

## Identification the Effect of Some Antibiotics on the Activities of *Brucella melitensis* that Isolated from Patients in Baghdad

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### Abstract:

Brucellosis constitutes a major health problem around the world, especially in developing countries. The sensitivity pattern of the *Brucella* isolates encountered in the most countries, numerous studies were indicated the sensitivity of *Brucella melitensis* to some antibiotics and resistance to another. The aim of this study to evaluated, in vitro, the effect of single and combination of some antibiotics on the activities of *Brucella melitensis*.

This study included collection of (100) clinical sample of blood from different hospital in Baghdad. Six isolates of *Brucella melitensis* were identified and determine according to their biotypes. The susceptibility to different antibiotics was evaluated by disk diffusion method and MICs for some antibiotics used for brucellosis treatment were applied. Some antibiotics combination affect at concentrations of sub MIC on the growth of *Brucella melitensis*.

Results showed that isolates of *Brucella melitensi* were sensitive completely to gentamycin, kanamycin and naldixic acid while it's resistant to lincomycin, rifampicin, ciprofloxacin, Levofloxacin and doxycyline.

The MIC to doxycycline and rifampicin were (40 and 5) µg/ml. respectively. There was an important effect on bacterial growth when the following antibiotics were mixed as (doxycycline+ rifampicin). The doxycycline changed the colonies from growth phase (pathogen phase) to intermediate phase while the rifampicin had no effect on colonies. The mixed of doxycycline with rifampicin have effect that change the pathogen phase to non pathogen phase (no growth phase).

By this study, we can conclude a combination of antibiotics should be used to treat Brucellosis, tetracyclines, quinolones, trimethoprim/sulfamethoxazole, rifampicin, and streptomycin are commonly used preparations for this treatment.

**Key words:** *Brucella*, MIC and Susceptibility.

تحديد تأثير بعض المضادات الحيوية على فعالية البروسيلاتا مالطيا  
المصابين في بغداد  
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تسبب البروسيلاتا مشكلة صحية كبيرة حول العالم وخاصة في الدول النامية. ساسية عزلات البروسيلاتا في قطار و دلت الدراسات على حساسية البروسيلاتا تجاه بعض المضادات الحيوية ومقاومتها للبعض الاخر. تهدف هذه الدراسة الى تقييم فاعلية بعض المضادات الحيوية بشكل مفرد و خليط على فاعلية بكتريا البروسيلاتا داخل

شملت هذا الدراسة (100) عينة سريرية من دم المرضى من مستشفيات مختلفة (6) نواع بكتريا البروسيلاتا وتحديد نماتها الحيوية وهي البروسيلاتا المالطية المعزولة من الانسان وتحديد

حساسيتها لمضادات الحيوية المختلفة من خلال طريقة نشر القرص والتركيز المثبط الأ  
الحمى المالطية  
أظهرت عزلات البروسيلا المالطية حساسة الى جنتاميسين (gentamycin) الكاناميسين (kanamycin)  
نالدكسيك (naldixic acid) بينما كانت مقاومة الى للينكوماميسين (lincomycin) ريفامبين (Rifampicin)  
دوكسيسيلين (Doxycyclin) وسبيروفلوكساسين (ciprofloxacin) واليفوفلوكساسين (Levofloxacin).  
بلغ التركيز المثبط الأدنى لكلا من لدوكسيسيكليين (Doxycyclin) وريفامبين (Rifampicin) (40 and5)  
ميكروغرام/ . كان هناك أثر مهم على نمو البكتيريا عند مزج المضادات الحيوية التالية (دوكسيسيلين +  
ريفامبين) (Doxycyclin+Rifampicin) حيث وجد ان الدوكسيسيكليين (Doxycyclin) غيرت المستعمر  
مرحلة وسيطة فيما لم يؤثر مضاد ريفامبين (Rifampicin) على نمو المستعمرات بينما غيرَ خلط مضادي  
(Doxycyclin+Rifampicin)  
نستنتج من الدراسة الحالية فاعلية خليط المضادات الحيوية (Doxycyclin+Rifampicin) تريد البروسيلا.

