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Original article

Optimization of the use of ciprofloxacin

Optimisation de l'utilisation de la ciprofloxacine

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Abstract

Aims. – To compare mutant prevention concentration (MPC) of ciprofloxacin and time-killing curve with regards to 11 genotyped *Escherichia coli.*

Method. – MICs were determined using the E-test method. Time-killing studies were performed in accordance with the NCCLS guidelines. The genes *gyr*A, *gyr*B, *par*C, *par*E and *mar*R were amplified by PCR and sequenced. The MPC was defined as the lowest antibiotic concentration preventing the growth of resistant colonies when 10^{10} CFU/mL were spread on a solid medium.

Results. – Strains with no genes *gyr*A, *gyr*B, *par*C, *par*E and *mar*R mutation presented MIC less or equal to 0.023 mg/L and MPC less or equal to 0.25 mg/L. Strains with two mutations (*gyr*A and *par*C) presented MIC equal to 1.5 mg/L and MPC equal to 4 mg/L. Strains with one mutation (*gyr*A) presented MIC less or equal to 0.75 mg/L, but MPC ranged from 0.5 to 6 mg/L depending of the MIC of ciprofloxacin. The time-killing curves for ciprofloxacin showed a bactericidal activity of 0.25 mg/L in 1 h for strains without mutation, compared with a bactericidal activity of 2 and 4 mg/L in 4 h for strains with one and two mutations, respectively.

Conclusion. – For strains of *E. coli* resistant to nalidixic acid, it was necessary to evaluate the MIC of ciprofloxacin in order to asses the optimal dosage of ciprofloxacin.

Résumé

Objectif. – Comparaison de la concentration prévenant la sélection de mutants résistants (CPM) de la ciprofloxacine et de la cinétique de bactéricidie vis-à-vis de 11 souches d'*Escherichia coli* génotypées.

Méthode. – Les CMI ont été déterminées par la méthode E-test. Les cinétiques de bactéricidie ont été réalisées selon les recommandations du NCCLS. Les gênes *gyr*A, *gyr*B, *par*C, *par*E et *mar*R ont été amplifiés par PCR et séquencés. La CPM a été définie comme la plus faible concentration d'antibiotique qui prévient la sélection de mutant résistant à la ciprofloxacine avec un inoculum de 10^{10} UFC/mL.

Résultats. – Les souches d'*E. coli* sans mutation dans les gènes *gyr*A, *gyr*B, *par*C, *par*E et *mar*R présentaient des CMI inférieures ou égales à 0.023 mg/L et des CPM inférieures ou égales à 0,25 mg/L. Les souches avec deux mutations (*gyr*A et *par*C) présentaient des CMI égales à 1,5 mg/L et des CPM égales à 4 mg/L. Les souches avec une mutation présentaient des CMI inférieures ou égales à 0,75 mg/L, mais les CPM variaient de 0,5 à 6 mg/L, dépendant de la CMI de la ciprofloxacine. La cinétique de bactéricidie montrait une activité bactéricide avec 0,25 mg/L de ciprofloxacine en une heure pour les souches sans mutation et une activité bactéricide en quatre heures pour des concentrations de 2 et 4 mg/L de ciprofloxacine pour les souches ayant respectivement, une et deux mutations.

Conclusion. – Afin d'optimiser la posologie de la ciprofloxacine, il est utile de réaliser les CMI de cet antibiotique pour les souches d'*E. coli* résistantes à l'acide nalidixique.

Keywords: Ciprofloxacin; Time-killing curve; Mutant prevention concentration; *E. coli*

Mots clés : Ciprofloxacine ; Cinétique de bactéricidie ; Concentration prévenant la sélection de mutants ; *E. coli*

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1. Introduction

The changing resistance of bacteria to antibiotics is an issue of vital clinical significance. The aim of all antibiotic bactericidal therapy is to achieve clinical cure of patients while avoiding selection of resistant mutant bacteria. For fluoroquinolones (FQ), determination of peak serum levels of the antibiotic in patients and of the MIC of the antibiotic is used to calculate the peak concentration ratio C_{max}/MIC. This pharmacodynamic (PD) parameter has been established for the clinical efficacy of dose-dependent antibiotics [1,2]. The mutant prevention concentration (MPC) is a novel concept evaluating the ability of an antibiotic to minimize or limit the development of resistant organisms [3]. For FQs, in vitro determination of MPC provides a therapeutic tool to help avoid selection of resistant mutants during clinical use and therapeutic failure [4,5]. The aim of our study was to compare MPC of ciprofloxacin and time-killing curve with regard to 11 clinical strains of *E. coli* as a function of three genotypes of resistance to FQ in order to formulate a rational therapeutic strategy suitable for use in routine practice.

