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Original Article

Dual versus triple therapy for uncomplicated brucellosis: A retrospective cohort study

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Abstract

Introduction: Brucellosis is a zoonotic disease caused by Brucella spp. affecting multiple body systems and may lead to complications. Saudi Arabia is a country where brucellosis is endemic. This study aimed to describe the epidemiological characteristics of uncomplicated brucellosis and to assess outcomes of different antibiotic regimens.

Methodology: A retrospective cohort study in a Saudi tertiary academic medical center. Adults with confirmed uncomplicated brucellosis between January 2008 and December 2018 who received antibiotics were included. The primary endpoint was clinical cure. Secondary endpoints included all-cause mortality and length of stay.

Results: Fifty-four patients met the inclusion criteria and were included in the study. Twenty five patients received a combination of doxycycline, rifampin, and aminoglycoside (group 1), whereas 29 patients received doxycycline and rifampin (group 2). There was no significant difference between the two groups in clinical cure, all-cause mortality, length of stay, and end of therapy parameters, including temperature, white blood cells count, C-reactive protein levels, and erythrocyte sedimentation rates.

Conclusions: Due to lack of differences in clinical outcomes, mortality, length of stay, and end of therapy parameters between the two groups, a regimen comprising two, rather than three, agents can be sufficient for uncomplicated brucellosis. This finding conforms to previous studies. Therefore, replacing rifampin with an aminoglycoside for its presumed superior efficacy as per the World Health Organization's guidelines is not substantiated by our study. Further studies with a larger sample size are required to confirm these findings.

Key words: brucellosis; *Brucella*; doxycycline; rifampin; aminoglycosides.

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Introduction

Brucellosis, also known as "Malta fever", is a zoonotic infectious disease caused by *Brucella* spp., Gram negative bacilli [1,2]. Ten *Brucella* spp. have been identified; of these only 4 have moderate to significant human pathogenicity and these include *B. melitensis* (transmitted from goats and sheep), *B. abortus* (transmitted from cattle), *B. suis* (transmitted from pigs), and *B. canis* (transmitted from dogs) [3-5]. It is transmitted from the infected animals by direct contact or by indirect methods, such as through the consumption of unpasteurized dairy products (e.g., milk and cheese) or by the inhalation of bacteria aerosolized from the excreta of infected animals [6]. It usually causes a systemic non-localized illness that typically manifests as fever, profuse sweating that has a characteristic wet-hay smell, general malaise, low backache, arthralgia, depression, and bacteremia. The infection may, however, affect specific body organs and cause localized brucellosis that may manifest as septic arthritis, spondylitis, endocarditis, orchitis, meningitis, dementia, and other neurologic symptoms (neurobrucellosis) [4,7]. The diagnosis of brucellosis is confirmed with blood cultures, antibody titer, and/or polymerase chain reaction (PCR) test [2].

Different regimens are currently recommended by the World Health Organization (WHO) for the treatment of brucellosis. The first-line regimen is composed of an aminoglycoside for 7-21 days plus doxycycline for 6 weeks. Alternative regimens include rifampin plus doxycycline for 6 weeks, and a fluoroquinolone or trimethoprim/sulfamethoxazole (TMP/SMX) plus doxycycline or rifampin for 6 weeks [4,8]. Similar regimens are also recommended by Brazilian guidelines [1].

Saudi Arabia, Iran, Palestine, Syria, Jordan, and Oman are Middle East countries with high incidence of brucellosis [9-11]. The annual cases per one million populations is 214.4 in Saudi Arabia [12]. In addition to being one of the most common zoonotic diseases in the world, brucellosis is a major health issue, as it negatively impacts the economy [8,12-14]. A study conducted in Al-Qassim region of Saudi Arabia describing the epidemiological characteristics of 4,283 cases of human brucellosis over five years showed that 51.1% of cases were Saudi citizens, and up to 46.5% were farmers and shepherds. The most common risk factor for the infection was direct contact with livestock animals followed by consumption of unpasteurized dairy products (80.7% and 55.6% respectively) [15].

As brucellosis causes major morbidity and possible mortality to humans if untreated, early diagnosis and management is important [16]. Based on the literature, the available data on treatment regimens for uncomplicated brucellosis are limited. Therefore, the objective of this study was to describe the epidemiology and compare dual- *vs*. triple-agent treatment regimens for uncomplicated brucellosis (defined as confirmed diagnosis of brucellosis without evidence of metastatic complication, such as endocarditis, neurobrucellosis, spondylitis, or orchitis) in our center and evaluate their efficacy.

