group X.

Enterobacteria, including _Escherichia coli_, are intrinsically resistant to low levels of macrolide, lincosamide, and streptogramin antibiotics (MICs of ≤500 µg/ml) (2). However, higher local antibiotic concentrations, in particular of erythromycin, are obtained in the lumen of the intestinal tract after oral administration of recommended therapeutic doses, and a reduction of the aerobic gram-negative flora of the intestinal tract is achieved (2). Thus, erythromycin has been successfully used for the prevention of travellers diarrhoea (2) and has been proposed for the microbial modulation of the intestinal tract in immunocompromised patients (1).

Under these conditions, strains of enterobacteirae resistant to these high levels of erythromycin can be selected. Mutants of _E. coli_ resistant to high concentrations of erythromycin and other macrolides have been isolated and shown to possess mutations affecting ribosomal protein L4 or L22 (28). Resistance of gram-positive cocci to macrolides is almost always associated with co-resistance to lincosamides and streptogramin B-type antibiotics (6, 27). Plasmid-mediated resistance to macrolides, lincosamides, or streptogramins has not yet been reported in enterobacteria. In this study we describe an _E. coli_ strain highly resistant (MIC ≥2 mg/ml) to erythromycin.

The properties of the strains used are listed in Table 1. Strain BM2195 was a blood isolate from a patient treated with erythromycin. _E. coli_ IGR42, which is highly resistant to erythromycin and does not inactivate the antibiotic, was of human fecal origin (1). The method of Steers et al. (25) was used to determine the MICs of the antibiotics on Mueller-Hinton agar. The upper limiting factor of MIC determination was the solubility of the drugs. The inactivation of erythromycin by growing (16) or resting (4) cells and assay for aminoglycoside-modifying enzymes by the phosphocellulose paper-binding technique were as described (17). Isoelectric focusing in polyacrylamide gels of S100 preparations (21), purification of high-molecular-weight plasmid DNA (19), and agarose gel electrophoresis (24) were as described.

_E. coli_ BM2195 encodes resistance to ampicillin, chloramphenicol, gentamicin, streptomycin, sulfonamide, tetracycline, and trimethoprim and to high levels of erythromycin (Table 1). In curing experiments with acridine orange and ethidium bromide (5), erythromycin and gentamicin resistances were lost en bloc (approximately 3% of 226 colonies tested). These two characters were also lost spontaneously at a very low frequency (0.12% of 1,617 colonies tested). One cured isolate was studied further (strain BM2195-1). The genes conferring resistance to ampicillin, erythromycin, gentamicin, and streptomycin were transferred en bloc from BM2195 to _E. coli_ BM694 by conjugation at a frequency of 10⁻⁴. Selection for transfer of erythromycin or gentamicin resistance revealed cotransfer of all four resistances (in the 10 clones studied). One transconjugant, strain BM2507, was studied further. The MICs of macrolide, lincosamide, and streptogramin antibiotics for strains BM2195, BM2195-1, BM694, and BM2507 are shown in Table 2. Plasmid DNA from strain BM2195 was purified by ultracentrifugation in cesium chloride-ethidium bromide and used to transform _E. coli_ HB101 (12). Three major classes of transformants were obtained: one had acquired resistance to ampicillin, erythromycin, gentamicin, and streptomycin (149 clones); another had acquired resistance to ampicillin, chloramphenicol, streptomycin, sulfonamide, and tetracycline (16 clones), and the third had acquired resistance to only ampicillin (937 clones). One transformant of each class (strains BM2526, BM2527, and BM2528 in Table 1) was studied further. Incompatibility testing (8) of the transconjugant BM2507 revealed that all of the acquired characters, i.e., resistance to ampicillin, erythromycin, gentamicin, and streptomycin, were borne by a single plasmid belonging to incompatibility (Inc) group X (18).

The plasmid DNA from strain BM2195, its cured derivative BM2195-1, transconjugant BM2507, and transformants BM2526, BM2527, and BM2528 was purified by ultracentrifugation and analyzed by agarose gel electrophoresis before and after digestion with _Hind_III endonuclease (data not shown). Comparative analysis of the phenotypes with the plasmid content in the individual strains led us to conclude that the wild-type strain BM2195 harbors three resistance plasmids (Table 1): plasmid pIP1100, encoding resistance to ampicillin, erythromycin, gentamicin, and streptomycin, with a molecular size of 61 kilobases and 11 _Hind_III-generated DNA fragments; pIP1101, encoding resistance to ampicillin, chloramphenicol, streptomycin, sulfonamide, and tetracycline, with a molecular size of approximately 123 kilobases; and pIP1102, encoding resistance to ampicillin, with a molecular size of 25 kilobases. Strain BM2195-1 was susceptible to erythromycin and gentamicin and had lost pIP1100. _E. coli_ strain BM2507 was resistant to ampicillin, erythromycin, gentamicin, and strep-

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tomycin after acquisition of pIP1100. Plasmids pIP1100, pIP1101, and pIP1102 were detected in strains BM2526, BM2527, and BM2528, respectively, after transformation (Table 1).

