Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Medicine

journal homepage:www.elsevier.com/locate/apjtm

Document heading doi:

# *In vitro* drug resistance of clinical isolated Brucella against antimicrobial agents

Xiu-Li Xu, Xiao Chen, Pei-Hong Yang, Jia-Yun Liu, Xiao-Ke Hao*

*Laboratory Department, Xijing Hospital, Fourth Military Medical University, Xi-an 710032, China*

ARTICLE INFO

*Article history:*
Received 10 August 2013
Received in revised form 15 September 2013
Accepted 15 October 2013
Available online 20 November 2013

*Keywords:*
Brucella
Antimicrobial agents
Minimal inhibitory concentration

ABSTRACT

**Objective:** To explore the antibiotic resistance of *Brucella melitensis* and instruct rational use of antimicrobial agents in clinical treatment of Brucella infection. **Methods:** Bacteria were cultured and identified by BACTEC9120 and VITEK Ⅱ automicrobic system. E-test was used to detect the minimal inhibitory concentration (MIC) of antimicrobial agents in the drug susceptivity experiment. **Results:** A total of 19 brucella strains (all *Brucella melitensis*) were isolated from 19 patients, who had fever between January 2010 and June 2012, and 17 samples were blood, one was bone marrow, the other sample was cerebrospinal fluid. The MIC range of ceftazidime was 2.0-8.0 mg/L, rifampicin was 0.06-2.0 mg/L, amikacin was 4.0-12.0 mg/L, levofloxacin was 2.0-8.0 mg/L, doxycycline was 8.0-32.0 mg/L, sulfamethoxazole-trimethoprim was 4.0-16.0 mg/L, ampicillin was 1.5-2.0 mg/L and gentamicin was 0.50-0.75 mg/L. **Conclusions:** The drugs used in this experiment cover common drugs for treating Brcella. Meanwhile, the results are consistent with clinical efficacy. It is suggested personalized regimen according to patients' status in treatment of Brucella.

## 1. Introduction

Brucellosis is an anthropo zoonosis which is distributed widely in the world. *Brucella melitensis* (*B. melitensis*), *Brucella bovis* and *Brucella suis* are three main epidemic brucelloses in China, among which *B. melitensis* is the most common[1]. *B. melitensis* can interfere with many organs through the infection by contact with skin mucous membranes, digestive tract and respiratory tract[2]. A total of 19 Brucella strains were separated from patients hospitalized in our hospital from January 2010 to June 2012, including 17 blood samples, 1 bone marrow sample and 1 cerebrospinal fluid sample, and they were all *B. melitensis*. The bacteriostatic actions of antimicrobial agents in common usage were reported in the following.

## 2. Materials and methods

### 2.1. Clinical data

A total of 19 patients admitted between January 2010 and June 2012 had fever in different degree with the body temperature of 37.9-40.9 ℃, averaged at 38.9 ℃. There were 12 cases with typical abortus fever, 2 with remittent fever and 5 with irregular fever. All the cases had joint pain. There were 12 cases with 3 months course, 6 cases with 3-6 months and 1 case with longer than 6 months, and 2 cases were once diagnosed as rheumatic arthritis. Among these 19 patients, 15 had the contact history of cattle and/or sheep, 2 took roast mutton, 1 drank goat's mild and 1 raised pet in

*Corresponding authors: Xiao-Ke Hao, Laboratory Department, Xijing Hospital, Fourth Military Medical University, Xi-an 710032, China.
Tel:+86-029-84775455
E-mail: haoxkg@fmmu.edu.cn
Foundation project: This project was supported by the Scientific Research Foundation of Shaanxi Province, China(Grant No.2009JQ4011).

the house. Brucella was cultured from al 19 patients.

### 2.2. Reagent and instrument

Gentamicin (GEN), ampicillin(AMP), rifampicin(RIF), amikacin (AMK), ceftazidime (CAZ), levofloxacin(LVF), sulfamethoxazole-trimethoprim (SXT), doxycycline (DOX); E-Test paper was purchased from Sweden AB-Biodisk Company. M-H agar was purchased from Beijing Aoboxing Bio-tech Co., LTD.

