Development of Plasmid-Mediated Resistance in *Vibrio cholerae* During Treatment with Trimethoprim-Sulfamethoxazole

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The persistence of *Vibrio cholerae*, biotype *el tor*, in a patient treated with trimethoprim-sulfamethoxazole was due to the acquisition of a conjugative resistance plasmid. The plasmid, with a molecular size of 72 megadaltons, belonged to incompatibility group 6-C and conferred resistance to ampicillin, chloramphenicol, sulfonamide, and trimethoprim.

The first strains of *Vibrio cholerae* with transmissible multiple resistance were isolated during 1964 and 1965 in the Philippines (6, 7). It seems, however, that the resistance factors acquired by these strains were maintained with difficulty and were not easily transmissible to enterobacteria. In contrast, a strain of *V. cholerae* isolated in Algeria in 1971 had a very stable R plasmid which was easily transferable to *Escherichia coli* K-12 (15). All *V. cholerae* plasmids later isolated in Algeria belonged to the 6-C incompatibility group (14).

On 27 August 1981, we isolated *V. cholerae* from stool samples of four Algerians returning from a stay in Algeria. The four strains were susceptible to most antibiotics and quickly disappeared from the stools of three of these patients after treatment with trimethoprim (8 mg/kg per day) and sulfamethoxazole (40 mg/kg per day). In contrast, in the fourth patient treated with the same antibiotics, the bacterium persisted for eight days and became resistant to ampicillin, chloramphenicol, sulfonamide and trimethoprim (Ap, Cm, Su, and Tp). Resistance was transferable by conjugation to *E. coli* with a low frequency (7 × 10⁻⁶). Conjugation was performed in a brain heart agar broth, with the resistant *V. cholerae* strain (2 × 10⁶ bacteria per ml) being mixed with recipient strains (*E. coli* K-12 UB1139 *leu met thy nal* or JC3272 *trp his lys str* [8 × 10⁶ bacteria per ml]) for 3 h at 37°C. The transconjugants UB1139 (Ap, Cm, Su, and Tp) and JC3272 (Ap, Cm, Su, and Tp) were then isolated on appropriate selective media. It was also possible to obtain transfer-of-resistance markers of UB1139 (Ap, Cm, Su, and Tp) to JC3272 by conjugation.

Isolation of the resistance plasmid, which we named pYMB2, was performed as described by Kado et al. (5). Examination under electron microscope by the modified Kleinschmidt method (3) allowed the visualization of a large plasmid (molecular size, 72 megadaltons) in the resistant *V. cholerae* strain. Susceptible strains examined by the same technique (9) were devoid of any extrachromosomal DNA as previously reported for *el tor* biotypes (1).

Production of beta-lactamase was investigated by the Novick iodometric method (11): a susceptible *V. cholerae* strain was devoid of beta-lactamase activity, and the resistant strain produced the enzyme.

This activity was also found in the transconjugants *E. coli* UB1139(pYMB2) and JC3272(pYMB2). The substrate profile of beta-lactamase coded by pYMB2 was determined (microacidimetric method at pH 7.0 and 37°C) (8) by using bacterial extracts prepared as previously reported (10). The maximal rates of hydrolysis were achieved with a saturated concentration of 0.24 mM for penicillins and 6.5 mM for cephalosporins. Results were suggestive of a TEM-type beta-lactamase. The nature of the enzyme was confirmed by analytical isoelectric focusing (5% polyacrylamide gel; pH gradient of 4 to 6.5; 400 V; 18 h at 10°C), with enzymatic activity being detected with nitrocefin (12). The *V. cholerae* beta-lactamase focused at pH 5.4, at the same point as the TEM-1 enzyme coded by plasmid R6K, and the TEM-2 enzyme coded by RP4 focused at pH 5.6 (Fig. 1).

Chloramphenicol was inactivated by bacterial extracts; thus, the resistance seemed due to an enzyme, probably an...
acetylttransferase (13). Trimethoprim resistance is presently under investigation (J. Burchall, unpublished data) to determine which of the various resistant dihydrofolate reductases is involved (16).

The V. cholerae plasmid was incompatible with group 6-C plasmids. This was shown by transferring reference plasmids of group 6-C, R55 (Ap', Cm', Gm', Km', and Su') or R16a (Ap', Km', and Su') from K-12 strains to JC3272(pYMB2). Transconjugants no longer had the markers of pYMB2, only those of the reference plasmid. Similarly, when pYMB2 was transferred to strains carrying R55 or R16a, the resident plasmids were eliminated, and pYMB2 was stably established.

The persistence of V. cholerae in the stool samples from patients during trimethoprim-sulfamethoxazole treatment was presumably due to the acquisition by the bacterium of a resistance plasmid pYMB2 which, like other plasmids isolated in V. cholerae in the last decade, belonged to compatibility group 6-C (4, 15). A study of the antibiotic resistance of the intestinal flora associated with the Vibrio strain might have allowed us to determine the origin of the plasmid. But because we were preoccupied by the clinical situation, the study was not performed. However, the beta-lactamase coded by this plasmid was of the TEM-1 type, as are the large majority of beta-lactamases coded by plasmids of enterobacteria (2).

The ability of V. cholerae to acquire an R plasmid during treatment and the subsequent dissemination of these strains selected by antibiotic therapy constitute an epidemiological danger, especially since sulfonamide chemoprophylaxis is used against cholera.

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LITERATURE CITED