Journal of Antibiotics Research
Volume 3 | Issue 1
ISSN: 2574-5980

Research Article Open Access

In Vitro Antimicrobial Susceptibility of *Brucella* Species Isolated from Human and Animals in India

Doimari S[1], Singh V[1], Kumari R[1], Kumar MS[2] and Singh DK[*1]

[1]Division of Veterinary Public Health, ICAR-Indian Veterinary Research Institute, Izatnagar, Uttar Pradesh, India
[2]Division of Nutrition, Feed & Products Technology, ICAR-Central Institute for Research on Goats, Mathura, Uttar Pradesh, India

[*]**Corresponding author:** Singh DK, Division of Veterinary Public Health, ICAR-Indian Veterinary Research Institute, Izatnagar 243 122 (UP), India, E-mail: dks@ivri.res.in; dks_bly@yahoo.com

Citation: Doimari S, Singh V, Kumari R, Kumar MS, Singh DK (2018) *In Vitro* Antimicrobial Susceptibility of *Brucella* Species Isolated from Human and Animals in India. J Antibioti Res 3(1): 102

Received Date: July 10, 2019 **Accepted Date:** August 24, 2019 **Published Date:** August 27, 2019

Abstract

Background: Brucellosis is a zoonotic disease that affects a wide range of animals, including domestic livestock, and human. It is considered one of the most common bacterial zoonoses in the world. Endemic in many parts of India little is known about the antibiotic susceptibility or resistance of *Brucella* isolates from India

Objective: The present study was aimed to find out the antibiotic susceptibility and resistance pattern, if any, of *Brucella* isolates of man and animals from different parts of India

Materials and Methods: A total of 45 *Brucella* isolates from man and animals (cattle, buffalo, sheep and goat) consisting of *B. abortus* (29) and *B. melitensis* (16) were tested for minimum inhibitory concentration (MIC) values for azithromycin, ciprofloxacin, co-trimoxazole, rifampicin, ofloxacin, streptomycin and tetracycline using HiComb strips (HiMedia) on Muller-Hinton agar (MHA; BD, BBL) supplemented with equine serum (5%)

Results: All the isolates were found sensitive to tetracycline, ciprofloxacin, ofloxacin, streptomycin and azithromycin. Of all (45) isolates, 15 (33.33%) and 20 (44.44%) were resistant to rifampicin and co-trimoxazole, respectively. Among 29 *B. abortus*, 10 (34.48%) were resistant to rifampicin while 14 (48.27%) were resistant to co-trimoxazole. Intermediate sensitivity to co-trimoxazole 4 (13.79%) *B. abortus* was also found. Among the 16 *B. melitensis* isolates, 5 (31.25%) and 6 (37.50%) were resistant to rifampicin and co-trimoxazole, respectively, while 6 (37.50%) isolates showed intermediate resistance to co-trimoxazole

Conclusion: The resistance to rifampicin and co-trimoxazole is matter of concern, which calls for judicious use of antibiotics and necessity for antibiotic sensitivity tests for *Brucella* isolates in endemic region

Keywords: *Brucella*; Brucellosis; Treatment; Antibiotic resistance; MIC

Introduction

Brucellosis in man and animals remains one of the most important veterinary, public health and economic issue across the world. Caused by the genus *Brucella* – an intracellular-extracellular Gram's negative bacteria, over half a million new cases of human brucellosis are reported every year [1]. Besides, the disease is one of the most common laboratory acquired infections [2]. Majority of the cases are reported in developing countries where brucellosis continues to be endemic [3]. Of the 12 recognized species of *Brucella, B. melitensis, B. abortus* and *B. suis* are implicated in majority of the cases with *B. melitensis* taking the major share [4].

A multisystemic disease in man, brucellosis presents with protean clinical manifestations following direct or indirect transmission with a long incubation period [5]. It is endemic in many parts of India though the exact incidence or prevalence is not known [6]. Nevertheless, human brucellosis cases are regularly reported, which may primarily be because of a) prevalence of brucellosis in livestock, b) close contact of people (especially the rural population) with animals and c) consumption of raw milk, which has been acerbated by incremental increase in intensive animal husbandry practices and trade resulting in increased animal movement.