## Introduction:

Historically, Brucellosis is an important public health problem, whose occurrence in human is related directly with the prevalence of the infection in animals [1]. *Brucella melitensis* is the main causative agent of brucellosis in both human and small ruminants [2]. The sensitivity pattern of the *Brucella* isolates encountered in the most countries, numerous studies were indicated to sensitivity of *Brucella melitensis* for some antibiotics and resistance to another. Doxycycline has become the most prescribed tetracycline derivative in the treatment of *Brucella* infection because of its superior pharmacokinetic nature [3]. Amongst the tested antimicrobial agents, the MIC breakpoint for both tetracycline and doxycycline was found to be within the susceptible category and among the lowest recorded [4].

Therefore, this drug is still a potent agent for the treatment of brucellosis. Similar findings were noted with its other derivative agent, tetracycline which displayed even a more potent activity compared to doxycycline as a good alternative agent for oral treatment. Value was also much lower than other studies conducted in area endemic of *Brucella melitensis* [5]. Aminoglycosides such as gentamicin, streptomycin, and its derivative were also reported to be effective drugs against brucellosis [6].

However, tetracycline, rifampicin, trimethoprim, gentamicin, and other aminoglycosides, separately or in combinations, are the most commonly used antimicrobial agents for brucellosis treatment. However, reduced susceptibility of the organisms to rifampicin has been reported recently warranting a proper investigation to assess the susceptibility pattern of the local isolates [7, 8]. The use of fluoroquinolone is a monotherapy in brucellosis patients has lead to frequent relapses and failures [9,10], and the combination of a fluoroquinolone with another antibiotic (especially rifampicin) is no more effective than currently available antibiotic [11]. Thus, fluoroquinolones are currently not recommended as a first-line drug to treat brucellosis patients [12].

The gold standard for brucellosis is doxycycline-rifampicin combination the recommended drug combination is widely used in most countries for treatment of *Brucella melitensis* without serious complication [13].

The aim of this study to evaluated, in vitro, the effect of single and combination of some antibiotics on the activities of *Brucella melitensis*.

## Materials and Methods:

### Bacterial isolates:

Total of (100) clinical blood samples were collected during period June 2014 till July 2014 from different hospitals in

Baghdad (medical city hospital, educational laboratories and al kadhimiya hospital). Among the all isolates six isolates of *Brucella melitensis* were identified. The isolates were identified according to colony characteristic, biotyp-ing, Gram-stain and confirmed by the pattern of biochemical profiles [14, 15].

**Antibiotic susceptibility testing:**

The antimicrobial susceptibility was done by using Kirby-Bauer disc diffusion technique on Mueller Hinton agar (Oxoid, England) following Clinical and Laboratory Standards Institute (CLSI) guidelines with commercially available antimicrobial discs. Isolates were tested against the following antimicrobial agents: Gentamycin, Kanamycin, Nalidixic acid, Doxycycline, Tetracycline, Rifampicin, Ciprofloxacin, levofloxacin, Lincomycin and Trimethoim.

**Values of MICs to some antibiotics against *Brucella melitensis*:**

This test used to determine the capability of *Brucella melitensis* resistance

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against some antibiotics by evaluating the MICs to antibiotics according to CLSI [14].

To evaluate MICs:

- \* Doxycyclin wasb belong to tetracycline group which has 40µg /ml value of MICs.
- \*\*Rifampicin has 5µg /ml value of MICs.

**The effect of antibiotics combination for *Brucella melitensis*:**

The MICs of Doxycycline and Rifampicin were determined by using Mueller-Hinton agar with antibiotic concentration (40, 5) µg/ml respectively according to the guidelines recommended by Colle *et al.* [16].

**Result and Discussion:**

Six isolates of *Brucella. Melitensis* were identified and determine according to their biotypes. The percentage of susceptibility patterns of isolates in this study were listed in table-1.

