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. 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 gentamycin, 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. neotomae* [1]. *Brucella abortus* is a Gram-negative bacteria produce infection is called Brucellosis. *Brucella abortus* is non-spore farming, 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 specie 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].

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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 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, rifapmicin 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 rifapmicin was weakly sensitive to Brucella abortus. It had been reported that chlorotetracycline is effective to Brucella abortus for the short period [16].
During the investigations, chloramphenicol was observed moderate effective to the isolated \textit{Brucella abortus} from milk and blood samples. Although, chloramphenicol had been found quite effective in control of \textit{Brucella} infections [17]. Overall, gentamycin and tobramycin were shown the highest antibiogram activity against the isolated \textit{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 kanamycin [18].

**CONCLUSION**

In conclusion, gentamycin and tobramycin demonstrated the highest antibacterial activity against the \textit{Brucella abortus} isolated from the bovine milk and blood samples whereas, the isolated organism was not sensitive to ampicillin.

**REFERENCES**


### Table 1: Antibiotic Sensitivity against the \textit{Brucella abortus} Isolated from Lohani and Holiest Frisian Breeds

<table>
<thead>
<tr>
<th>Inhibition disc used</th>
<th>Sensitivity %</th>
<th>Indication of sensitivity</th>
<th>Degree of sensitivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gentamicin</td>
<td>72.74</td>
<td>+++</td>
<td>Highly sensitive</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>63.13</td>
<td>+++</td>
<td>Moderately sensitive</td>
</tr>
<tr>
<td>Streptomycin</td>
<td>32.45</td>
<td>++</td>
<td>Week sensitive</td>
</tr>
<tr>
<td>Kanamicin</td>
<td>31.37</td>
<td>++</td>
<td>Week sensitive</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>59.34</td>
<td>+++</td>
<td>Moderate sensitive</td>
</tr>
<tr>
<td>Tobramycin</td>
<td>96.79</td>
<td>+++</td>
<td>Highly sensitive</td>
</tr>
<tr>
<td>Rifampin</td>
<td>64.65</td>
<td>+++</td>
<td>Moderate sensitive</td>
</tr>
<tr>
<td>Enrofloxacin</td>
<td>57.3</td>
<td>+++</td>
<td>Moderate sensitive</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>0</td>
<td>0</td>
<td>Not sensitive</td>
</tr>
</tbody>
</table>

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**CONCLUSION**

In conclusion, gentamycin and tobramycin demonstrated the highest antibacterial activity against the \textit{Brucella abortus} isolated from the bovine milk and blood samples whereas, the isolated organism was not sensitive to ampicillin.
Antibiotic Susceptibility of Brucella abortus Isolated from Milk


Received on 15-06-2016
Accepted on 06-10-2016
Published on 03-02-2017

https://doi.org/10.6000/1927-5129.2017.13.02

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