Methodology

Study design and patients

This was a retrospective cohort study conducted at King Abdulaziz University Hospital, a tertiary-care academic medical center in Jeddah, Saudi Arabia. The study was approved by the Biomedical Research Ethics Unit, Faculty of Medicine, King Abdulaziz University (reference No. 128-18). From January 2008 to December 2018, all patients aged 18 years or older who received treatment for blood culture- and/or serology-confirmed brucellosis were included. Patients with suspected brucellosis who received no treatment in our institution and patients with missing follow up data to determine outcomes were excluded. Included patients were divided on the basis of the most common regimens prescribed.

Laboratory tests

In order to establish microbiological diagnosis for brucellosis, blood samples labelled with "*Brucella*" are collected in BD BACTEC™ blood culture media bottles (Becton, Dickinson and Company, Franklin Lakes, NJ, USA) and incubated in BD BACTEC™ FX system at 37°C for up to 14 days. If the system generates an alarm, the positive sample is subcultured on blood and chocolate agar plates, which are incubated in 5-10% CO_2 for 24-48 hours. Once growth is observed on the plate, the bacteria is identified via the morphology of the colonies, Gram staining, and biochemical testing using urease and oxidase (*Brucella* produce both). This process provides identification to the genus level. According to the microbiology lab protocol, species identification is no longer carried out using Matrix-Assisted Laser Desorption/Ionization-Time Of Flight (MALDI-TOF) mass spectrometry due to biohazard risk. Alternatively, species are identified using serology.

The serological test used in our hospital is the standard tube agglutination test (SAT) which measures total antibodies (IgG and IgM) and provides semi-quantitative results (titers). Two types of febrile antigens are used in the test, one for *B. melitensis* and the other for *B. abortus*. After mixing with serum, the tubes are incubated at 37°C for up to 48 hours. The test is validated using the positive and negative controls provided with the test kit (different commercial tests have been used). SAT has a reported sensitivity of 95.6-100% and a specificity of 96-100% [17,18]. Given the endemic nature of brucellosis in Saudi Arabia, an antibody titer of at least 1:640 of either *Brucella* spp. is needed to confirm the serological diagnosis, especially in the absence of positive *Brucella* culture or classic clinical symptoms suggestive of acute brucellosis.

Data collection

A standardized data collection form was used to collect data including patients' demographics, epidemiological features, clinical manifestations, laboratory and microbiological results (at baseline and end of therapy), antibiotic regimens, medication side effects, response to therapy, complications, and outcomes. Both electronic and paper medical records of patients were used for data collection. For the small fraction of patients who were lost to follow-up, the last available data were included and assessed.

Endpoints

The primary endpoint was clinical cure, defined as resolution of the fever and leukocytosis or leukopenia and normalization of the C-reactive protein levels and erythrocyte sedimentation rates. Conversely, treatment failure was defined as persistence of clinical symptoms despite adequate treatment or relapse within 12 weeks after complete recovery (total follow up duration was 18 weeks considering the 45-day duration of therapy). Secondary endpoints included all-cause mortality

(death due to any cause), length of stay (for admitted patients), and adverse effects of therapy.

Statistical analysis

Continuous variables were described using mean ± standard deviation or median (interquartile range, IQR) for normally and non-normally distributed data, respectively. For continuous variables, means were compared using Student's *t*-test while medians were compared using Mann-Whitney U test. Shapiro-Wilk test for normality was used to determine normal distribution. Categorical data were compared using chi-square test. An *a priori* P value of < 0.05 was considered statistically significant. Multivariable logistic regression was used for further analysis to control for confounding factors. Variables were considered for the regression analysis if P values were < 0.2 in the univariate analysis. Statistical analysis was performed using SPSS version 24.0 software (SPSS, Inc., Chicago, Illinois, USA).

Results

Patients and regimens

After screening for patients who had blood culture and/or serological tests for *Brucella*, 54 patients met the inclusion criteria of confirmed uncomplicated brucellosis and were included in the study. Several antibiotic regimens were used to treat brucellosis. However, the most commonly utilized regimens were doxycycline-rifampin-aminoglycoside (DRA) in 25 patients and doxycycline-rifampin (DR) in 29 patients. Doxycycline was dosed at 100 mg orally every 12 hours whereas rifampin was dosed at 300 mg orally every 8 hours (or 900 mg orally once daily). Both were given for at least 45 days. In the DRA group, the most commonly prescribed aminoglycoside was streptomycin given daily at 1 g intramuscularly (n = 23 of 25). The other two patients in the DRA group received amikacin 7.5 mg/kg intravenously every 12 hours with a target peak of 20-30 µg/mL and a target trough of < 4 µg/mL. Aminoglycosides were

Table 1. Baseline characteristics of uncomplicated brucellosis patients.

