In vitro Activities of Various Antimicrobials against *Brucella melitensis* Strains in the Aegean Region in Turkey

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Key Words

Agar dilution method · Antibiotic susceptibility · *Brucella melitensis*

Abstract

Objective: To study in vitro activities of three quinolones (ciprofloxacin, levofloxacin, moxifloxacin), four macrolides (erythromycin, dirithromycin, azithromycin, clarithromycin) and doxycycline against 44 clinical isolates of *Brucella melitensis*. **Materials and Methods:** Forty-four *B. melitensis* strains were isolated from blood cultures of adult patients with acute brucellosis who were hospitalized in the clinical ward of the Department of Clinical Microbiology and Infectious Diseases. The minimum inhibitory concentrations (MICs) of the tested antimicrobials were measured by the agar dilution method. MIC₉₀ and MIC₅₀ values were defined as the lowest concentration of the antibiotic at which 90 and 50% of the isolates were inhibited, respectively. **Results:** Doxycycline (MIC₅₀: 0.25 µg/ml, MIC₉₀: 0.50 µg/ml) had the lowest MIC in vitro against the *B. melitensis* strains. Among the quinolones, ciprofloxacin and levofloxacin had similar activities (MIC₅₀: 0.5 µg/ml, MIC₉₀: 2 µg/ml), whereas

MIC of moxifloxacin (MIC₅₀: 1 µg/ml, MIC₉₀: 8 µg/ml) was higher than both antibiotics in this group. Clarithromycin and azithromycin were the most active macrolides (MIC₅₀: 8 µg/ml and MIC₉₀: 32 µg/ml), followed by erythromycin (MIC₅₀: 16 µg/ml, MIC₉₀: 32 µg/ml) and dirithromycin (MIC₅₀: 64 µg/ml and MIC₉₀: 64 µg/ml). **Conclusion:** The results indicate that the conventional agent doxycycline is more active than quinolones and macrolides against the *B. melitensis* in vitro.

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Introduction

Human brucellosis in Turkey is a serious public health problem in countries where *Brucella melitensis* is prevalent in sheep and other domestic animals, and it is one of the most common infectious conditions contracted by man [1]. Brucellosis is a long-term disease with frequent relapses. The major antimicrobials used in the treatment of brucellosis worldwide are doxycycline, rifampicin, streptomycin and trimethoprim-sulfamethoxazole [2]. Recently fluoroquinolones and newer macrolides have been used in combination with these major microbials [3,

4]. Despite having alternative therapies, treatment of brucellosis is still problematic due to factors such as side effects of some antibiotics, poor compliance of patients, and the high relapse rate with some of the combination regimens [5].

The purpose of this study was to compare the in vitro susceptibility of clinical isolates of *B. melitensis* to three quinolones (ciprofloxacin, levofloxacin, moxifloxacin), four macrolides (erythromycin, dirithromycin, azithromycin, clarithromycin) and the conventional agent doxycycline.

Materials and Methods

The study was performed on 44 *B. melitensis* strains, which were isolated from blood cultures of hospitalized adult patients with acute brucellosis between 1998 and 2003. *B. melitensis* strains were identified by conventional methods (gram staining, no CO₂ requirement, production of H₂S, and other biochemical properties). Stocked strains were stored in protect bacterial preservers (Technical Service Consultant Ltd., Lancashire, England) at -80°C and were subcultured twice a week prior to the study.

The antibiotics were provided by the following suppliers in powder form: erythromycin (Sigma, Istanbul, Turkey); dirithromycin (Abdi İbrahim İlaç Sanayi ve Ticaret AŞ, Istanbul, Turkey); azithromycin and clarithromycin (Adilna Sanovel İlaç Sanayii, Istanbul, Turkey); ciprofloxacin and moxifloxacin (Bayer, Istanbul, Turkey); levofloxacin and doxycycline (Fako İlaçları AŞ, Istanbul, Turkey).

The agar dilution method as previously described [6] was used to determine the minimum inhibitory concentrations (MICs) of the antimicrobial agents. Mueller-Hinton agar (Oxoid Ltd., Basingstoke, Hampshire, England) supplemented with 1% hemoglobin (bioMérieux, Lyon, France) and 1% PoliVitex (bioMérieux) was used as the culture medium. The stock solutions of the antibiotics were prepared in 100 ml of sterile distilled water and stored frozen in small volumes, in sterile polypropylene vials at -70°C. Serial twofold dilutions of the antimicrobial agents were prepared in Mueller-Hinton broth supplemented with 1% PoliVitex. The concentration range was 0.125–64 µg/ml for the macrolides, and 0.03–16 µg/ml for the quinolones and doxycycline.

The bacterial inoculum was prepared by suspending smooth *B. melitensis* strains cultivated in Brucella agar (Difco), in brain-heart infusion broth. Following 48 h incubation, the suspensions were adjusted to 0.5 McFarland turbidity to yield a final inoculum of 10⁵–10⁶ cfu/ml. The inoculations were made by a multipoint inoculator. Plates were incubated at 35°C in ambient conditions. The results were evaluated after 48 h. MIC values were defined as the lowest concentration of the antibiotic that completely inhibited growth. MIC₉₀ and MIC₅₀ values were defined as the lowest concentration of the antibiotic at which 90 and 50% of the isolates were inhibited, respectively. All measurements were performed in duplicate. *Escherichia coli* ATCC 25922 and *Staphylococcus aureus* ATCC 29213 and plates of Mueller-Hinton agar without antibiotics were used as controls.

