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# Inducible High Resistance to Colistin in *Proteus* Strains

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Wild-type *Proteus* strains are usually resistant to colistin. We have observed an increase in the level of colistin resistance in four clinical isolates of *P. vulgaris*, *P. morganii*, and *P. rettgeri* by prior treatment with subinhibitory concentrations of the drug. The enhanced resistance in these strains was lost after cultivating them in colistin-free medium, indicating that the enhanced resistance in the *Proteus* group is inducible.

Colistin (CL) and polymyxin B (PL) exhibit a wide spectrum of antibacterial activity against gram-negative organisms and lesser activity against gram-positive organisms and fungi (10). Among the *Enterobacteriaceae*, only *Proteus* species and *Serratia marcescens* are resistant to the CL and polymyxin antibiotics (2, 7, 8). Sud and Feingold (11) reported that PL resistance in *P. mirabilis* is due to the cell envelope, which prevents access of the drug to the susceptible lipid target sites. Teuber (12) reported that penicillin-induced *P. mirabilis* spheroplasts were lysed by PL whereas the intact organisms were resistant to the bactericidal effect of the drug.

In the course of investigations on the mechanism of resistance to CL in *Proteus* strains isolated from clinical sources, we have observed an inducible enhanced resistance to CL and PL in *Proteus* strains. The present paper deals with kinetic studies of the induction of this enhanced resistance.

## MATERIALS AND METHODS

**Bacterial strains.** *P. vulgaris* GN2330 and GN2318, *P. morganii* GN2717, and *P. rettgeri* GN2594 are the stock cultures of this laboratory that were isolated from clinical sources.

**Media.** Brain heart infusion (BHI) broth (Eiken Tokyo), peptone broth, and heart infusion agar (Eiken, Tokyo) were employed for liquid and solid cultures. Peptone broth consisted of 10 g of peptone (Oxoid), 5 g of NaCl, and distilled water in a final volume of 1 liter (pH 7.2).

**Drugs and drug resistance.** CL and PL were supplied by the Kayaku Antibiotic Research Co., Ltd., and the Taito Pfizer Co., Ltd., respectively. Drug resistance was determined by the agar dilution method.

**Determination of bacterial growth.** Growth in liquid culture was photometrically assayed at 540 nm with a Hitachi 101 spectrophotometer.

**Induction of CL resistance.** An overnight culture in BHI broth was diluted 100-fold in fresh BHI broth and shaken in a water bath at 37°C. After 2 h of incubation, a 0.35-ml sample of the culture that reached the middle of exponential growth was inoculated in 9.65 ml of BHI broth containing various concentrations of CL as an inducer and shaken in a water bath at 37°C. After appropriate time intervals of incubation, 0.2-ml samples were withdrawn, inoculated in 9.8 ml of BHI broth containing various concentrations of CL, and shaken in a water bath at 37°C. At appropriate time intervals, the ability of the bacteria to grow in this medium was photometrically assayed. In control experiments, 0.35-ml samples in the mid-exponential phase were inoculated in 9.65 ml of BHI broth without CL. Similarly, the growth of the noninduced cells in BHI broth containing various concentrations of CL was photometrically assayed at appropriate time intervals.

## RESULTS

**Inhibitory effect of CL on bacterial growth.** We examined the concentrations of CL that inhibited the growth of the four *Proteus* strains (Fig. 1). Two strains of *P. vulgaris*, GN2330 and GN2318, and a *P. morganii* strain (GN2717) were completely inhibited at concentrations of 400 μg, 6.4 mg, and 12.8 mg of CL per ml, respectively, and the growth of *P. rettgeri* GN2594 was strongly inhibited at a concentration of 51.2 mg of CL per ml.

**Rise in the level of colistin resistance by prior treatment with the drug.** It was found that prior treatment of strain GN2330 with subinhibitory concentrations of CL enhanced the level of CL resistance. Cells pretreated with CL were capable of growing on agar plates containing 25.6 mg of CL per ml. The nontreated cells did not grow on a plate containing 3.2 mg of CL per ml. To determine the pretreatment time required for the complete enhancement of CL resistance, strain GN2330 was incubated with shaking in BHI broth containing 0.2 μg of CL per ml, a concentration of CL in which the growth of GN2330 was not affected (Fig. 1). At