Brucella being intracellular pathogen, treatment of human brucellosis cases requires antibiotics that can enter the cells and be active inside the intracellular milieu of the cells. WHO has recommended tetracycline (doxycycline) with rifampicin and tetracycline with streptomycin as the treatment of choice for brucellosis. In certain cases, especially the young ones, co-trimoxazole in combination

with rifampicin is recommended. Besides these, other drugs used are fluoroquinolones and gentamycin. Brucellae are generally not prone to antibiotics resistance and same treatment regimens are given in cases of relapse [7]. However, recent time has seen some reports describing development of resistance to some of the antibiotics of choice [8,9].

WHO recommended use of doxycycline along with rifampicin and streptomycin to treat brucellosis in man [10]. Since then, it is in vogue even today as brucellae are generally considered as susceptible to these antibiotics. But of late, reports of antibiotic resistance has been reported from various parts of world [9]. Little literature is available on this aspect. It has been suggested that development of antibiotic resistance is less likely to be observed in case of *Brucella* notwithstanding the fact that each regimen recommended is fraught with one or other drawbacks. However, no standard antimicrobial susceptibility assay protocol is available. Many methods have been used for *Brucella* resistance/susceptibility assessment, with no significant variation in results, with E-test protocol reported as reliable, reproducible, low labor and time-intensive besides lowered chance of infection to laboratory personals [11,12].

Only scanty reports on the antibiotic susceptibility or resistance are available with respect to *Brucella* isolates of India. The present study was aimed to find out the antibiotic susceptibility and resistance pattern, if any, of *Brucella* isolates of man and animals from different parts of India.

Material and methods

A total of 45 *Brucella* isolates from man and animals available in the *Brucella* Laboratory, Division of Veterinary Public Health, ICAR-Indian Veterinary Research Institute were included in this study. The laboratory does routinely receive from different laboratories suspected *Brucella* isolates for confirmation. The isolates were randomly selected and included in the present study to generate preliminary data on the susceptibility/resistance pattern of *Brucella* isolates circulating in India. Of these, 29 were identified as *B. abortus* while 16 were *B. melitensis* isolated from man and animals. There were 10 *B. melitensis* isolated from man (Karnataka: 8; Maharastra: 1; Tamil Nadu: 1) while only one *B. abortus* (Punjab) was isolated from man. Similarly, 6 *B. melitensis* (Punjab: 3; Himanchal Pradesh: 3) and 28 *B. abortus* (Jammu & Kashmir: 2; Punjab: 9; Assam: 13; Meghalaya: 3; West Bengal: 1) from different species of livestock, *viz.* cattle (22), buffalo (6), sheep (3) and goat (3) were isolated. These isolates were identified based on bacteriological methods and PCR using *bcsp31* gene [13,14]. The bacteriological examination included colony morphology, Gram's staining, CO_2 requirement, production of nitrate, urease, catalase, oxidase, agglutination with *Brucella* positive serum and sensitivity to dyes (basic fuchsin and thionin). Isolates were maintained at 4°C on glycerine dextrose agar (GDA) slants by monthly serial passage.