Bact/Alert 3D automatic blood culture system and VITEK Ⅱ automatic Bacteria analyzer were purchased from the French BioMérieux Company.

### 2.3. Methods

#### 2.3.1. Culture and identification of bacteria

A total of 16-20 mL blood was extracted from patients and injected into the blood culture aerobic bottle and anaerobic bottle specialized for Bact/Alert 3D instrument. When the instrument indicated positive, the positive bottle was taken out for film preparation, Gram's staining and subcultivation in blood agar plate, chocolate plate and MacConkey plate. Then the plates were cultured in the 5% $CO_2$ environment. The acquired pure bacteria were identified by VITEK Ⅱ automatic Bacteria analyzer.

#### 2.3.2. Susceptibility test

The minimal inhibitory concentration (MIC) of strain was detected by E-Test method. The bacteria solution in 0.5 McFarland units was evenly spread in the M-H blood agar plate. After 5 min, E-Test paper was attached on it. After 10 min at room temperature, the plate was then cultured in the environment containing 5% $CO_2$ at 35 ℃ for 24 h. The rate of bacteriostasis was determined according to the CLSI M100-22 standard (MIC explanation for bacteria not belonging to *Enterobacteriaceae*).

## 3. Results

### 3.1. Growth characteristics and identification results of bacteria

The mean alarm time of the 19 blood cultures was 52.7 h with a minimum of 39.3 h and a maximum of 87.9 h. After subcultivation in blood plate, chocolate plate and MacConkey plate and cultured for 48 h at 35 ℃, the round, protuberant, ivory-white, moist and anhemolytic bacterial colonies with 1-2 mm in size can be found in blood plate and chocolate plate, and no colony can be found in the MacConkey plate. Gram staining was Gram-negative small ball bacili, weak and distributed like silver sand. Brucella diagnosis indicated the positive serum. The results were positive in the tests of oxidase and catalase and tolerance tests of sulfur and fuchsine. The identification result of VITEK Ⅱ automatic bacteria analyzer was *B. melitensis*.

### 3.2. MIC

The MIC range of CAZ was 2.0-8.0 mg/L, RIF was 0.06-2.0 mg/L, AMK was 4.0-12.0 mg/L, LVF was 2.0-8.0 mg/L, DOX was 8.0-32.0 mg/L, SXT was 4.0-16.0 mg/L, AMP was 1.5-2.0 mg/L and GEN was 0.50-0.75 mg/L (Table 1, 2).

**Table 1**

MICs of 19 *B. melitensis* strains against 8 antimicrobial agents (mg/L).

