

Investigation of the in vitro activities of various antibiotics against *Brucella melitensis* strains

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Aim: To assess the antimicrobial susceptibility of *Brucella melitensis* strains in our region. Brucellosis is a common disease in Turkey. Moreover, difficulties are encountered in the treatment process, such as long-lasting therapy, relapses, and side effects of drugs. Hence, novel treatment approaches and the susceptibility of current antibiotics are vital.

Materials and methods: Thirty-four *Brucella* spp. isolated from blood samples of patients with brucellosis were included. Minimal inhibitory concentrations for tetracycline, rifampicin, streptomycin, ciprofloxacin, doxycycline, ceftriaxone, and levofloxacin were detected by broth microdilution.

Results: Tetracycline had the lowest and streptomycin the highest MIC₅₀ and MIC₉₀ values, respectively. A total of 5 strains were intermediate-susceptible and 1 strain was resistant to rifampicin.

Conclusion: Doxycycline and tetracycline seem to be quite effective antibiotics against *Brucella melitensis* strains. Although streptomycin and rifampicin have high MIC levels against *Brucella melitensis* strains in our region, inconsistencies between in vitro susceptibility and in vivo activity should be considered.

Key words: *Brucella*, MIC, susceptibility

Çeşitli antibiyotiklerin *Brucella melitensis* suşlarına karşı in vitro etkinliklerinin araştırılması

Amaç: Bruselloz, Türkiye’de sık görülen bir hastalıktır. Bunun yanında tedavisinde zorluklarla karşılaşmaktadır (örneğin tedavi süresinin uzun olması, relapslar, ilaç yan etkileri). Bu yüzden yeni tedavi seçenekleri ve mevcut antibiyotiklerin duyarlılıkları önemlidir. Bu kapsamda yöremizde *Brucella melitensis* suşlarının antimikrobiyal duyarlılıklarının araştırılmasını amaçladık.

Yöntem ve gereç: Brusellozlu olguların kan kültürlerinden izole edilen toplam 34 *Brucella* spp. izolatu çalışmaya alınmıştır. Tetrasiklin, rifampisin, streptomisin, siprofloksasin, doksisisiklin, seftriakson, levofloksasinin MİK düzeyleri broth mikrodilüsyon yöntemiyle saptanmıştır.

Bulgular: Tetrasiklin en düşük, streptomisin ise en yüksek MİK₅₀ ve MİK₉₀ düzeylerine sahipti. Beş suşun rifampisine orta duyarlı, bir suşun dirençli olduğu saptanmıştır.

Sonuç: Bu sonuçlara göre doksisisiklin ve tetrasiklinin *Brucella melitensis* suşlarına karşı oldukça etkili antibiyotikler oldukları görülmektedir. Yöremizde streptomisin ve rifampisinin *Brucella melitensis* suşlarına karşı yüksek MİK değerleri olsa da in vitro duyarlılıkla in vivo etkinlik arasında farkında olabileceği göz önünde bulundurulmalıdır.

Anahtar sözcükler: *Brucella*, MİK, duyarlılık

Introduction

Brucellosis is a zoonosis caused by gram-negative microorganisms, *Brucella* spp. Human brucellosis remains the most common zoonotic disease worldwide, with more than 500,000

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new cases annually. It is hyperendemic in many Mediterranean countries, including Turkey (1-3). The etiologic agent is usually transmitted through the gastrointestinal tract, excoriations in skin and mucosa, or inhalation. As brucellosis is a multisystem disease, clinical findings might be diverse, leading to intricacy in diagnosis. Therefore, treatment can be delayed and may fail.

Brucella strains are intracellular pathogens that infect host macrophages. Hence, the antibiotics to be used for treatment should penetrate adequately into the cell. Furthermore, a combination of antibiotics should also be used to prevent relapse. Tetracyclines, quinolones, trimethoprim/sulfamethoxazole, rifampicin, and streptomycin are commonly used preparations for this treatment. Unfortunately, despite these combinations, the relapse rate is almost 30% (4). Therefore, new treatment approaches and more data on susceptibility are needed. In our study, we aimed to examine the in vitro susceptibility of certain antibiotics for the treatment of brucellosis.

Materials and methods

Bacteria

The isolates were collected between 1999 and 2005 from patients hospitalized at Süleyman Demirel University's Faculty of Medicine, Department of Infectious Diseases and Clinical Microbiology. In total, 34 isolates were obtained from blood cultures. The BACTEC 9120 automated blood culture system (Becton, Dickinson and Company; Franklin Lakes, NJ, USA) was used for blood cultures. *Brucella* species were identified according to the gram-staining properties of the bacteria, urease positivity, oxidase testing, H₂S production, dye sensitivity (basic

fuchsin and thionine), and CO₂ requirements. The strains were stored at -80 °C in brain heart infusion broth media containing 20% glycerol until studied. The stored isolates were dissolved on the study day and subcultures were made in duplicate. All tests were carried out in a class II biological safety cabinet.

Antibiotics

The tested antibiotics, tetracycline (Sigma®, St. Louis, MO, USA), doxycycline (Fako®, İstanbul, Turkey), rifampicin (Sigma®), streptomycin (Sigma®), levofloxacin (Aventis®, Frankfurt, Germany), ciprofloxacin (Bayer®, İstanbul, Turkey), and ceftriaxone (Sigma®), were prepared in accordance with the recommendations of the Clinical and Laboratory Standards Institute (CLSI) with relevant solvents and diluents (5). Determination of the minimal inhibitory concentration (MIC) was done with 96-well plates containing *Brucella* broth. The serial dilution of all of the antibiotics was done in these wells.