In Vitro Susceptibility to Antibiotics

Antibiotics commonly indicated/used for treatment of brucellosis in man were selected for the study. The minimum inhibitory concentration (MIC) values for azithromycin, ciprofloxacin, co-trimoxazole, rifampicin, ofloxacin, streptomycin and tetracycline were determined by HiComb strips procured form HiMedia on Muller-Hinton agar (MHA; BD, BBL) supplemented with equine serum (5%). *Brucella* culture was grown on trypticase soy agar (TSA; BD, BBL) containing equine serum (5%) for 48h at 37°C with or without CO_2 (5-10%) as per requirement of the isolate. The growth was suspended in normal saline solution (NSS) or phosphate buffered saline (PBS 0.01M; pH 6.8) to match with 0.5 McFarland opacity tube for lawning the MHA plates. The plates were lawned with culture suspension within 20 min of its preparation and left for 15-20 min for drying. Each antibiotic strip was individually placed onto the inoculated MHA plates and incubated for 48h at 37°C with or without CO_2 (5-10%) as required. The MIC was interpreted as values showing the inhibition zones intercepting the scale on the HiComb strip. MIC value interpretation for all antibiotics was based on breakpoints for slow growing bacteria (*Haemophilus* spp.) as per the Clinical and Laboratory Standards Institute (CLSI) guidelines since break points for *Brucella* have not been established [15]. Appropriate controls were included in the study.

Results

The results of the MIC (Figure 1) estimation were recorded as per the literature supplied by the manufacturer in accordance to CLSI guidelines for slow growing fastidious bacteria and the results have been presented in (Table 1) [16]. All the isolates of *B. abortus* and *B. melitensis* were sensitive to tetracycline, ciprofloxacin, ofloxacin, streptomycin and azithromycin. These isolates were also considered sensitive to doxycycline on the basis of their sensitivity to tetracycline [16]. Resistance to rifampicin and co-trimoxazole was observed where 15 and 20 were resistant to rifampicin and co-trimoxazole, respectively. Of the 29 *B. abortus*, 10 were resistant to rifampicin while 14 were resistant to co-trimoxazole. *B. abortus* (4) were also found to have intermediate sensitivity to co-trimoxazole. Among the 16 *B. melitensis* isolates, 5 and 6 were resistant to rifampicin and co-trimoxazole, respectively, with 6 isolates showing intermediate resistance to co-trimoxazole. The isolates of *Brucella* were from different parts of the country and resistance to rifampicin and co-trimoxazole was observed among isolates from all regions and across the species from which they were isolated.

