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Short Communications

Influence of chloramphenicol on the sensitivity of *Pseudomonas aeruginosa* to chloroxylenol and crystal violet

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Summary

Gustafson and co-workers (1973) in a study on *Escherichia coli* show that the chloramphenicol inhibition on protein synthesis opens also the outer membrane barrier to crystal violet.

There is evidence that crystal violet and chloroxylenol, two hydrophobic molecules (Nikaido, 1976; Kropinski et al., 1978) cross the outer membrane of *Pseudomonas aeruginosa* by a particular pathway. These observations were made in our study on the sensitivity of *Pseudomonas aeruginosa* to antibacterials according to the growth conditions. The data show a resistance to chloroxylenol and crystal violet when the organism is grown in tryptic soy broth (T.S.B. Difco) and a sensitivity to these products when the bacteria is grown in brain heart infusion (B.H.I. Difco) (Devleeschouwer et al., submitted). It seemed thus interesting to look if a pretreatment of the strain with chloramphenicol would restore the sensitivity to chloroxylenol and crystal violet of the bacteria grown in T.S.B.

The method used for the evaluation of the sensitivity to disinfectants has been previously described (Devleeschouwer and Dony, 1981).

Briefly, *Pseudomonas aeruginosa* ATCC 15442 is cultured for 18 h, either in tryptic soy broth (Difco) or in brain heart infusion (Difco). After culture, chloramphenicol at a final concentration of 100 μg/ml is added to the two bacterial suspensions which are then reincubated for 1 h at 37°C. A test of the evaluation of the effectiveness of chloroxylenol and crystal violet is performed on the treated as well as on the untreated cultures. The results given in Table 1 are expressed as logarithms of the reduction of the initial number of bacteria/ml.

When looking to the results of the trial with chloroxylenol on untreated cells, there is a significant difference in sensitivity of *Pseudomonas aeruginosa* according to the culture medium, the bacteria being much more resistant when grown in tryptic soy broth. The difference between the two media reaches 2 or 3 log phases depending of the presence or not of horse serum (10%). This difference of more than 2 log phases (2.7–2.8) between the two media remains even after the treatment with chloramphenicol, but in each particular medium the bacteria are more resistant to the action of chloroxylenol than without contact with chloramphenicol. For example, in T.S.B. there is a reduction of 4.3 log of the initial number of bacteria without treatment and only 2.4 log when

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TABLE 1

SENSITIVITY TO CHLOROXYLENOL AND CRESYL VIOLET OF *PSEUDOMONAS AERUGINOSA* ATCC 15442 TREATED AND UNTREATED WITH CHLORAMPHENICOL

Antibacterial agent	Without serum			With serum		
	TSB	BHI	BHI-TSB	TSB	BHI	BHI-TSB
Chloroxylenol (0.24%) (U)	4.3 *	7.4	3.1	3.2	5.4	2.2
Chloroxylenol (0.24%) + chloramphenicol (T)	2.4	5.2	2.8	1.7	4.4	2.7
U–T	1.9	2.2	–	1.5	1.0	–
Crystal violet (12.5 mg/ml) (U)	−0.2	0.5	0.7	−0.4	0.6	1.0
Crystal violet (12.5 mg/ml) + chloramphenicol (T)	−0.4	−0.45	−0.05	−0.6	−0.65	−0.05
U–T	0.2	0.95	–	0.2	1.25	–

* Reduction of the initial number of organisms in 10 minutes expressed as logarithms.
A negative logarithm is the expression of a growth of the microorganisms.

the bacteria are treated one hour with chloramphenicol. The same kind of results are obtained in brain heart infusion, a reduction of 7.4 log without chloramphenicol and only 5.2 log with chloramphenicol treatment. The case of crystal violet is not clear due to an insufficient bactericidal action in 10 min of the highest possible concentration.

However, the same kind of results are recorded, that is to say, bacteria more resistant to the dye when cultured in tryptic soy broth. Again, a treatment with chloramphenicol before the action of the dye makes the bacteria more resistant in the two media. This fact is not due to a selection by chloramphenicol of resistant bacteria as a consequence of the killing of the more sensitive ones. Indeed, countings performed before and after the chloramphenicol treatment give the same numbers of bacteria/ml in each medium. There was no decrease of the bacteria after treatment by chloramphenicol.

In conclusion, our experiences give evidence that in our particular conditions the action of chloramphenicol does not open the outer membrane barrier in *Pseudomonas aeruginosa*. In contrast, a treatment of 1 h with chloramphenicol produces bacteria more resistant to crystal violet and chloroxylenol. It is likely that chloramphenicol prevents these products from entering through the cell wall.

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