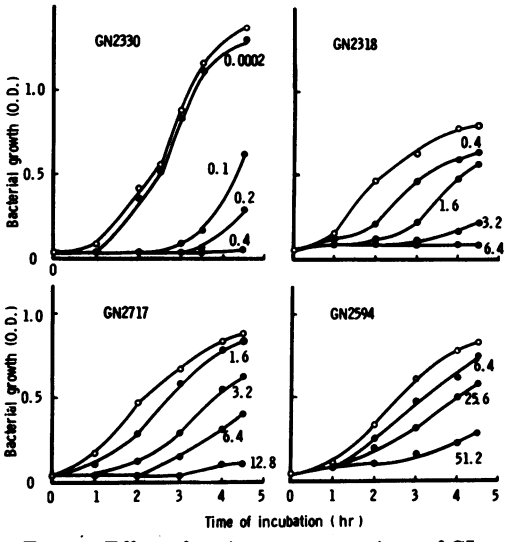


FIG. 1. *Effect of various concentrations of CL on the growth of Proteus strains. An overnight culture in BHI broth was diluted 10-fold in fresh BHI broth containing various concentrations of CL and shaken at 37°C. Numbers indicate the concentrations of CL (milligrams per milliliter). Symbols: ●, bacterial growth in media containing CL; ○, bacterial growth in CL-free medium. O.D., Optical density.*

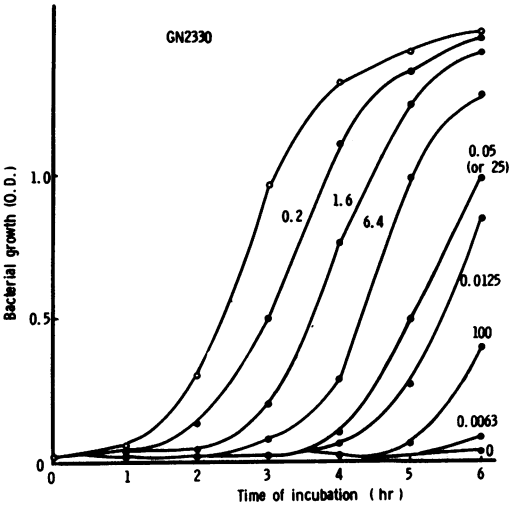


FIG. 3. *Effect of CL concentrations on the rise in level of CL resistance. Induction of CL resistance was carried out in BHI broth containing various concentrations of CL. After 1 h of incubation, the culture was inoculated in BHI broth containing 400 μg of CL per ml to examine the rise in level of CL resistance. Numbers indicate the concentrations of CL (micrograms per milliliter) in the induction culture. P vulgaris GN2330 was used as a test organism. For symbols, see the legend of Fig. 1. O.D., Optical density.*

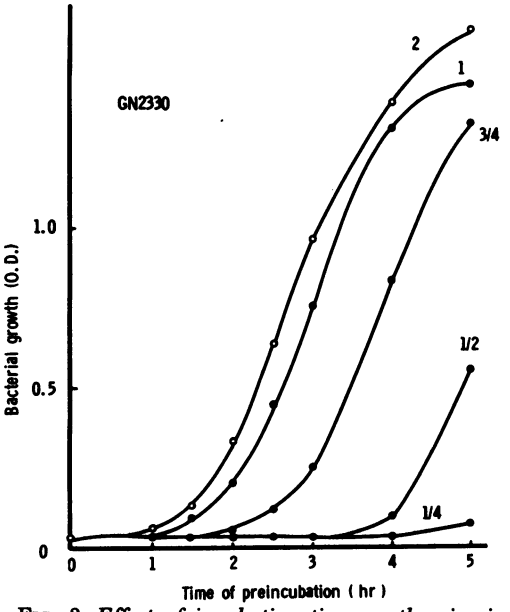


FIG. 2. *Effect of incubation time on the rise in level of CL resistance. A 0.35-ml sample was withdrawn from the induction culture containing 0.2 μg of CL per ml at various time intervals and inoculated in BHI broth containing 400 μg of CL per ml to determine the rise in level of CL resistance. Details are described in the text. P. vulgaris GN2330 was used as a test organism. For symbols, see the legend of Fig. 1. Numbers indicate the incubation time (hours) in induction medium. O.D., Optical density.*