| No | GEN | AMP | RIF | AMK | CAZ | LVF | SXT | DOX |
|---|---|---|---|---|---|---|---|---|
| 1 | 0.50 | 2.0 | 0.06 | 12.0 | 2.0 | 2.0 | 16.0 | 32.0 |
| 2 | 0.75 | 2.0 | 0.06 | 12.0 | 8.0 | 8.0 | 4.0 | 16.0 |
| 3 | 0.75 | 1.5 | 0.06 | 4.0 | 4.0 | 2.0 | 8.0 | 16.0 |
| 4 | 0.50 | 2.0 | 2.00 | 8.0 | 2.0 | 2.0 | 8.0 | 8.0 |
| 5 | 0.75 | 1.5 | 0.75 | 8.0 | 4.0 | 8.0 | 8.0 | 8.0 |
| 6 | 0.50 | 2.0 | 0.75 | 8.0 | 2.0 | 2.0 | 4.0 | 32.0 |
| 7 | 0.50 | 2.0 | 0.06 | 4.0 | 8.0 | 2.0 | 4.0 | 16.0 |
| 8 | 0.50 | 1.5 | 2.00 | 8.0 | 2.0 | 2.0 | 8.0 | 16.0 |
| 9 | 0.50 | 1.5 | 0.75 | 4.0 | 8.0 | 4.0 | 16.0 | 8.0 |
| 10 | 0.75 | 2.0 | 0.75 | 8.0 | 2.0 | 4.0 | 4.0 | 32.0 |
| 11 | 0.75 | 1.5 | 0.06 | 12.0 | 4.0 | 4.0 | 4.0 | 16.0 |
| 12 | 0.75 | 1.5 | 0.06 | 4.0 | 4.0 | 8.0 | 8.0 | 16.0 |
| 13 | 0.75 | 1.5 | 2.00 | 4.0 | 2.0 | 2.0 | 16.0 | 8.0 |
| 14 | 0.75 | 1.5 | 0.06 | 4.0 | 4.0 | 8.0 | 4.0 | 32.0 |
| 15 | 0.75 | 2.0 | 2.00 | 4.0 | 2.0 | 4.0 | 4.0 | 16.0 |
| 16 | 0.50 | 2.0 | 0.06 | 4.0 | 8.0 | 2.0 | 8.0 | 8.0 |
| 17 | 0.50 | 2.0 | 2.00 | 8.0 | 4.0 | 8.0 | 16.0 | 8.0 |
| 18 | 0.75 | 2.0 | 0.06 | 4.0 | 2.0 | 8.0 | 4.0 | 8.0 |
| 19 | 0.75 | 2.0 | 2.00 | 8.0 | 4.0 | 2.0 | 8.0 | 8.0 |

**Table 2**

Results of MIC range, $MIC_{50}$ and $MIC_{90}$ (mg/L).

| Antimicrobial agent | MIC range | $MIC_{50}$ | $MIC_{90}$ | Bacteriostatic rate (%) |
|---|---|---|---|---|
| GEN | 0.50-0.75 | 0.75 | 0.75 | 100.00 |
| AMP | 1.50-2.00 | 2.00 | 2.00 | 100.00 |
| RIF | 0.06-2.00 | 0.75 | 2.00 | -- |
| AMK | 4.00-12.00 | 8.00 | 12.00 | 100.00 |
| CAZ | 2.00-8.00 | 4.00 | 8.00 | 100.00 |
| LVF | 2.00-8.00 | 4.00 | 8.00 | 68.42 |
| SXT | 4.00-16.00 | 8.00 | 16.00 | 0.00 |
| DOS | 8.00-32.00 | 16.00 | 32.00 | 42.11 |

## 4. Discussion

Brucella is a Gram-negative *Bacillus pumilis* which can cause infectious abortion of female animal, and cattle, sheep

and swine are most susceptible to Brucella. Brucella is a zoonotic pathogen[3]. The infected animals continuously excrete Brucella through secretion and excretion substance (milk, sperm, vaginal secretion, dung and urine), especially the numerous pathogenic bacteria excreted with aborted fetus, afterbirth and amniotic fluid. Brucella becomes the most dangerous communicable disease[4]. Since Brucella was firstly formally reported by Boone in Chongqing in 1905, Brucellosis has been found in 170 of the more than 200 countries and regions in all continents[5-9]. The epidemic situation of Brucellosis in human and animals shows the ascending tendency in domestic and abroad[10,11]. The development of Brucellosis is very complex. Brucellosis is very complex and hard to be cured for the following reasons. Firstly, Brucellosis is associated with bacteremia, toxemia and allergy. Secondly, Brucella invades many organs. Thirdly, antibacterials and antibody can hardly enter the cell[12-14].

According to the course of disease, Brucellosis can be divided into acute stage (within 6 months) and chronic phase (more than 6 months)[15]. Acute brucellosis would cause the systemic proliferation of reticuloendothelial cells accompanied by sepsis and the damage on nervous, circulating and genital system, especially on bone joint system. The current treatment of Brucellosis in acute stage generally adopts the internationally recommended oral administration of Tetracycline and intramuscular injection of streptomycin[16]. However, the chronic phase is characterized by proliferation of tissue cells and formation of granuloma. Fibrosclerosis of granulation tissue in some patients is the basis of having sequela[17].

