Investigation of Antibiotic Resistance *Brucella* spp. Isolated from Aborted Animal Fetuses

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**Abstract**

Antibiotic resistance is a global threat to human and animal health especially when it related with a zoonotic disease like brucellosis which treated with the same bunch of antibiotic for long decades. This issue raised a question of whether *Brucella* spp. have gain resistance to the commonly used antibiotics. To study *Brucella* spp. antibiotic susceptibility, samples from (20) aborted fetuses (12 sheep and 8 buffalo) were used. It submitted to PCR and culture methods for detection and microbial isolation and (7) kinds of antibiotic for the susceptibility test. Fifteen *Brucella* culture was isolated (9) from sheep fetuses and (6) from buffalo. The biotyping of the isolates revealed that they were Br. melitensis and Br. abortus respectively. The overall agreement between PCR and culture reached (95%) with the superiority of PCR over microbiological culture. Susceptibility tests indicate that (tetracycline, ciprofloxacin and gentamicin) still efficient while rifampicin and cephalexin show moderate results at last comes neomycin and erythromycin which were not efficient.

In conclusion, *Brucella* spp. did not gain resistance trait to the main antibiotic used in it treatment.

**Keywords:** Brucellosis, animal fetuses, antibiotic resistance

1. Introduction

Since 1978, the World Health Organization (WHO) expressed its concern about the worldwide antibiotic resistance associated with the growing and frequently indiscriminate use of antibiotic in man and animals (WHO, 1978). From that time and forward, growing discoveries were made of many drug-resistance microorganisms, including bacteria, viruses, fungi, and parasites. (Pasmatzi *et al*., 2012; Hussain *et al*., 2022; Oluranti *et al*., 2023 and Bloland, 2001).

Brucellosis on the other hand constitutes a major health and economic problem in many parts of the world, including our country (Iraq), where the disease is endemic (Albayatti *et al*., 2004). It caused by *Brucella* spp. which is primarily contagious disease of domestic animals. But it is also zoonotic, which means that it is transmittable to humans where it shows a high degree of morbidity according to WHO, (1997). To treat human brucellosis, antimicrobial regimens containing quinolones, doxycycline, rifampcin, streptomycin and aminoglycosides are being used alone or in combination (Ariza *et al*., 2007 and Khan *et al*., 2019). While the most common antibiotics used to treat animal brucellosis are tetracycline, oxy-tetracycline, gentamicin and doxycycline. Recently, several sporadic cases of relapse, therapeutic failure, and antibiotic resistance of animal and human isolates have been reported (Wareth *et al*., 2022). This finding is alarming as WHO recommended treatment regimes obviously failed in many cases, and ten thousands of new human cases are to be expected annually posing remarkable political and socio-economic impacts on
Middle East and North African countries where the disease is endemic (Wareth et al., 2022).

As Iraq is one of the Middle East countries where the disease is endemic, a need for regular evaluation and update for the current treatment regimes is necessary, and to investigate if any local Brucella isolates has ever acquired resistance to any of the antibiotic that commonly used to treat it. Thus the aim of this study is to isolate and genetically identified local Brucella isolates obtained from aborted sheep fetuses, then to see if they had acquired any antibiotic resistance.

2. Material and Methods

Twenty aborted fetuses (12 sheep and 8 buffalos) collected from flocks infected with contagious abortion in over than 20% of their pregnant females were used. Those fetuses collected from different villages around Baghdad. Samples from its stomach contents, liver, lung, and spleen were cultured in duplication each, one in normal air incubation and the other in 5-10% Co₂ add atmosphere as described by Alton et al. (1988).

- Identification of Brucella organisms: The positive culture with typical Brucella colonies morphological characteristics were submitted to Gram stain and biochemical tests to further emphasize the genus and biotype (Alton et al., 1988).

- Molecular identification of Brucella spp.: Stomach contents from all the 20 samples were subjected to PCR, to further confirm the above results as described by Marques et al. (2001) and using Promega purification and amplification kits. A sample was considered positive when a specific fragment of 223bp was detected (Baily et al., 1992).

- Susceptibility of Brucella species to various antibiotics: The Kirby-Bauer technique used to assess Brucella species vulnerability and resistance to 7 antibiotics (tetracycline, ciprofloxacin, gentamicin, rifampicin, cephalexin, neomycin and erythromycin) according to Hsueh et al. method (2010).