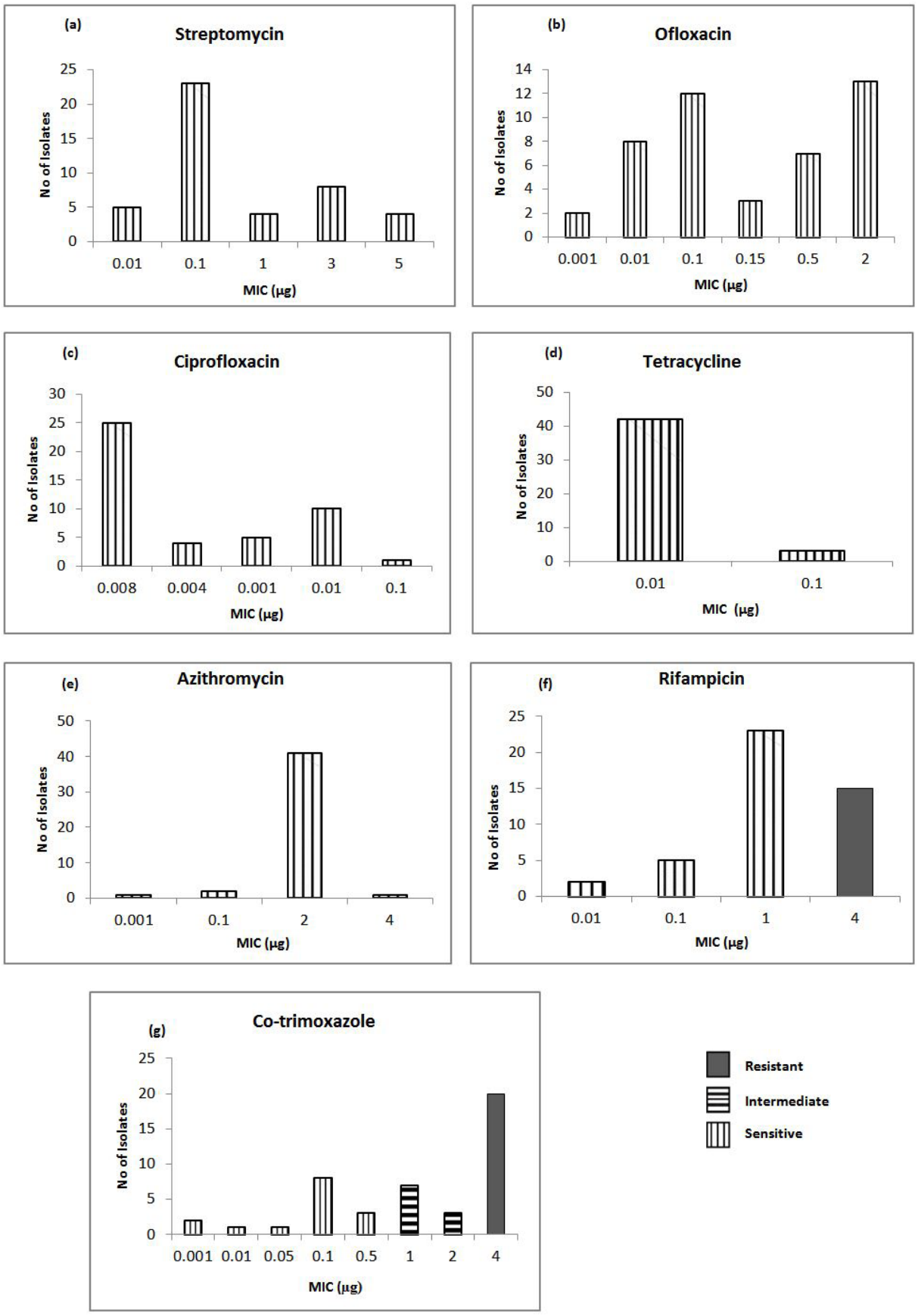

Figure 1: Distribution of MIC (µg) values of different antibiotics for *Brucella* isolates

Antibiotics	MICs range (µg/ml)	CLSI Breakpoints (µg/ml)[a]			MIC_{50} (µg/ml)		MIC_{90} (µg/ml)		*B. melitensis* (n=16)			*B. abortus* (n=29)		
		S	I	R	Value	No	Value	No	S	I	R	S	I	R
Rifampicin	0.01-4	≤ 1	2	≥4	1	30	NA	-	11 (68.75%)	-	5 (31.25%)	19 (65.51%)	-	10 (34.48%)
Azithromycin	0.001-4	≤ 4	-	-	2	44	2	44	16	-	-	29	-	-
Tetracycline	0.01-0.1	≤ 2	4	≥8	0.01	42	0.01	42	16	-	-	29	-	-
Ciprofloxacin	0.008-0.1	≤ 1	-	-	0.008	25	0.01	44	16	-	-	29	-	-
Co-trimoxazole	0.001-4	≤ 0.5	1-2	≥ 4	NA	-	NA	-	4 (25.0%)	6 (37.5%)	6 (37.5%)	11 (37.93%)	4 (13.79%)	14 (48.27%)
Ofloxacin	0.001-2	≤ 2	-	-	0.1	22	2	45	16	-	-	29	-	-
Streptomycin	0.01-5	≤ 8	-	-	0.1	29	3	41	16	-	-	29	-	-

S: Sensitive, I: Intermediate sensitive, R: Resistant, [a] for slow growing bacteria
Sensitivity to tetracycline indicates sensitivity to doxycycline [16]

Table 1: Ranges of MICs, MIC_{50} and MIC_{90} and antibiotic resistance in *Brucella* (n=45) isolates

Discussion

India is an agrarian country with >70% of human population living in rural areas in very close association with livestock. Socio-cultural and economic situations prevalent allow for frequent contact of man with animals providing ample opportunities for contracting zoonotic infections like brucellosis. It is in this context that the present study was undertaken to assess the antibiotic sensitivity/resistance profile of *Brucella* isolates where isolates from human as well as animals were used from different parts of the country.