Characteristic	Total (n = 54)	DRA group (n = 25)	DR group (n = 29)	P value
Age, years (mean ± SD, range, median)	52 ± 21.4, 21-70, 58.5	49.1 ± 20, 20-82, 51	49.3 ± 19.3, 21-88, 48	0.97
Sex, male, n (%)	29 (53.7)	14 (56)	15 (51.7)	0.75
Race, n (%)				0.64
White	49 (90.7)	23 (92)	26 (89.7)	
Black	1 (1.9)	0 (0)	1 (3.4)	
Asian	4 (7.4)	2 (8)	2 (6.9)	
Location, n (%)				0.05
Outpatient	26 (48.1)	8 (32)	18 (62.1)	
Inpatient medical ward	26 (48.1)	15 (60)	11 (37.9)	
Intensive care unit	2 (3.7)	2 (8)	0 (0)	
Charlson comorbidity index (mean ± SD)	2.5 ± 2.1	1.8 ± 1.9	1.7 ± 1.8	0.83
Temperature, °C (mean ± SD)	37.3 ±1.1	37.4 ± 1.3	37.1 ± 0.9	0.37
White blood cells count, cells/mm^3 (mean ± SD)	5.6 ± 1.7	5.6 ± 2.3	6.8 ± 4.7	0.28
C-reactive protein, mg/L (mean ± SD)	41 ± 47.7	32.1 ± 31.7	24 ± 23.1	0.35
Erythrocyte sedimentation rate, mm/hr (median [IQR])	16.5 [12.1-28]	14.2 [6.5-35.5]	15.5 [12.8-22]	0.68
Risk factors for infection, n (%)				0.85
Consumption of unpasteurized dairy products	25 (46.3)	11 (44)	14 (48.3)	
Direct contact with animals	2 (3.7)	1 (4)	1 (3.4)	
Both	6 (11.1)	2 (8)	4 (13.8)	
Unknown	21 (38.9)	11 (44)	10 (34.5)	
Diagnostic test positivity, n (%)				0.51
Brucella serology alone	22 (40.7)	9 (36	13 (44.8)	
Both *Brucella* serology and culture	32 (59.3)	16 (64)	16 (55.2)	
***Brucella* spp., n (%)**				0.44
B. melitensis	5 (9.3)	3 (12)	2 (6.9)	
B. abortus	1 (1.9)	1 (4)	0 (0)	
Both	48 (88.9)	21 (84)	27 (93.1)	
Presence of coinfection, n (%)	6 (11.1)	2 (8)	4 (13.8)	0.5
Presence of arthralgia, n (%)	21 (38.9)	8 (32)	13 (44.8)	0.34

DRA: doxycycline-rifampin-aminoglycoside; DR: doxycycline-rifampin; IQR: interquartile range.

administered for seven days, whereas doxycycline and rifampin were given for 45 days. Treatment was extended as needed in case of lack of clinical improvement. This was observed in seven patients, five in the DRA group compared with two in the DR group.

Baseline characteristics of the study patients, risk factors for acquiring the infection, laboratory features, species of *Brucella* causing the infection, and diagnostic tests are summarized in Table 1. No difference was observed in baseline characteristics of either group and none of the included patients was pregnant.

Endpoints

No statistically significant difference was observed between the two groups in all outcomes, clinical cure, all-cause mortality, length of stay, and end of therapy parameters including temperature, white blood cells count, C-reactive protein levels, and erythrocyte sedimentation rates (Table 2). While the median duration of therapy was equal in both groups, the IQR in the DRA group was larger resulting in a statistical significance (P = 0.006) probably since more patients in this group were treated as either inpatients (60%) or in the intensive care unit (8%) rather than outpatients compared with 37.9% and 0% in the DR group, respectively (though the difference in patients' distribution was not significant; P = 0.05). Two patients in the DR group, one due to septic shock and pulmonary embolism and the other due to peritonitis that resulted from an infected peritoneal dialysis catheter.