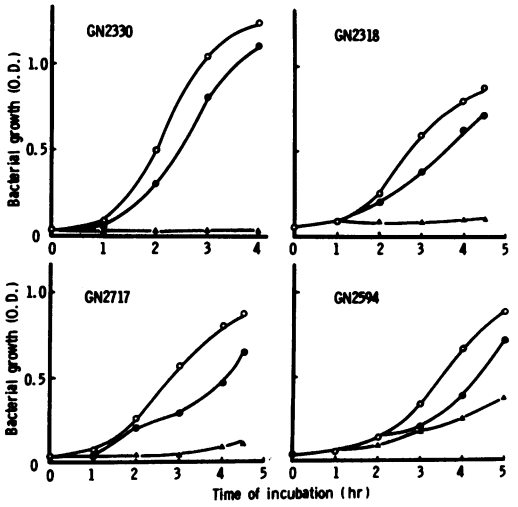


FIG. 4. *Induction of CL resistance in Proteus strains. Induction of CL resistance was carried out by incubation of strains GN2330, GN2318, GN2717, and GN2594 in BHI broth containing 0.2 μg of CL per ml. After 1 h of incubation, the cultures were inoculated in BHI broth containing the following selective concentrations of CL (milligrams per milliliter) against strains: GN2330, 0.4; GN2318, 6.4; GN2717, 12.8; GN2594, 51.2. For symbols, see the legend of Fig. 1 and the following: ●, after induction; ▲, without induction. O.D., Optical density.*

appropriate time intervals, bacterial cells were withdrawn and inoculated in BHI broth containing 400 μg of CL per ml to see whether they were capable of growing in this medium. As shown in Fig. 2, the enhancement of CL resistance was found to be completed at 1 h of incubation of bacterial cells. Similarly, prior incubation with PL also raised the level of resistance to CL under the same conditions. The optimal concentrations of CL for the enhancement of CL resistance in strain GN2330 are shown in Fig. 3. A concentration of 0.2 μg of CL per ml in broth was found to be sufficient to complete the enhancement of CL resistance after 1 h of incubation, and the bacterial cells could grow in the medium containing 400 μg of CL per ml. Strains GN2330, GN2318, GN2717, and GN2594 all acquired a high resistance to CL after pretreatment with CL (Fig. 4).

**Loss of CL resistance after incubation in drug-free medium.** Loss of CL resistance of induced populations was observed after growth of the cells in CL-free medium. The growth of induced strain GN2330 cells in a medium containing 400 μg of CL per ml was retarded with an increasing time of prior incubation of the bacteria in CL-free medium (Fig. 5). The CL resistance of the induced populations was lost after 1 to 4 h of prior incubation in CL-free medium. The same results were observed with the other three strains (GN2318, GN2717, and GN2594).

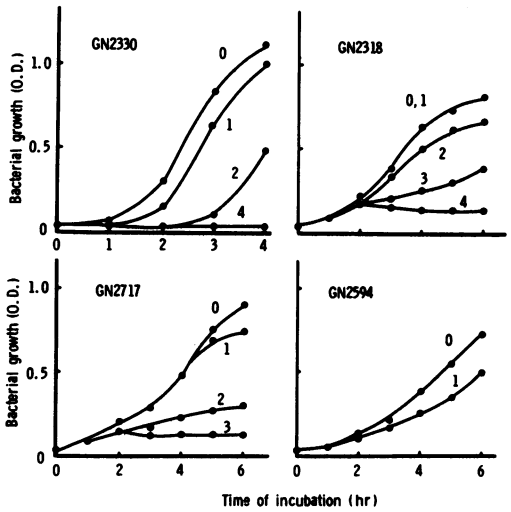


FIG. 5. *Loss of CL resistance in induced populations of Proteus strains after incubation in CL-free medium. Induction of CL resistance was carried out by incubation of strains GN2330, GN2318, GN2717, and GN2594 in BHI broth containing 0.2 μg of CL per ml. After incubation for 1 h at 37°C, bacterial cells were harvested by centrifugation, washed twice with BHI broth, and suspended in the original volume of BHI broth. After various time intervals of incubation, the loss of CL resistance was examined by inoculating the cells in BHI broth containing 400 μg (GN2330), 6.4 mg (GN2318), 12.8 mg (GN2717), and 51.2 mg (GN2594) of CL per ml. Numbers indicate the incubation time in CL-free medium. O.D., Optical density.*

## DISCUSSION

It has been reported that the resistance to various drugs, such as tetracycline, macrolide antibiotics, chloramphenicol, and β-lactam antibiotics, was inducible in gram-positive or gram-negative bacteria (1, 3, 4–6, 9). By the same criteria, the rise in level of CL resistance in *Proteus* strains by prior treatment with subinhibitory concentrations of the drug is also considered to be due to an induction. A brief period of prior treatment of microorganisms with low concentrations of CL is enough to complete the rise of CL resistance. Second, there is rapid loss of the CL-enhanced resistance in induced populations after incubation in CL-free medium.

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