2. Materials and method

2.1. Bacterial strains

We studied nine clinical strains of *E. coli* isolated in the bacteriology laboratory of Bellevue hospital in Saint-Étienne, France: eight strains resistant to nalidixic acid and one sensitive according to the antibiogram method (API bioMerieux, France). Reference strains *E. coli* ATCC 25992 (American Type Culture Collection) sensitive to nalidixic acid and IP K12 resistant to nalidixic acid were tested in parallel. Ciprofloxacin was kindly provided by Bayer, France.

2.2. MICs

The MICs of ciprofloxacin were determined by E-test in accordance with the manufacturer's instructions (AB-Biodisk, Sweden). Etest was used with an adjusted inoculum (0.5 McFarland) and with Mueller–Hinton (Becton Dickinson, France) agar plates incubated for 18 h at 37 °C.

2.3. Time-killing curves

Bactericidal activity was evaluated by plotting time-killing curves for the *E. coli* strains as described in the NCCLS guidelines [6]. Sterile ciprofloxacin solution (0.1 mL) was added to 9.9 mL of the broth cultures (10^7 UFC/mL) giving final drug concentrations equivalent to 1 × MIC to 32 × MIC. Antibiotic-free growth controls were also included. Flasks were incubated aerobically at 37 °C with mechanical agitation. Viable cell counts were performed on Mueller–Hinton plates at 0, 1, 2, 4, 6 and 24 h after adding the antimicrobial agent and the results plotted on semi logarithmic graph paper. All tests were performed in triplicate. A bactericidal effect was defined as a 4 $\log_{10}$ decrease CFU/mL.

2.4. PCR amplification and DNA sequencing

The oligonucleotide primers used for PCR amplification and DNA sequencing were described by Lindgren et al. for amplification of the quinolone-resistance-determining region (QRDR) of the *gyr*A, *gyr*B, *par*C and *par*E genes and the entire *mar*R gene [7]. Amplification was performed as described by Lindgren. DNA sequencing was carried out using the dideoxy chain-termination method by CEQ2000 dye terminator cycle sequencing (Beckman Coulter).

2.5. Mutant prevention concentration

MPC was defined as the lowest concentration preventing recovery of colonies when more than 10^{10} cells were tested [3]. For determination, an overnight culture was grown in liquid medium followed by a 10-fold dilution series and 4 h of incubation with shaking at 37 °C. Cells were concentrated by centrifugation. The 10^{10} cells were plated onto different concentrations of ciprofloxacin. Plates were incubated up to 72 h and periodically assayed to determine the concentrations preventing growth of resistant mutants and killing all cells. All procedures were performed in triplicate to ensure reproducibility.

3. Results

No changes were seen in the QRDR of *gyr*B, *par*E or in the *mar*R gene. Screening for mutations in the *gyr*A and *par*C genes revealed three genotypes of *E. coli*: two strains with no mutation (the two strains sensitive to nalidixic acid), six strains with a mutation in *gyr*A (S83L or D87G) and three strains with two mutations, one in *gyr*A (S83L) and one in *par*C (S80I or S80R). Each of these mutations at positions 83 and 87 on *gyr*A and position 80 on *par*C has previously been found in FQ-resistant *E. coli* strains [7]. The MPC values were two to 10 times in greater than the MIC values. There is only a weak correlation between MIC and MPC with a linear regression R^2 value of 0.7. However, for the strains without mutation, MPC was less than 0.5 mg/L, while for strains with one mutation, *gyr*A, MPC ranged from 0.5 to 6 mg/L and for strains with two mutations, *gyr*A and *par*C, MPC was equal to 4 mg/L (Table 1).

Table 1
MIC and MPC of ciprofloxacin on the 11 genotyped *E. coli* strains

Strain number	Mutations		Nalidixic acid	Ciprofloxacin	
	gyrA	*parC*	MIC (mg/L)	MIC (mg/L/)	MPC (mg/L)
1			3	0.012	0.12
2			2	0.023	0.25
3	S83L		> 64	0.094	0.5
4	S83L		> 64	0.125	0.5
5	S83L		> 64	0.25	1
6	S80I		> 64	0.5	4
7	S83L		> 64	0.5	2
8	S83L		> 64	0.75	6
9	D87G	S80I	> 64	1.5	4
10	S83L	S80I	> 64	1.5	4
11	S83L	S80R	> 64	1.5	4