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NORFLOXACIN RESISTANCE IN PROVIDENCIA STUARTII: MODIFICATIONS IN DNA-GYRASE AND PERMEABILITY

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INTRODUCTION

It has recently been found that several strains of Providencia stuartii present a high level of resistance to 4-quinolones, compounds which have shown strong antibacterial activity against both gram-positive and gramnegative bacteria.
The target of their action is the bacterial essential enzyme DNA-gyrase, whose activity affects superhelical conformation of DNA, mainly by introduction of negative superhelical turns.(1)
Resistance to 4-quinolones, in these strains of P. stuartii, such as in resistant strains of E. coli and Klebsiella pneumoniae was usually coupled by appreciable levels of resistance to unrelated classes of antibiotics, above all β-lactams and aminoglycosides.(2) Cross resistance between antibiotics with different targets seemed to involve cell permeability mechanisms: in most of these strains it was possible to demonstrate that porins, outer membrane proteins which control permeability, were affected by such mutations to change their polyacrilamide gel electrophoresis pattern.(3)
Specific studies on strains resistant to norfloxacin, a member of 4-quinolones family which has shown a peculiar efficacy in therapy, showed that mutations in outer membrane proteins could be coupled by mutations in DNA-gyrase, the target of the drug. These two kinds of mutations, together, were able to give the strain a high level of resistance to norfloxacin.(4)
We obtained a strain of Providencia stuartii, clinically isolated, which presented high levels of resistance to 4-quinolones norfloxacin and ciprofloxacin (Mic were 200 μg and 80 μg, respectively) and was also resistant to B-lactams and aminoglycosides antibiotics. Our first step was to detect possible modifications in outer membrane proteins electrophoretical pattern, which could be responsible of a modification in cell permeability.

RESULTS AND DISCUSSION

The extraction of outer membrane proteins of P. stuartii was performed using the methods generally accepted for other Enterobacteria.(5)
Proteins were analysed on polyacrilamide gel which showed two main proteins with a molecular weight of 35-40 Kdal corresponding to E. coli and other Enterobacteria porins.(6) These two proteins represented the best part of the pattern and did not show any difference between resistant and susceptible strains. On the other hand, differences in electrophoretical mobility were found in proteins whose molecular weight was just above 45Kdal. We can not exclude that these changes, even if slight, can be responsible of modifications in cell permeability.
Role of DNA-gyrase mutations which could be involved in raising 4-quinolones resistance level was detected performing assays for supercoiling activity of DNA-gyrase in presence of norfloxacin and ciprofloxacin.
We reported an inhibiting concentration of 5 μg for both the compounds (fig.1): this value, compared to the inhibiting concentrations for a standard DNA-gyrase, which is 1 μg, indicates that the enzyme is mutated, but this mutation can not be held responsible, on his own, of strain's high level of resistance.
It has recently been described that DNA-gyrase action is affected by DNA structural modifications, and that 4-quinolones inhibition is due to the binding of these compounds to DNA and consequent distorsion of the double helix.(7)
According to these data, we performed supercoiling assays in presence of norfloxacin having as substrate a plasmid coupled with DNA packaging proteins extracted from the same strain of P. stuartii. Our intent was to find out any possible change in norfloxacin inhibition of DNA-gyrase due to the structural modifications of DNA induced by the packaging proteins. Results did not show any appreciable difference.

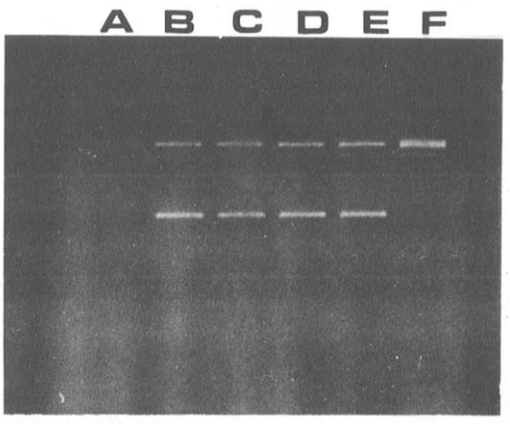

Figure 1. Assay of DNA-gyrase activity in presence of ciprofloxacin with plasmid pAT153 as substrate. Lane A): relaxed pAT153; B): pAT153 supercoiled by DNA-gyrase; C) reaction performed with 1 μg/ml of ciprofloxacin; D): 2 μg/ml; E): 3 μg/ml; F): 5 μg/ml.

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