Brucellosis is one of the most common zoonosis exhibiting strong correlation between human and animal brucellosis, endemicity of livestock brucellosis highly influences the incidence and prevalence of disease in human population [5]. Infection by *Brucella* in man is treatable provided care is taken to choose a combination of drugs with intracellular penetration to attain brucellacidal concentration inside the cell since it is an intracellular pathogen. Human brucellosis treatment is accomplished with tetracycline, rifampicin, doxycycline, streptomycin since long and has also been recommended by WHO [17]. However, neither treatment for animal is recommended nor antibiotic sensitivity for *Brucella* isolates is performed as a routine - the later primarily because antibiotic resistance has not been widely reported for *Brucella* [11].

In the present study, all *B. abortus* and *B. melitensis* isolates were found to be sensitive to tetracycline, ciprofloxacin, ofloxacin, azithromycin and streptomycin (Table 1). Similar observations have been reported earlier by various workers from different parts of world [11,12,15,18]. Susceptibility to tetracycline indicates susceptibility to doxycycline [16]. Tetracycline and its derivatives has long been the drug of choice to treat cases of human brucellosis [9,17]. Doxycycline among these have become one of the most commonly prescribed drugs as treatment of brucellosis cases owing to its better/efficient pharmacokinetic properties [19]. A lower MIC for tetracycline has been reported although high MIC values have also been observed [9,20]. Such variations could be because of geographical origin of the *Brucella* isolates as have also been suggested by others [9,15]. This susceptibility of *Brucella* isolates to tetracycline/doxycycline augurs good as these are the most commonly prescribed drugs for treatment of brucellosis in man and, therefore, can be continued to be prescribed with no concern whatsoever.

Among other drugs of choice to treat brucellosis, co-trimoxazole and rifampicin were found to exhibit high degree of resistance indicating a reason for concern. These drugs have also been reported to show resistance by other workers in different countries [15,21]. It is distressing to note such high resistance among *Brucella* isolates from man as well as animals representing both *B. abortus* and *B. melitensis* in the present study. Speculations regarding high resistance to rifampicin has been made in the occurrence of tuberculosis (TB) especially the multidrug resistant (MDR) in the area with reports of high degree of rifampicin resistance among *Brucella* isolates in areas with high prevalence of MDR-TB [21-23]. It would be interesting to analyze the situation in India in light of the present findings and reported high prevalence of MDR-TB in India with forecast to show increasing trend in future [24-26]. Alongside rifampicin, *Brucella* isolates were also found to have high resistance to co-trimoxazole - another oral anti-*Brucella* drug of choice particularly in cases of relapse and child brucellosis. Relapse and treatment failure has been reported in cases of brucellosis treated with conventional regimen [27]. Such high resistance has also been reported by various researchers from regions endemic for brucellosis [28,29]. Ilhan *et al*. reported a very high resistance of 46.3% to this drug [5]. The increased resistance of *Brucella* isolates from animals to two choice drugs/ antibiotic indicated for treatment of human brucellosis has been observed in the present study. It is to be noted that these two antibiotics are not in routine use in livestock sector, at least in this country. This is interesting and difficult to explain how *Brucella* isolates of animals could develop resistance to rifampicin and co-trimoxazole when these are not used to treat livestock.

These evidences raise concern for the non-testing of *Brucella* isolates for antibiotic sensitivity based on the past evidences and experiences of susceptibility for the antibiotics recommended for treatment of brucellosis by WHO [17]. These evidences indicate for a rational use of antibiotics as well as antibiotic sensitivity testing of *Brucella* isolates on regional basis. Apart from this, it is

also becoming imperative to develop and standardize a uniform protocol for assessing sensitivity/resistance of *Brucella* against the antibiotics of choice. Recently, augmentation of treatment effectiveness to brucellosis has been reported [27]. The veracity of such studies needs to be established through extensive randomized trials in endemic zones for brucellosis. Such studies would open a novel treatment regimen with better curative efficacy and hopefully lowered treatment failure as well as relapse cases.