3. Results

3.1 Culture and PCR

Fifteen Brucella culture was isolated successfully, as shown in Table-1. The obtained isolates were Gram-negative, coccobacilli, show no haemolysis on blood agar, and no growth was obtained at MacConkey agar. They were negative for nitrate reduction and indol production, while they were positive for oxidase and catalase. The biotyping characteristics of the sheep fetuses were negative for H₂S production, positive for urease, and grow well without Co₂ requirement, On the other hand the isolates form the buffalo fetuses were positive for H₂S and do need Co₂ to grow. On the basis of biochemical results, the clinical isolates from the sheep fetuses were identified as Brucella melitensis, while the isolates from the buffaloes identified as Brucella abortus. The overall agreement between the microbiological culture and PCR test reached 95% with the advantage of PCR over the microbiological culture because PCR succeeded in detect 16 positive cases from stomach content of the aborted fetuses while the culture was 15.

Table 1: Culture and PCR Results of the Stomach Content.

<table>
<thead>
<tr>
<th>Groups</th>
<th>No. of Samples</th>
<th>Culture</th>
<th>PCR</th>
<th>Total Agreement %*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Pos.</td>
<td>%</td>
<td>Pos.</td>
</tr>
<tr>
<td>Sheep</td>
<td>12</td>
<td>9</td>
<td>3</td>
<td>75</td>
</tr>
<tr>
<td>Buffalo</td>
<td>8</td>
<td>6</td>
<td>2</td>
<td>75</td>
</tr>
<tr>
<td>Total</td>
<td>20</td>
<td>15</td>
<td>5</td>
<td>75</td>
</tr>
</tbody>
</table>

The total agreement % is the proportional similarity of the results of both tests. (Showman.1986)
3.2 Antibiotic Susceptibility Test Results

Table 2, reveals susceptibility of (15) local *Brucella* isolates obtained from both (sheep and buffalo) fetuses to the seven most commonly used antibiotics as animal medication.

Table 2: Antibiotic Susceptibility Results.

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Disk Potency</th>
<th>Sensitive</th>
<th>Intermediate</th>
<th>Resistant</th>
<th>Antimicrobial Sensitivity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>No.</td>
<td>%</td>
<td>No.</td>
<td>%</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>30μ</td>
<td>10</td>
<td>66.66</td>
<td>4</td>
<td>26.66</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>5μ</td>
<td>10</td>
<td>66.66</td>
<td>3</td>
<td>20</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>10μ</td>
<td>10</td>
<td>66.66</td>
<td>3</td>
<td>20</td>
</tr>
<tr>
<td>Rifampicin</td>
<td>5μ</td>
<td>5</td>
<td>33.33</td>
<td>3</td>
<td>20</td>
</tr>
<tr>
<td>Cephalexin</td>
<td>30μ</td>
<td>5</td>
<td>33.33</td>
<td>-</td>
<td>0</td>
</tr>
<tr>
<td>Neomycin</td>
<td>30μ</td>
<td>5</td>
<td>33.33</td>
<td>5</td>
<td>33.33</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>15μ</td>
<td>-</td>
<td>0</td>
<td>4</td>
<td>26.66</td>
</tr>
</tbody>
</table>

*Antimicrobial sensitivity zone done according to (Clinical and Laboratory Standard Institute, 2009)*

4. Discussion

4.1. Culture and PCR

Fifteen *Brucella* culture isolated from the twenty aborted fetuses used in this study in total percentage 75% which indicate that brucellosis is still no. 1 cause of abortion in animals as found before in Iraq (Al-Bayatti et al., 2004; Al-Bayatti et al., 2005 and Al-Bayatti et al., 2006) and in Middle East and Africa (Wareth et al., 2022 and Joint FAO/WHO, 1986).

Nine *Brucella melitensis* isolated from sheep fetuses and this finding agree with what was concluded in the FAO/WHO experts report (1986) as the sheep is the main host for *Br. melitensis*, however both *Br. melitensis* and *Br. abortus* can infect sheep as was found by (Al-Bayatti et al., 2005) and even human can be infected in both species (Joint FAO/WHO, 1986). Six *Br. abortus* isolated from buffalo fetuses and this result was expected as *Br. abortus* is very well known as the main cause for abortion in cattle and buffaloes (Al-Bayatti et al., 2006) and Joint FAO/WHO, 1986).