E. coli BM2195 and its derivative BM2195-1, strain BM694 and the transconjugant BM2507, and strain HB101 and transformants were examined for erythromycin-modifying activity by microbiological techniques (Fig. 1 and 2). Strains harboring plasmid pIP1100 were found to inactivate erythromycin and oleandomycin but none of the other commercially available macrolide, lincosamide, or streptogramin antibiotics. The inactivating enzyme conferred detectable resistance to erythromycin only (Table 2). Because of the high intrinsic resistance to oleandomycin, we did not see any difference in MICs for this compound. The modifying activity was found both in growing (Fig. 1) and in resting (Fig. 2) cells. The enzyme appeared extremely efficient with erythromycin as a substrate (half-time decay of 30 min). We did not find any inactivating activity in the culture medium. In addition, strains harboring plasmid pIP1100 were found to contain aminoglycoside acetyltransferase and adenylyltransferase activities but no phosphotransferase activity.

Resistance to gentamicin and structurally related antibiotics was due to synthesis of a 3-acyetyltransferase, and resistance to streptomycin and spectinomycin was due to the production of a 3',9-adenylyltransferase (data not shown) (11, 20). Plasmid pIP1100 also encoded a TEM-1 beta-lactamase with an isoelectric point of about 5.4 (data not shown).

We have demonstrated that the gene conferring resistance to high levels of erythromycin in E. coli BM2195 is borne on a 61-kilobase plasmid, pIP1100, which belongs to incompatibility group X. The natural resistance of gram-negative bacteria to low levels of macrolides, lincosamides, and streptogramins appears to be due to cellular impermeability, t-forms are much more susceptible to macrolides than the corresponding normal bacteria (26), and E. coli mutants which are more than usually susceptible to these antibiotics are also permeable to other agents which do not normally affect E. coli (10). Until now, microbial degradation of erythromycin has been detected in steroid-transforming strains of Streptomyces spp. (14), in Lactobacillus spp. (15), and in Pseudomonas spp. (15) (for a recent review see reference 9). However, the biochemical and genetic bases of bacterial detoxification of the antibiotic have been poorly studied. E. coli BM2195 resisted high levels of erythromycin but remained susceptible to other macrolide, lincosamide, and streptogramin antibiotics (Table 2). This new resistance phenotype was due to the constitutive synthesis of an enzyme which inactivates the antibiotic (Fig. 1 and 2). We have determined the structure of the modified drug by physicochemical techniques (4), and the results obtained indicate that the new enzyme is an erythromycin esterase which hydrolyzes the lactone ring of the antibiotic. We have cloned and sequenced a pIP1100 DNA fragment conferring high-level resistance to erythromycin, and the resistance gene, designated ereA, was located in an open reading frame of 1,032 base pairs (23). The distribution of this new character in enterobacteria highly resistant to erythromycin which were isolated from human feces was studied by colony hybridization with an intragenic probe. The gene for

### Table 1. Properties of the bacterial strains used

<table>
<thead>
<tr>
<th>E. coli strain</th>
<th>Relevant characteristics and plasmid content*</th>
<th>Source (reference)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BM2195</td>
<td>Prototroph, mucoid, pIP100, pIP1101, pIP1102</td>
<td>Wild-type strain</td>
</tr>
<tr>
<td>BM2195-1</td>
<td>Prototroph, mucoid, pIP1101, pIP1102</td>
<td>Curing of BM2195</td>
</tr>
<tr>
<td>BM694</td>
<td>Prototroph, gyrA</td>
<td>Spontaneous mutant of Cla (19)</td>
</tr>
<tr>
<td>HB101</td>
<td>hsdR + hsdM + recA13 supE44 lac -24 leuB6 proA2 thi-1 rpsL20</td>
<td>Boyer and Roulland-Dussoix, 1969 (12)</td>
</tr>
<tr>
<td>IGR42</td>
<td>Prototroph, Em</td>
<td>Wild-type strain</td>
</tr>
<tr>
<td>BM2507</td>
<td>Prototroph, gyrA, pIP1100</td>
<td>Conjugation BM2195 × BM694</td>
</tr>
<tr>
<td>BM2526</td>
<td>hsdR + hsdM + recA13 supE44 lac -24 leuB6 proA2 thi-1 rpsL20, pIP1100</td>
<td>Transformation pIP1100 × HB101</td>
</tr>
<tr>
<td>BM2527</td>
<td>hsdR + hsdM + recA13 supE44 lac -24 leuB6 proA2 thi-1 rpsL20, pIP1101</td>
<td>Transformation pIP1101 × HB101</td>
</tr>
<tr>
<td>BM2528</td>
<td>hsdR + hsdM + recA13 supE44 lac -24 leuB6 proA2 thi-1 rpsL20, pIP1102</td>
<td>Transformation pIP1102 × HB101</td>
</tr>
</tbody>
</table>

* Genetic symbols are as described by Bachmann (3) and Novick et al. (22). pIP1100 encodes Tra + IncX Ap Em Gm Sm; pIP1101 encodes Tra + Ap Cm Sm Su Tc; pIP1102 encodes Tra + Ap.

### Table 2. MICs of various macrolide, lincosamide, and streptogramin antibiotics against E. coli strains

<table>
<thead>
<tr>
<th>Strain</th>
<th>MIC (µg/ml) of:</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Erythromycin</td>
</tr>
<tr>
<td>BM2195</td>
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</tr>
<tr>
<td>BM2195-1</td>
<td>128</td>
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<tr>
<td>BM694</td>
<td>64</td>
</tr>
<tr>
<td>BM2507</td>
<td>2.048</td>
</tr>
<tr>
<td>IGR42</td>
<td>1.024</td>
</tr>
</tbody>
</table>
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LITERATURE CITED
23. Ounissi, H., and P. Courvalin. 1985. Nucleotide sequence of the...