This is a preliminary study, which runs with certain limitations the most important being the small number of isolates especially from human source used. Nevertheless, it indicates to the presence of rifampicin/co-trimoxazole resistance among ≃ 50% isolates circulating among human as well as animals. It would have been an interesting study to look into the molecular aspects of the resistant isolates especially the rifampicin resistance. It is interesting to observe that all the isolates resistant to rifampicin were of smooth morphology while this antibiotic has been reported to induce alteration of the pathogen besides making them rough through disruption of genes involved in lipopolysaccharide (LPS) synthesis [29,30].

Conclusion

Brucellosis is endemic in many parts of world including India. Treatment for human brucellosis requires combination drug selection for 6 weeks or longer. *Brucella* isolates of man and animal origin from different parts of India showed high resistance to rifampicin and co-trimoxazole, which indicate periodic testing for antibiotic sensitivity particularly in endemic areas.

Acknowledgments

Authors extend thanks to the Head, Division of Veterinary public Health and the Director, ICAR-Indian Veterinary Research Institute, Izatnagar, India for providing facilities to conduct the study.

References

1. World Health Organization (1997) Fact sheet N173, Geneva: World Health Organization.

2. Bouza E, Sanchez-Carrillo C, Hernangomez S, Gonzalez MJ (2005) Spanish Co-operative Group for the Study of Laboratory-acquired Brucellosis (2005) Laboratory-acquired brucellosis: a Spanish national survey. J Hosp Infect 61: 80-3.

3. Pappas G, Papadimitriou P, Akritidis N, Christou L, Tsianos EV (2006) The new global map of human brucellosis. Lancet Infect Dis 6: 91-9.

4. Galinska EM, Zagorski J (2013) Brucellosis in humans-etiology, diagnostics, clinical forms. Ann Agric Environ Med 20: 233-38.

5. Ilhan Z, Solmaz H, Ekin IH (2013) *In vitro* antimicrobial susceptibility of *Brucella melitensis* isolates from sheep in an area endemic for human brucellosis in Turkey. J Vet Med Sci 75: 1035-40.

6. Boral R, Singh M, Singh DK (2009) Status and strategies for control of brucellosis: A review. Indian J Anim Sci 79: 1191-9.

7. Ariza J, Bosilkovski M, Cascio A, Colmenero JD, Corbel MJ, et al. (2007) Perspectives for the treatment of brucellosis in the 21st century: the Ioannina recommendations. PLoS Med 4: 1872-78.

8. Motamedi H, Darabpour E, Gholipour M, Nejad SM (2010) *In vitro* assay for the anti-*Brucella* activity of medicinal plants against tetracycline-resistant *Brucella melitensis*. J Zhejiang Univ Sci B 11: 506-11.

9. Bayram Y, Korkoca H, Aypak C, Parlak M, Cikman A, et al. (2011) Antimicrobial susceptibilities of *Brucella* isolates from various clinical specimens. Int J Med Sci 8: 198-02.

10. FAO/WHO Expert Committee on Brucellosis (1986) Sixth Report. WHO Technical Report Series No. 740.

11. Hashim R, Ahmad N, Zahidi M, Tay BY, Mohd Noor A, et al. (2014) Identification and *in vitro* antimicrobial susceptibility of *Brucella* species isolated from human brucellosis. Int J Microbiol 2014: doi: 10.1155/2014/596245.

12. Marianelli C, Graziani C, Santangelo C, Xibilia MT, Imbriani A, et al. (2007) Molecular epidemiological and antibiotic susceptibility characterization of *Brucella* isolates from humans in Sicily, Italy. J Clin Microbiol 45: 2923-8.

13. Alton GG, Jones LM, Pietz DE (1975) Laboratory Techniques in Brucellosis (2nd edn), World Health Organization. Monograph Series No 55.

14. Baily GG, Krahn JB, Drasar BS, Stoker NG (1992) Detection of *Brucella melitensis* and *Brucella abortus* by DNA amplification. Trop Med Int Health 95: 271-5.