In this experiment, LVF, GEN, RIF and CAZ belong to bactericidal agents. LVF is a kind of quinolone drugs whose mechanism is inhibiting the gyrase of DNA to make the DNA unable to form superhelix which can result in irreversible damage on chromatosome, inhibiting the cell division of bacteria and producing rapid bactericidal effect. Belonging to rifomycins, RIF can block the synthesis of bacteria RNA and protein, having obvious killing effect on the bacteria inside and outside the cell[18]. CAZ has a high effect on bacteria in idophase and mainly acts on the bacterial cell wall, but it has no influence on the already synthesized cell wall in the quiescent condition[19]. From the results of this study, the MIC of RIF was in low level with good bactericidal activity, which is consistent with the report[16]. RIF has long been regarded as the first choice in treating Brucella infection, and its clinical therapy is generally satisfactory. But some patients cannot use RIF for a long time because of the obvious gastrointestinal reaction. In this case, other alternative medicines or combined use of other drugs should be adopted to reduce patients' discomfortableness. The MIC values of LVF and CAZ were in medium level, but the bacteriostatic rate of CAZ was better. There is a report on the successful treatment of brucellosis by LVF and CAZ[20], and therefore, they can be the second choice. AMK and GEN belong to aminoglycoside antibiotics. They have high bactericidal activity to Br.melitensis like AMP, but there are only a few case reports on treatment of Brucella infection by GEN or AMP[21,22]. The MICs of DOX to 19 strains were all above 8mg/L with the highest of 32 mg/L, which was consistent with what reported several years ago[23]. The bacteriostatic rate of SXT was low, and it can not be selected as the therapeutic medicine from the antibacterial activity.

Among the 19 Br.melitensis patients observed in this study, 15 hospitalized patients received the therapy of oral administration of RIF and intramuscular injection of streptomycin (course of treatment: 4 weeks) after diagnosed as Brucella infection. After medication for 2 weeks, the patients got better in different degrees. The other 4 cases are out-patients who cannot be traced and followed-up, and the treatment and prognosis remain unknown. The authors believe that Brucella infection would invade many systems of body; each patient would show different clinical symptoms and physical signs according to different physical quality and immune competence; the tolerance of drug is also different from each other. Therefore, the therapeutic regimen of RIF combined with streptomycin or DOX is far from enough. Through this experiment, it can be clearly seen that AMP and aminoglycosides drugs are also good choices. For the early rehabilitation of patients in clinic, drugs should be flexibly chosen according to the individual difference and results of susceptibility test.

## Conflict of interest statement

We declare that we have no conflict of interest.

## References

[1] Kasymbekov J, Imanseitov J, Ballif M, et al.Molecular epidemiology and antibiotic susceptibility of livestock *Brucella melitensis* isolates from Naryn Oblast, Kyrgyzstan. *PLoS Negl Trop Dis* 2013; **7**(2): e2047.

[2] Tian ZM, Zhao W, Cui YP, ZuLuBiYa. Detection and analysis of 16 suspected brucella. *Chin J Nosocomiol* 2012; **3**(12): 15-16.

[3] Kim JY, Her M, Kang SI, et al. Epidemiologic relatedness between

*Brucella abortus* isolates from livestock and wildlife in South Korea. *J Wildl Dis* 2013; **49**(2): 451-454.

[4] Cai YF, Zhong Q, Yi RG, Zhang XY, Wang KC, Huang BX, et al. Isolation and identification of Brucella in two dairies and comparison of four serological tests. *Chin J Zoonoses* 2009; (5): 456-459.

[5] Havas KA, Ramishvili M, Navdarashvili A, Imnadze P, Salman M. The human-animal interface of domestic livestock management and production and its relationship to brucellosis in the country of Georgia 2010: a rapid assessment analysis. *Prev Vet Med* 2012; **105**(1-2): 10-16.

[6] Abo-Shehada MN, Abu-Halaweh M. Risk factors for human brucellosis in northern Jordan. *East Mediterr Health* 2013; **19**(2): 135-140.