The overall agreement between the microbiological culture and PCR reached 95%
with superiority of PCR over culture because the last failed in detect one positive case in PCR. This result agrees with other researchers (Marques et al., 2001; Al-Bayatti & Al-Thwani, 2009 and Scarcelli et al., 2004).

4.2. Antibiotic sensitivity and resistance

The results showed in table 2 clearly indicate that tetracycline which record (66.66 %) still the most suitable antibiotic for brucellosis treatment, although one isolate showed resistance and 4 intermediate but, in compare with other antibiotic it was the best (table 2). Each of ciprofloxacin and gentamicin share tetracycline its rate, because they also record (66.66%) as well this provide suitable alternatives for treatment. Rifampicin and cephalaxin each record (33.33%) sensitive, while neomycin and erythromycin were resistant. These results mean that Brucella isolates which used in this study never acquired resistance to tetracycline in spite of long history of using it for it treatment and the resistance to each of neomycin and erythromycin may be due it its compositions rather than resistance acquirement. These results agree with what was found by Abbas and Aldeewan in Basrah Provence who run their tests on Brucella ovis and also found that tetracycline and gentamicin were susceptible while is erythromycin not (Abbas and Aldeewan, 2010). From Egypt Khan et al. (2019) agree with our results for the same mentioned antibiotics. Wareth et al. (2021) from Egypt and Lounes et al. (2018) from Algeria also agree with our results for each of tetracycline and gentamicin. Saeed et al., (2019) from Iran agree with our results in regard with gentamicin and rifampicin. And agree with (Elbehiry et al., 2022) in regard to rifampicin.

In other word our results agree with all the above researchers for the similar used antibiotic. In conclusion Brucella spp. did not generate important antibiotic resistance traits and the resistance to neomycin and erythromycin may be due to some other reasons other than acquiring resistance traits. This result agree with Johansen et al.(2018) study how did not found any mutation for antibiotic resistance , and concluded that there is an overestimation of resistance based on phenotype results of antibiotic susceptibility tests.

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التحري عن جراثيم البروسيلا المقاومة للمضادات الحيوية في عزلات اجنة الحيوانات
المجهضة

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الخلاصة

تشكل المقاومة للمضادات الحيوية تهديد للصحة البشرية والحيوانية خاصة لو كانت متعلقة بالأمراض المشتركة مع الحيوانات مثل داء البروسيلا، والذي كان ولايزال يعالج بنفس المجموعة من المضادات ولعقود من الزمن. مما يثير السؤال فيما إذا اكتسبت جراثيم البروسيلا مقاومة لهذه المضادات. لدراسة حساسية جراثيم البروسيلا للمضادات تم استخدام (20) جنين مجهض وواقع 12 من الأغنام و 8 من الجاموس. خضعت هذه الاجنة لفحص الـ PCR والزرع الجرثومي وتم استخدام سبعة أنواع من المضادات الحيوية لفحص المقاومة والحساسية. تم عزل جراثيم البروسيلا من (15) جنين مجهض وواقع (9) من الأغنام و (2) من الجاموس. نتائج اختبارات التنميط الحيوي أظهرت كونها تنتمي للبروسيلا المالطية والمجهضة وكلا النوعين من الحيوانات على التوالي. نسبة المنوية للتوافق بين اختبار الـ PCR والزرع الجرثومي بلغ 95% مع تفوق الـ PCR على الزرع الجرثومي. فحص الحساسية والمقاومة أظهر أنه لازال كل من الـ سائرباتسكين والسايبروفلوكساسين والجناماسين كفوأة في علاج البروسيلا وبنفس المستوى فيما أظهر كل من الريفامبسين والسيفالكسين نتائج معتدلة ولم يظهر أي من النيومايسين أو الأريثرومايسين أي كفاءة ضد جراثيم البروسيلا. نستنتج مما سبق بأن جراثيم البروسيلا لم تكتسب مناعة ضد المضادات الحيوية التي لطالما استخدمت في علاجها.