Table 1. Susceptibility of 44 *B. melitensis* isolates to several antibiotics

Antimicrobial agents	MIC, µg/ml ¹		
	range	50%	90%
Ciprofloxacin	0.25–16	0.5	2
Levofloxacin	0.25–16	0.5	2
Moxifloxacin	0.5–16	1	8
Erythromycin	2–64	16	32
Azithromycin	1–32	8	32
Clarithromycin	0.125–32	8	32
Dirithromycin	8–64	64	64
Doxycycline	≤0.03–1	0.25	0.50

¹ MIC₅₀ and MIC₉₀, at which 50 and 90% of the isolates are inhibited, respectively.

Results

The MIC values of the antimicrobial agents studied by the agar dilution method are listed in table 1. Doxycycline had the lowest MIC in vitro against the *B. melitensis* strains. Among the quinolones, ciprofloxacin and levofloxacin had similar activities (MIC₅₀: 0.5 µg/ml; MIC₉₀: 2 µg/ml), whereas the MIC of moxifloxacin (MIC₅₀: 1 µg/ml; MIC₉₀: 8 µg/ml) was higher than that of both antibiotics in this group. Among the macrolides, clarithromycin and azithromycin (MIC₅₀: 8 µg/ml and MIC₉₀: 32 µg/ml) were the most active macrolides, followed by erythromycin (MIC₅₀: 16 µg/ml; MIC₉₀: 32 µg/ml) and dirithromycin (MIC₅₀ and MIC₉₀: 64 µg/ml).

Discussion

When compared with previous studies, the strains examined in this study had higher MIC values for all drugs except doxycycline [7, 8]. This result may be due to the regional differences in the resistance rates of *Brucella* strains in Turkey, where brucellosis is an endemic disease. The considerably high MICs for macrolides are of specific importance. Although it is forbidden by the Ministry of Agriculture, some antibiotics (naracin, avoparcin, erythromycin and other macrolides) are found in animal feed [personal commun., Prof. Figen Kırkpınar, Ege University, Agriculture Faculty, Department of Animal Science]. This may explain the high resistance rates to macrolides among *B. melitensis* in our region. Inappropriate quinolone and macrolide usage is somewhat higher than

that of tetracyclines in Turkey, hence the fact that all the strains were clinical isolates may also contribute to the higher macrolide and quinolone resistance [9].

The combination of doxycycline and rifampin is currently recommended by the World Health Organization for patients with acute brucellosis [10], but the limited number of effective antibiotics, adverse effects, therapeutic failures and the necessity to use antimicrobials for long periods of time in patients with localized brucellosis have led to a search for new drugs to combat the disease. Quinolones are among the leading drugs for future potential therapeutic options. Quinolones are widely used in the treatment of brucellosis in our country because they possess excellent bioavailability after oral intake and achieve high concentrations in tissues, macrophages and leukocytes [11]. However, their reduced activity in acidic pH, the development of resistance in *B. melitensis* during ciprofloxacin therapy and cross-resistance to other fluoroquinolones made them unfavorable in brucellosis therapy in a short time [12, 13].

In the present study, while the MIC₅₀ and MIC₉₀ values for ciprofloxacin and levofloxacin were similar (0.5 and 2 µg/ml, respectively), the MIC₉₀ value for moxifloxacin (8 µg/ml) was twofold higher than those of the other quinolones, and these were significantly higher than previously reported [8, 14]. Martin et al. [14] reported MIC₉₀ 0.06 µg/ml for sitafloxacin against *B. melitensis* strains in vitro.

Although the value of MIC₉₀ for doxycycline (0.5 µg/ml) and ciprofloxacin (2 µg/ml) in this study is higher than (doxycycline: 0.064 µg/ml and ceftriaxone: 0.25 µg/

ml) that reported by Bodur et al. [8], the magnitude of the values is approximately the same (fourfold).

Macrolides are generally perceived as safe and effective antibiotics, with good activity against *Brucella* spp. However, the MIC values of the isolates in this study were higher compared to those previously reported [15–17]. Of the four macrolides tested in this study, dirithromycin was the least active agent (64 µg/ml), thereby supporting our previous in vivo findings in a murine brucellosis model, in which dirithromycin had the lowest cure rate alone or in combination with rifampicin [18].

Doxycycline had the lowest MIC₉₀ value in our study. This finding is consistent with data reported in previous studies [8, 13, 19, 20] involving the cyclines.

Conclusion

The antimicrobial susceptibility of *B. melitensis* isolates in our region showed that the conventional agent doxycycline is more active than quinolones and macrolides. Our findings further indicate that there is an increasing resistance among *B. melitensis* strains against macrolides and quinolones.

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