15. Abdel-Maksoud M, House B, Wasfy M, Abdel-Rahman B, Pimentel G, et al. (2014) *In vitro* antibiotic susceptibility testing of *Brucella* isolates from Egypt between 1999 and 2007 and evidence of probable rifampin resistance. Ann Clin Microbiol Antimicrob 11: 24.

16. Clinical Laboratory Standards Institute (2014) Laboratory Testing for the Lupus Anticoagulant; Approved Guideline. CLSI document H60-A. Wayne, PA: Clinical and Laboratory Standards Institute.

17. Corbel M J (2006) Treatment of brucellosis in human. In: Brucellosis in Humans and Animals (M.J. Corbel, S.S. Elberg and O Cosivi, eds).World Health Organization, Geneva, Switzerland.

18. Gur D, Kocagoz S, Akova M, Unal S (1999) Comparison of E test to microdilution for determining *in vitro* activities of antibiotics against *Brucella melitensis*. Antimicrob Agents Chemother 43: 2337.

19. Madkour MM (2001) Treatment. In: Madkour's Brucellosis. Springer, Berlin, Heidelberg.

20. Lopez-Merino A, Contreras-Rodriguez A, Migranas-Ortiz R, Orrantia-Gradin R, Hernandez-Oliva GM, et al. (2004) Susceptibility of Mexican *Brucella* isolates to moxifloxacin, ciprofloxacin and other antimicrobials used in the treatment of human brucellosis. Scand J Infect Dis 36: 636-8.

21. Shevtsov A, Syzdykov M, Kuznetsov A, Shustov A, Shevtsova E, et al. (2017) Antimicrobial susceptibility of *Brucella melitensis* in Kazakhstan. Antimicrob Resist Infect Control 6: 130.

22. Pauletti RB, Stynen AP, da Silva Mol JP, Dorneles EM, Alves TM, et al. (2015) Reduced susceptibility to Rifampicin and resistance to multiple antimicrobial agents among *Brucella abortus* isolates from cattle in Brazil. PloS ONE 10: e0132532.

23. Denk A, Demirdag K, Kalkan A, Ozden M, Cetinkaya B, et al. (2015) *In vitro* activity of *Brucella melitensis* isolates to various antimicrobials in Turkey. J Infect Dis(Lond) 47: 364-9.

24. Fojo AT, Dowdy DW (2017) Multidrug-resistant tuberculosis in India: looking back, thinking ahead. Lancet Public Health 2: e8-9.

25. Prasad R, Singh A, Balasubramanian V, Gupta N (2017) Extensively drug-resistant tuberculosis in India: Current evidence on diagnosis & management. Indian J Med Res 145: 271-93.

26. Sharma A, Hill A, Kurbatova E, van der Walt M, Kvasnovsky C, et al. (2017) Estimating the future burden of multidrug-resistant and extensively drug-resistant tuberculosis in India, the Philippines, Russia, and South Africa: a mathematical modelling study. Lancet Infect Dis 17: 707-15.

27. Majzoobi MM, Hashemi SH, Mamani M, Keramat F, Poorolajal J, et al. (2018) Effect of hydroxychloroquine on treatment and recurrence of acute brucellosis: a single-blind, randomized clinical trial. Int J Antimicrob Agents 51: 365-9.

28. Baykam N, Esener H, Ergonul O, Eren S, Celikbas AK, et al. (2004) *In vitro* antimicrobial susceptibility of *Brucella* species. Int J Antimicrob Agents 23: 405-7.

29. Marianelli C, Ciuchini F, Tarantino M, Pasquali P, Adone R (2004) Genetic bases of the rifampin resistance phenotype in *Brucella* spp. J Clin Microbiol 42: 5439-43.

30. Adone R, Ciuchini F, Marianelli C, Tarantino M, Pistoia C, et al. (2005) Protective properties of rifampin-resistant rough mutants of *Brucella melitensis*. Infect Immun 73: 4198-204.