Preparation of inoculum

The prepared bacterial suspensions (adjusted to 0.5 McFarland) were added to each well to achieve a final concentration of 5 × 10⁵ cfu/mL (5 × 10⁴ cfu/well), and the plates were incubated at 35 °C in a 5% CO₂ atmosphere for 48 h. The *Escherichia coli* ATCC 25922 strain was used as the standard strain.

Results

All of the isolates were identified as *Brucella melitensis*. The MIC₅₀ and MIC₉₀ values of the isolates are given in the Table. Tetracycline had the lowest and streptomycin the highest MIC₅₀ and MIC₉₀ values. For rifampicin, 5 of the strains (14.7%) were

Table. Antimicrobial susceptibility of 34 *Brucella melitensis* isolates.

Antibiotics	Range (µg/mL)	MIC ₅₀ (µg/mL)	MIC ₉₀ (µg/mL)
Doxycycline	0.016-0.064	0.032	0.064
Tetracycline	0.008-0.64	0.016	0.032
Rifampicin	0.5-4	1	2
Ciprofloxacin	0.25-1	0.5	1
Levofloxacin	0.25-1	0.5	0.5
Streptomycin	1-8	4	8
Ceftriaxone	0.125-2	0.5	1

intermediate-susceptible, and 1 (2.94%) strain was resistant, according to the interpretive criteria for slow-growing bacteria (*Haemophilus*).

Conclusion

Brucellosis constitutes a major health problem around the world, especially in developing countries. Doxycycline-streptomycin and doxycycline-rifampicin combinations are recommended for treatment of the disease by the World Health Organization (6). Despite appropriate drug selection, there are difficulties because of the long duration of treatment, the necessity of giving some drugs parenterally, and possible relapses. Therefore, novel treatment approaches and susceptibility of present antibiotics need to be adequately defined.

Tetracyclines have been generally used for the treatment of brucellosis. In our study, MIC₅₀ and MIC₉₀ values were 0.032 µg/mL and 0.064 µg/mL for doxycycline, and 0.016 µg/mL and 0.032 µg/mL for tetracycline, respectively. These results would be “susceptible” according to CLSI guidelines. Other data included from Turkey have revealed similarly low MIC values (7-14). Therefore, the tetracycline group of drugs for brucellosis treatment still seems to be relevant. Although we found a lower MIC value for tetracycline compared to doxycycline, the latter is still preferable because it has fewer side effects and a favorable pharmacological profile.

Rifampicin is an antibiotic with good penetration into the cell and increased activity against *Brucella* strains in an acidic pH environment (for example, in the phagolysosomes of macrophages) (15). In 2 recent Turkey-based investigations, intermediate susceptibility against *Brucella* strains was reported at a rate of 9.5%-23% for rifampicin, and no rifampicin resistance was detected (8,9). However, another report by Memish et al. (16) revealed an in vitro resistance rate of 3.5% for this antibiotic. In our study, 5 strains (14.7%) were intermediate-susceptible and 1 strain (2.94%) was resistant, with a similar resistance rate reported by Memish et al. (16). There are no interpretive criteria for rifampicin against *Brucella* strains in the CLSI guide. However, if the MIC values of rifampicin are examined according to the interpretive criteria of slow-growing bacteria,

1 resistant and 5 intermediate-susceptible strains can be found. Reduced susceptibility to rifampicin may be due to 2 factors: the low pH value of the medium and inappropriate use of rifampicin leading to resistance.

Regional antimicrobial susceptibility of the causative agent is an important factor in the treatment of infectious diseases. Rifampicin is important in the treatment of brucellosis, but its resistance rates may pose a problem for our region in the future. However, antibiotic susceptibility tests should be considered where there is recurrent brucellosis (8). Therefore, periodic assessment of the susceptibility of strains to rifampicin in our region should ideally be conducted.

Quinolones penetrate well into leukocytes and macrophages, making them suitable agents for the treatment of intracellular infections (17,18). The MIC values for ciprofloxacin against *Brucella* strains in various studies were between 0.19 and 1 µg/mL (7-12,14,19). In the present study, the MIC₉₀ value for ciprofloxacin was 1 µg/mL, and the MIC₉₀ value of levofloxacin was 0.50 µg/mL, parallel to the findings of other investigations (14,19). Hence, quinolones might be preferred in the treatment of brucellosis due to their low MIC levels against *Brucella* spp. and their good intracellular penetration.

Of the aminoglycoside antibiotics, streptomycin and gentamicin are the preferred preparations for the treatment of brucellosis. Despite the recognized side effects of this group of drugs, they give particularly good results in brucellosis with bone-joint involvement (3). In various studies, the MIC₉₀ values for streptomycin were found to be between 0.5 and 8 µg/mL (7,10,12,14,20). In the present study, the MIC₉₀ value for streptomycin was 8 µg/mL; according to CLSI guidelines, all of the strains were interpreted as “susceptible.”

In the clinical treatment of patients with neurobrucellosis, ceftriaxone is used in combined treatment due to high concentrations in the cerebrospinal fluid (2,3). Therefore, ceftriaxone susceptibility in *Brucella* strains has been examined in several studies. Data from Turkey revealed MIC₉₀ values of 0.38-0.50 µg/mL (7,8,11). In the present study, the MIC₉₀ value of ceftriaxone was 1 µg/mL, and, according to CLSI guidelines, all strains were “susceptible” to ceftriaxone.

In conclusion, susceptibility tests for *Brucella* strains are not routinely carried out due to variable test results, secondary to various factors such as pH and inoculum amount (7,15). In this study, although our data indicated certain problems with rifampicin

and streptomycin in our region, the MIC values of both antibiotics were still high. However, it should be considered that there may be discrepancies between in vitro susceptibility and in vivo activities.

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