[7] Eini P, Keramat F, Hasanzadehhoseinabadi M. Epidemiologic,clinical and laboratory findings of patients with brucellosis in Hamadan,west of iran. *J Res Health Sci* 2012; **12**(2): 105-108.

[8] Liu J, Fu CT, Chen XH. Clinical analysis of 229 cases of brucellosis. *Zhejiang Da Xue Xue Bao Yi Xue Ban* 2012; **41**(6): 677-680.

[9] Sun MY. Analysis of the clinical features of 38 brucellosis cases. *Chin J Difficult Complicated Cases* 2011; **10**(2): 145-146.

[10]Zhao YL, Wang DL, Jiang SL. The monitoring report of brucellosis of the year 2005/2006 in China. *Chin J Control Endemic Dis* 2008; **23**(1): 38-40.

[11]Andriopoulos P, Tsironi M, Deftereos S, Aessopos A, Assimakopoulos G. Acute Brucellosis: presentation, diagnosis and treatment of 144 cases. *Int J Infect Dis* 2007; **11**(1): 52-57.

[12]Ko J, Splitter GA. Molecular host-pathogen interaction in brucellosis: current understanding and future approaches to vaccine development for mice and humans. *Clin Microbiol Rev* 2003; **16**(1): 65-78.

[13]Oliveira SC, de Oliveira FS, Macedo GC, de Almeida LA, Carvalho NB. Recent advances in understanding immunity against Brucellosis: application for vaccine development. *Open Vet Sci J* 2010; **4**: 102-108.

[14]Chen HZ, Lin G. *Practice of internal medicine*. The 13th edition. Beijing: People's Medical Publishing House; 2009, p. 545-548.

[15]Turkulov V, Madle-Samardzija N, Canak G, Gavrancic C, Vukadinov J, Doder R. Various clinical manifestations of brucellosis infenction. *Med Pregl* 2008; **61**(9-10): 517-520.

[16]Jian SL, Wang DL, Li TF, Zhao YL, Zhang ZG, Wang JQ, et al. Study of treatment method for acute brucellosis. *Chin J Control Endemic Dis* 2008; **23**(6): 401-403.

[17]Zou Y, Feng ML, Wang F, Wang L, Li XL, Zheng XY, et al. Current situation of brucellosis treatment. *Chin General Pract* 2012; **15**(7B): 2332-2335.

[18]Gattringer KB, Suchomel M, Eder M, Lassnigg AM, Graninger W, Presterl E. Time-dependent effects of rifampicin on staphylococcal biofilms. *Int J Artif Organs* 2010; **33**(9): 621-626.

[19]Eumkeb G,Sakdarat S, Siriwong S. Reversing β-lactam antibiotic resistance of *Staphylococcus aureus* with galangin from *Alpinia officinarum* Hance and synergism with ceftazidime. *Phytomedicine* 2010; **18**(1): 40-45.

[20]Hashemi SH, Gachkar L, Keramat F, Mamani M, Hajilooi M, Janbakhsh A, et al. Comparison of doxycycline-streptomycin,doxycycline-rifampin,and ofloxacinrifampin in the treatment of brucellosis: a randomized clinical trial. *Int J Infect Dis* 2012; **16**(4): e247-e251.

[21]Skalsky K, Yahav D, Bishara J, Pitlik S, Leibovici L, Paul M. Treatment of human brucellosis: systematic review and meta-analysis of randomized controlled trials. *BMJ* 2008; **336**: 701-704.

[22]Solera J, Espinosa A, Martínez-Alfaro E, Sánchez L, Geijo P, Navarro E, et al. Treatment of human brucellosis with doxycycline and gentamicin. *Antimicrob Agents Chemother* 1997; **41**(1): 80-84.

[23]Xu XL, Zhang JF, Zhang L, Fan X, Sun YQ. Study of routine antimicrobial susceptibility of clinical isolated *Brucella melitenesis*. *Chin J Inf Control* 2006; **5**(1): 54-55.