**Table-1: Susceptibility of *Brucella melitensis* isolates to antibiotics**

Antibiotics	<i>Brucella melitensis</i>						Percentage of sensitivity test
	1	2	3	4	5	6	
<b>Gentamycin (oxid)</b>	S	S	S	S	S	S	100%
<b>Kanamycin (oxid)</b>	S	S	S	S	S	S	100%
<b>Nalidixic acid (oxid)</b>	S	S	S	S	S	S	100%
<b>Doxycycline (oxid)</b>	R	S	S	S	S	S	87.5%
<b>Tetracycline (oxid)</b>	R	S	S	S	S	S	87.5%
<b>Rifampicin (oxid)</b>	R	S	S	S	R	R	50%
<b>Trimethoprim (oxid)</b>	R	R	S	R	S	R	37%
<b>Lincomycin (oxid)</b>	R	R	R	R	R	R	100%
<b>Ciprofloxacin (oxid)</b>	R	S	R	R	R	R	14%
<b>Levofloxacin (oxid)</b>	R	R	R	R	R	R	100%

In this study, the percentage of *Brucella melitensis* isolates showed a varied levels of sensitivity to gentamycin, kanamycin and nalidixic acid (100%), Tetracycline and doxycycline (87.5%), Rifampicin (50%), trimethoprim (37%), Ciprofloxacin (14%), while, all isolates of

*Brucella melitensis* were resistant to lincomycine and Levofloxacin (100%) as in table-1. Minimum Inhibitory concentrations (MICs) for doxycycline and rifampicin were determined (40, 5) µg/ml respectively. The result of this study is agree with result obtained by Al-neami, who found that

(100%) of *Brucella melitensis* isolates were sensitive to gentamycin and kanamycine<sup>[17]</sup>.

Current study agree with a study done by N.Ravanel *et al*, who showed that clinical isolates of *Brucella melitensis* were high resistant to ciprofloxacin and Levofloxacin<sup>[18]</sup>. Whereas, the results about sensitivity to tetracycline and trimethoprim were disagree with that presented by Ziyallhan *et al*.<sup>[19]</sup>. In which all isolates were found to be sensitive to Tetracycline (100%) and highly sensitive (73.1%) to Rifampicin, while, it is agree it about all isolates of *Brucella melitensis* were highly resistance (100%) to lincomycine<sup>[19]</sup>.

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Brucellosis constitutes a major health problem around the world, especially in developing countries<sup>[2]</sup>. Doxycyclin was found to be the most effective antibiotic against *Brucella* strains<sup>[3]</sup>.

Despite, it's appropriate drug selection but there are difficulties because of the long duration of treatment (adults with acute infection), combined with either rifampicin or streptomycin for six-week duration<sup>[13]</sup>. This recommendation is still being used till today and the result in this study were confirmed these facts as in table-2.

**Table-2: The effect MICs of antibiotics on growth of *Brucella melitensis* by using single and combination antibiotics (doxycycline, rifampicin).**

MICs (Mg/ml) of antibiotics	Growth of <i>Brucella melitensis</i> isolates
Control	Growth
Doxycyclin(40µg /ml)	Intermediate growth
Rifampicin(5µg /ml)	Growth
Rifampicin(5µg/ml)+Doxycyclin(40µg/ml)	No growth

Table-2 indicates the effect of doxycycline + rifampicin combination improved the results obtained with doxycycline alone but resulted in similar efficacy to rifampicin given alone. This result was agreed to M. J. Grillo', et al<sup>[20]</sup>.

There are three principle components for treatment of brucellosis, adequate intracellular concentration of antibiotics should be achieved, the antibiotics combination should be chosen because of its synergetic effect and thirdly, in vitro susceptibility of antibiotics should be evaluated<sup>[2]</sup>.

A combination of rifampin and doxycycline is the best oral therapy for brucellosis nowadays. In previous studies, rifampin has been presented together with less active antibiotics and existence of intermediate-sensitive strains has been reported<sup>[21]</sup>. Doxycycline + rifampicin combinations are recommended for treatment of

Brucellosis by the World Health Organization<sup>[6]</sup>, as show in table-2. Despite appropriate drug selection, there are difficulties because of the long duration of treatment (adults with acute infection, combined with either rifampicin or streptomycin for six-week duration<sup>[13]</sup>. This recommendation is still being used till today.

**Conclusion:**

By this study, we can conclude that *Brucella* strains are intracellular pathogens that infect host macrophage. 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 tetracycline's, quinolones, trimethoprim/sulfameth-oxazole, rifampicin, and streptomycin are commonly used preparations for this treatment.

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