

# Antibiotic Susceptibility of *Brucella abortus* Isolated from Milk and Blood Samples of Cattle

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**Abstract:** The bacterial species belongs to genus *Brucella* produce infections in humans, farm and wild animals are known as Brucellosis. In normal routine there is rational misuse of drugs especially broad spectrum poses a great concern for the treatment of Brucellosis in cattle. Therefore, this study was designed to evaluate the efficacy of locally available various antibiotics against the *Brucella abortus* isolated from Lohani and Holstein cattle breeds. Various antibiotics such as kanamycin, chloramphenicol, rifampicin, gentamycin, enrofloxacin, tetracycline, streptomycin, tobramycin and penicillin were tested against the isolated *Brucella abortus*. *Brucella abortus* was found highly sensitive to gentamicin, tobramycin and penicillin G with sensitivity percentage 75, 100 and 100%, respectively. The antibiogram results revealed gentamycin and tobramycin were highly effective antibiotics against the *Brucella abortus*. The organism was moderately sensitive against tetracycline, chloramphenicol, rifampicin and enrofloxacin with sensitivity recorded of 65, 60, 60 and 90%, respectively. However, the organism was weakly sensitive against streptomycin and kanamycin 50 and 40%, respectively. The organism confirmed its resistance against the ampicillin. Overall, gentamycin and tobramycin were shown the highest antibiogram activity against the isolated *Brucella abortus* from the bovine milk and blood samples. While, the isolated organism was not sensitive to ampicillin.

**Keywords:** Bovine, *Brucella abortus*, antibiotics, resistance.

## INTRODUCTION

The bacterial species belongs to genus *Brucella* produce infections in humans, farm and wild animals. The *Brucella* genus classified based on the host species and comprised of six species including *B. abortus*, *B. melitensis*, *B. canis*, *B. suis*, *B. ovis* and *B. neotomea* [1]. *Brucella abortus* is a Gram-negative bacteria produce infection is called Brucellosis. *Brucella abortus* is non-spore forming, aerobic and non-motile coccobacilli or rod shape [2]. Generally, the organism is transmitted through contaminated fetal, vaginal fluids, placenta, fetus laceration or abrasion of mucus membrane of genital organs from infected animal. The organism may occur in semen, feces and milk of diseased animals. The infected animal is reservoir of the bacterial species shed and transfers the infection to other healthy animals. Genus *Brucella* produce disease is known as Brucellosis. Clinically infections are manifested by full term parturition or abortion in cattle [3].

In normal routine there is rational misuse of drugs especially broad spectrum poses a great concern for the treatment of infectious diseases. However, various antibiotics have been effective to *Brucella* species. In routine treatment, the use of rifampin, tetracycline, trimethoprim/sulfamethoxazole, deoxycycline, quinolones, streptomycin and ceftriaxone were shown good therapy results. Guidelines of World Health Organization recommended combination of deoxycycline along with streptomycin or rifampin is suitable combination for the treatment of *Brucella* infections [4, 5]. Various antibiotics individually or in combination were shown efficacy against the *Brucella abortus* infections [6]. *In vitro* bovine cell culture, the growth of the organism was inhibited when penicillin synergistically acts with tetracycline and streptomycin [7]. Development of resistance to different drugs is an emerging problem for *Brucella* infections in dairy animals. Therefore, this study was designed to evaluate the efficacy of various antibiotics against the *Brucella abortus* isolated from Lohani and Holstein cattle breeds.

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## MATERIAL AND METHODS

### Sample Collection, Culture and Biochemical Characterization

A total of four hundred samples which included blood (n = 200) and milk (n = 200) were obtained from local Lohani and Holiest Frisian breeds of cattle of District Loralai, Baluchistan, Pakistan. The blood samples (n = 200) were obtained from jugular vein with disposable sterilized syringes. The sample site of the animal was cleaned using spirit. The collected samples were taken to the Disease Investigation Livestock & Dairy Development Department Baluchistan, for further investigation of *Brucella* species in the milk and blood samples. Initially, the samples were screened for *Brucella abortus* using Milk Ring Test, Serum Agglutination Test and Rose Bengal Plate Test. *Brucella abortus* was further identified and biochemically characterized using cultural, staining and biochemical characteristics. Biochemical tests e.g. coagulase, oxidase, indole production, triple sugar iron, triple sugar iron, catalase, Voges-Proskauer, methyl red, Simmon citrate were carried-out using standard procedures as prescribed by Abro *et al.* (2009); Khalil and Gabbar (1992) [8, 9].

