

In vitro activities of new quinolones against *Brucella melitensis* isolated in a tertiary-care hospital in Turkey

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We have evaluated the in vitro activities of seven fluoroquinolones against 69 strains of *Brucella melitensis*. According to their minimum inhibitory concentration for 90% growth (MIC₉₀) values, the most active agent was found to be sparfloxacin (MIC₉₀ 0.12 mg/L) followed by levofloxacin, ciprofloxacin, ofloxacin (MIC₉₀ 0.50 mg/L) and grepafloxacin (MIC₉₀ 1 mg/L), gemifloxacin (MIC₉₀ 2 mg/L) and gatifloxacin (MIC₉₀ 4 mg/L).

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Brucellosis is a frequently encountered zoonosis in various regions of the world, including the Mediterranean basin. It is one of the major health problems in rural Turkey. The pathogen is able to survive and multiply within the phagocytic cells of the host. Intracellular killing is inhibited by mechanisms that are still only partially understood. The most effective treatment is the combination of doxycycline with either streptomycin or rifampin. However, relatively frequent side-effects may restrict the use of these combinations [1]. In addition, long-term usage of oral doxycycline in combination with parenteral administration of streptomycin usually leads to poor patient compliance. An ideal regimen should provide high penetration rates into macrophages plus greater stability and activity inside the acidic environment of phagolysosomes. Fluoroquinolones have been shown to have good intracellular penetration and in vitro activity against *Brucella* spp. However, their reduced activity in acidic pH could pose a problem.

The current study evaluates the in vitro activities of gatifloxacin (Grünenthal, Aachen, Germany), gemifloxacin (Smithkline Beecham, Brentford, UK), grepafloxacin (GlaxoWellcome, Stevenage, UK), levofloxacin (Hoechst Marion Roussel, Istanbul, Turkey), sparfloxacin (Rhone-Poulenc Rorer, Istanbul, Turkey), ciprofloxacin (Bayer, Wuppertal-Elberfeld, Germany), and

ofloxacin (Hoechst Marion Roussel) against 69 *Brucella melitensis* isolates that were collected between 1991 and 1999 from blood and bone-marrow cultures of adult patients with acute brucellosis at Hacettepe University Hospital, Ankara, Turkey. Only one isolate per patient was included. The organisms were identified to species level by conventional methods and on the basis of not requiring CO₂ and not producing H₂S [2]. They were stored at –70 °C and subcultured twice before susceptibility testing and a Class II biological safety cabinet was used. Minimum inhibitory concentrations (MICs) were determined by the agar dilution technique with Mueller–Hinton agar (Merck, Darmstadt, Germany), supplemented with 1% hemoglobin and 1% PoliviteX (bioMérieux, Lyon, France), using an inoculum of 10⁴ colony-forming units (CFU) per spot. The inoculations were made by a multipoint inoculator (Denley, Nivelles, Belgium) and the MIC values were defined as the lowest concentration of the antibiotic that completely inhibited growth.

The MIC₅₀ and MIC₉₀ values of antibiotics for these isolates are shown in Table 1. According to their MIC₉₀ values, the most active quinolone was sparfloxacin, followed by levofloxacin, ciprofloxacin, ofloxacin, grepafloxacin, gemifloxacin and gatifloxacin. Sparfloxacin's activity was four-fold to 32-fold greater than the other fluoroquinolones tested.

Table 1 In vitro activities of fluoroquinolones against *Brucella melitensis* isolates

| Antibiotic | Range (mg/L) | MIC ₅₀ (mg/L) | MIC ₉₀ (mg/L) |
|---------------|--------------|--------------------------|--------------------------|
| Sparfloxacin | 0.03–4 | 0.06 | 0.12 |
| Levofloxacin | 0.03–8 | 0.12 | 0.50 |
| Ciprofloxacin | 0.06–2 | 0.25 | 0.50 |
| Ofloxacin | 0.06–2 | 0.25 | 0.50 |
| Grepafloxacin | 0.015–2 | 0.12 | 1 |
| Gemifloxacin | 0.015–2 | 0.50 | 2 |
| Gatifloxacin | 0.015–8 | 0.50 | 4 |

A limited number of studies have examined the activity of new fluoroquinolones against *Brucella* isolates. Qadri et al. [3] reported that the MIC₉₀ of lomefloxacin (0.5 mg/L) was similar to those of ciprofloxacin, tetracycline and gentamicin. Garcia-Rodriguez et al. [4] found clinafloxacin to be the most active compound (MIC₉₀ 0.06 mg/L) when compared to four other experimental quinolones and ciprofloxacin. Trujillano-Martin et al. [5] tested the in vitro activities of sitafloxacin, ciprofloxacin, ofloxacin, levofloxacin, trovafloxacin, moxifloxacin, grepafloxacin and gatifloxacin against 160 *B. melitensis* strains. They found sitafloxacin to be the most active, with an MIC₉₀ value of 0.12 mg/L. Similar to our results, the MIC₉₀ values of the other tested fluoroquinolones were as follows: levofloxacin (0.5 mg/L), ciprofloxacin, trovafloxacin and moxifloxacin (1 mg/L); ofloxacin, grepafloxacin and gatifloxacin (2 mg/L).

Although the emergence of resistance to antibacterial agents is not a known factor relating to relapses of brucellosis, it has been shown that factors such as pH, inoculum size and medium have significant effects on susceptibility results [6–8]. The activities of study drugs at pH 5.0 were not tested in the present work. However, we have previously shown that both ciprofloxacin and ofloxacin were less active at pH 5.0 against *B. melitensis* [6]. Fluoroquinolone monotherapy was found to be disappointing for the treatment of brucellosis in clinical trials [9,10]. This may well be correlated with their reduced activity at acidic pH in phagolysosomes.

In a previous trial, we reported that ofloxacin in combination with rifampin was as effective as doxycycline plus rifampin in the treatment of brucellosis, despite the fact later shown that less synergy existed between rifampin and ofloxacin

than rifampin and doxycycline at either pH 7.0 or pH 5.0 [1,6]. The discrepancy between in vitro and in vivo results could be explained by the fact that quinolones and rifampin may produce antibacterial activity at different sites in vivo (i.e. rifampin is most active in phagolysosomes with acidic pH and quinolones are highly active in the serum where pH is >7.0), hence eluding the in vitro antagonism [6].

This study provides further support for our belief that the activities of new quinolones against *B. melitensis* are similar to those of older quinolones. Based on these results and those of other trials it seems that the newer quinolones, either alone or in combination with rifampin, should not replace the established regimens for the treatment of brucellosis. However, such combinations may provide an alternative, especially in cases of intolerance to either doxycycline or streptomycin.

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