Adverse effects

A total of 17 (31.5%) patients developed side effects related to the medications, 7 in the DRA group and 10 in the DR group. The most common side effects were gastric discomfort, esophagitis, and heartburn due to doxycycline which occurred in 4 patients (7.4%); only one in the DRA group discontinued doxycycline due to severe pain and continued streptomycin instead along with rifampin. On the other hand, elevation of liver transaminases, probably attributed to rifampin, was observed in both groups in 11 patients (20.4%), 5 patients in the DRA group and 6 patients in the DR group. (P = 0.95) Additionally, one patient reported gastrointestinal upset presumed to be due to rifampin. No adverse effects due to aminoglycoside therapy were reported in any patient.

Controlling for confounding factors

The only variable that had a P value < 0.2 was the patient location. Compared with the DRA group and after adjusting for the location of inpatient *vs*. outpatient in multivariable logistic regression analysis, the DR group was not found to be an independent predictor of clinical cure (adjusted OR = 1.85; 95% CI, 0.40–8.53). This model was statistically significant (χ^2, 0.872; df, 2; P = 0.027) and good fit was indicated by a lack of statistical significance in the Hosmer-Lomeshow goodness of fit test (P = 0.365). When other factors were added to the model, the quality of the model was compromised as the goodness of fit was lost (Hosmer-Lomeshow goodness of fit test was statistically significant), thus it wasn't feasible to proceed and include other factors in the model.

Discussion

Our study included 54 patients with confirmed uncomplicated brucellosis. There was no statistically significant difference between male and female gender in our study, likely because most infections were acquired via consumption of raw milk or cheese and not via direct contact with infected animals. Several other studies showed that men were more commonly infected than women likely because of their animal-related occupations [11,15]. The mean age of the patients in our study was 49 ± 20 year which is higher than the mean age reported in another study in Saudi Arabia [19]. This is likely because the practice of drinking raw milk and eating raw dairy products is more common among older

Table 2. Clinical outcomes of uncomplicated brucellosis patients.

Outcome	Total	DRA (n = 25)	DR (n = 29)	P value
Duration of therapy, median, (IQR)	45 (45-60)	45 (45-90)	45 (45-45)	0.006
Clinical cure, n (%)	45 (83.3)	20 (80)	25 (86.2)	0.54
All-cause mortality, n (%)	2 (3.7)	0 (0)	2 (6.9)	0.18
Length of stay, days (mean ± SD)[a]	3 ± 2.9	8.8 ± 6.3	9.7 ± 12	0.8
Temperature, °C (mean ± SD)[b]	36.5 ± 0.4	36.7 ± 0.3	36.6 ± 0.6	0.73
White blood cells count, cells/mm^3 (mean ± SD)[b]	6 ± 1.8	5.6 ± 1.6	7.3 ± 5.4	0.21
C-reactive protein, mg/L, median (IQR)[b]	3.2 (3.2-3.6)	3.2 (3.2-3.7)	3.2 (3.2-7.8)	0.46
Erythrocyte sedimentation rate, mm/hour (mean ± SD)[b]	10.3 ± 9.7	23.7 ± 25.1	10.5 ± 9.1	0.1

EOT: end of therapy; [a] Data for patients admitted to the hospital (i.e., excluding outpatients); [b] Data available for the majority, but not all, of the patients.

individuals [20]. Similar to previous studies, consumption of unpasteurized raw dairy products and direct contact with infected animals were the most common risk factors associated with the disease in our patient population [14,21,22]. Both *B. melitensis and B. abortus* were serologically identified in most of our patients. Previous reports indicated that *B. melitensis* was endemic in Saudi Arabia and Egypt [19,23].

Based on WHO recommendations, tetracyclines (tetracycline or doxycycline) are the backbone antibiotics for the treatment of uncomplicated brucellosis. However, tetracyclines should not be used alone due to their high relapse rates of 10-20%. Therefore, dual or triple therapy is recommended. Additional agents that can be used in combination with tetracyclines include an aminoglycoside (streptomycin or gentamicin) and/or rifampin [4].

The first-line therapy recommended by the WHO for uncomplicated brucellosis is doxycycline-streptomycin [4]. Several studies have compared antibiotic regimens using different combinations to determine the most favorable therapeutic regimens and the optimal duration of therapy with the lowest relapse rate. The results of these studies have been controversial [24-29]. Some studies have shown doxycycline-aminoglycoside combination to be superior to doxycycline-rifampin or doxycycline-trimethoprim-sulfamethoxazole combinations in terms of therapeutic failure and relapse rates [4,30,31]. Comparable studies showed that fever resolved more rapidly with the triple (DRA) than the dual (DR) regimen [32,33]. However, in our study; symptoms resolution and clinical cure were equal among the two groups which is similar to the findings of a study by Ulu-Kilic et al, showing that either the dual or triple therapy had the same clinical outcomes in both complicated and uncomplicated brucellosis [34]. Similarly, a prospective non-randomized study by Mile, *et al.* in patients with uncomplicated brucellosis demonstrated lack of difference in time to defervescence and total therapeutic unresponsiveness (therapeutic failure and relapse) between a dual DR and a triple DRA (gentamicin as the aminoglycoside) therapies (P = 0.58 and 0.097, respectively) [29]. Relapse rates were only numerically lower in the DRA group *vs*. the DR group (4.6% *vs*. 13.8%).