### Antibiotic Sensitivity of *Brucella abortus*

Antibiotic sensitivity of the provide insight for the choice of antibiotic for the treatment of *Brucella abortus* infection. The antibiogram of the isolated organism was performed as reported by Bauer *et al.* (1996) [10]. The different antibiotics such as gentamycin, tetracycline, streptomycin, tobramycin and pencillin were against the isolated organism.

Muller Hinton agar's surface was dried and incubated at 37 °C - 15-20 minutes. Some of pure colonies from culture were suspended evenly in sterile normal saline solution in order to distribute barium chloride standard for the antibiogram. A sterile cotton swab dipped into suspended and culture was smeared on the surface of Muller Hinton agar in such a way that all agar surfaces was covered evenly with the bacterial suspension, and incubated at 37°C for 15-20 minutes. The common commercially available antibiotic disc were applied on the surface of Muller Hinton agar with the help of disc dispenser and slightly pressed in order to fix at particular position on agar surface. The plates were covered in plastic bag and incubated for 24 hours at 37°C. The inhibition zones were measured for the clear zone from the bacterial colonies of surface of

agar. The clear inhibition zone formed against the *Brucella abortus*. The efficacies of antibiotics were observed in millimeters from the zone to center of disc. The antibiogram of the sensitive zone was recorded as high, moderate, weak and resistant depending on the antibiotic applied and size of zone created. The symbols were kept for evaluating the drug efficacy.

No clear zone around discs = Not sensitive (-)

Apparent zone in 1-2mm = Weak sensitive (+)

Apparent zone in 2-5mm diameter around discs = Moderate sensitive (++)

Apparent zone in 5-10mm diameter around discs = Quite sensitive (+++)

Apparent zone in 10-15mm diameter around discs = High sensitive (++++)

## RESULTS AND DISCUSSION

The intracellular localization of *Brucella abortus* in the mono-nuclear phagocytic cells tends to resist the transportation of antibiotics from cell membrane. Therefore, *Brucella* species hampers the efficiency of various antibiotics, due to their prolong development of resistance [11]. In this study, the *Brucella abortus* was found highly sensitive to gentamicin, tobramycin and pencillin G with sensitivity percentage 75, 100 and 100%, respectively. The antibiogram results revealed gentamycin and tobramycin were highly effective antibiotics against the *Brucella abortus* (Table 1). Liposome-containing gentamycin has been proved to effective against the *Brucella abortus* infected bovine cells [12]. Similarly, the certain strains of *Brucella* species were sensitive to pencillin G [13]. While, thiazole derivatives; pencillin and gentamycin were observe less effective against the organism [14]. The organism was moderately sensitive against tetracycline, chloramphenicol, rifampicin and enrofloxacin with sensitivity recorded of 65, 60, 60 and 90%, respectively. However, the organism was weakly sensitive against streptomycin and kanamycin 50 and 40%, respectively. The organism confirmed its resistance against the ampicillin. The findings of current study are in accordance with previous observations reported by [15]. However, they described that rifampicin was weakly sensitive to *Brucella abortus*. It had been reported that chlorotetracycline is effective to *Brucella abortus* for the short period [16].

**Table 1: Antibiotic Sensitivity against the *Brucella abortus* Isolated from Lohani and Holiest Frisian Breeds**

Inhibition disc used	Sensitivity %	Indication of sensitivity	Degree of sensitivity
Gentamicin	72.74	++++	Highly sensitive
Tetracycline	63.13	+++	Moderately sensitive
Streptomycine	32.45	++	Week sensitive
Kanamycin	31.37	++	Week sensitive
Chloramphenicol	59.34	+++	Moderate sensitive
Tobramicin	96.79	++++	Highly sensitive
Rifapmicin	64.65	++++	Moderate sensitive
Enroflaxcin	57.3	+++	Moderate sensitive
Ampicillin	0	0	Not sensitive

During the investigations, chloramphenicol was observed moderate effective to the isolated *Brucella abortus* from milk and blood samples. Although, chloramphenicol had been found quite effective in control of *Brucella* infections [17]. Overall, gentamycin and tobramicin were shown the highest antibiogram activity against the isolated *Brucella abortus*. While, in this study the organism did not exhibit sensitivity against ampicillin. Interestingly, it has been reported recombination and deletion in the plasmid and chromosome was associated to development of resistance to ampicillin and kenamycin [18].

## CONCLUSION

In conclusion, gentamycin and tobramicin demonstrated the highest antibacterial activity against the *Brucella abortus* isolated from the bovine milk and blood samples whereas, the isolated organism was not sensitive to ampicillin.

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