While a meta-analysis by Skalsky, *et al.* showed that the inclusion of an aminoglycoside in a dual or triple therapy would be beneficial, the meta-analysis by Meng, *et al.* recommended replacing rifampin with streptomycin in the case of dual therapy [35,36]. When the two studies that evaluated dual versus triple therapy that were included in these meta-analyses were examined, we found that one was conducted in patients with complicated brucellosis (who would actually need a triple drug regimen with an aminoglycoside) while the other was a study with no difference in the overall failure or relapse rates between the DR and the DRA regimens but only a small significant difference in the symptoms relief by the end of therapy (88.2% *vs*. 96.4%; P = 0.04) [37,38]. A study by Vrioni, *et al.* was similar to the former study where the triple therapy group (streptomycin for two weeks plus doxycycline and rifampin for six weeks) suffered from complicated brucellosis but had significantly lower DNA loads of *Brucella* by the end of therapy than patients treated with a dual regimen comprised of streptomycin for two weeks with doxycycline for six weeks (P = 0.026) [39]. A potential explanation for this difference and the lack of difference in outcomes between the dual and triple therapy groups in our study is that in the study by Vrioni, *et al.* rifampin that was given as the third drug in the triple regimen group is bactericidal and was given for six weeks as in the case of both the DRA and DR groups of our study. In contrast, the dual therapy group in the study by Vrioni, *et al.* received doxycycline alone for the remining four weeks post completion of streptomycin therapy unlike the dual therapy group in our study which had both doxycycline and rifampin for at least six weeks. Overall, the use of triple therapy is recommended by the WHO for complicated brucellosis cases [4]. In our study, this regimen was used in patients with uncomplicated form of the disease probably because numerically more patients in this group were critically ill and were admitted to the intensive care unit (but didn't have complicated brucellosis in other organs) as demonstrated in Table 1 (60% *vs*. 37.9% in the DR group). While this difference was not statistically significant, this may have masked he potential benefit of using triple therapy (or lack of thereof with the dual therapy).

The optimal duration of therapy has been assessed in a randomized clinical trial, in which an 8-week duration of the triple regimen doxycycline-rifampicin-streptomycin did not decrease the rate of relapse in comparison to a 6-week duration of therapy [27]. In our study, different regimens with different durations were prescribed for patients in inpatient or outpatient settings. While median duration of therapy in both the DRA and DR group was equal at 45 days, the DRA group had a longer duration based on the IQR reaching 90 days at the 75th percentile. This can probably be attributed to the numerical high proportion of patients in the DRA group who had a more severe disease.

Nonetheless, this proportion was not significantly different compared with the proportion of critically ill patients in the DR group (Table 1). Given the lack of difference in the outcomes between the two major regimens in our study, longer durations of therapy did not generally add any benefit even though the difference in durations of therapy was statistically significant.

Doxycycline was the most common antibiotic with reported adverse effects, ranging from epigastric discomfort and heartburn to esophagitis. Although some clinicians may prefer prescribing an alternative to doxycycline due to its adverse effects profile, compliance has been shown to improve when patients are counseled regarding appropriate use (to abstain from lying down for 30-60 minutes post dose administration) [40].

Our study has a few limitations. Overall, the retrospective nature of the study can be limiting especially with a relatively small sample size. Thus, a study with a larger sample size, possibly multi-center, may be needed to confirm the findings. Furthermore, some patients were lost during follow-up due to their transfer to other hospitals or not reporting to the outpatient clinic appointments post-discharge. Lastly, the duration of therapy was not accurately captured for a few patients due to some missing notes, as the older hospital system was paper-based.

Conclusion

In conclusion, in view of the lack of differences in clinical outcomes, all-cause mortality, length of stay, and end of therapy parameters between the two groups, a regimen comprised of two (namely doxycycline and rifampin), rather than three agents is sufficient to treat uncomplicated brucellosis.

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