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

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Received: November 18, 2023; Revised: December 04, 2023; Accepted: January 13, 2024

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% Co_2 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 H_2S production, positive for urease, and grow well without Co_2 requirement, On the other hand the isolates form the buffalo fetuses were positive for H2S and do need Co_2 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.

Groups	No. of Samples	Culture			PCR			Total Agreement %*
		Pos.	Neg.	%	Pos.	Neg.	%	
Sheep	12	9	3	75	10	2	83.3	91.66
Buffalo	8	6	2	75	6	2	75	100
Total	20	15	5	75	16	4	80	95

* The total agreement % is the proportional similarity of the results of both tests. (Showman.1986)

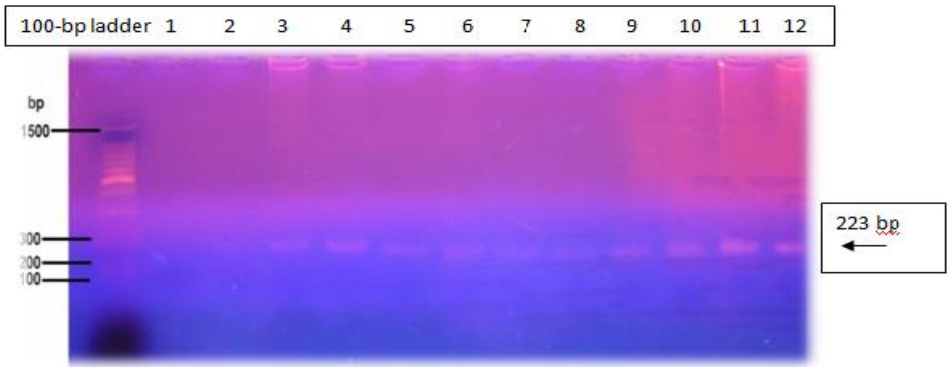

Figure 1: Results of PCT Products from the Stomach of the Aborted Sheep Fetues. The Amplified Fragments Were Separated by Electrophoresis on 2 % Agarose gel, Stained Ethidium Bromide at 100 volts for 25 min.

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: Anitbiotic Susceptibility Results.

Anitbiotic	Disk potency	Sensitive		Intermediate		Resistant		Antimicrobial* sensitivity		
		No.	%	No.	%	No.	%	S	I	R
tetracycline	30µ	10	66.66	4	26.66	1	6.66	≥19	13-15	≤14
ciprofloxacin	5 µ	10	66.66	3	20	2	13.33	≥21	16-20	≤15
gentamicin	10 µ	10	66.66	3	20	2	13.33	≥15	13-14	≤12
rifampicin	5 µ	5	33.33	3	20	7	46.66	≥20	17-19	≤16
cephalexin	30 µ	5	33.33	-	0	10	66.66	≥18	15-17	≤14
neomycin	30 µ	-	0	5	33.33	10	66.66	≥17	13-16	≤12
erythromycin	15 µ	-	0	4	26.66	11	73.33	≥23	14-22	≤13

*Antimivrobial sensitivity zone done according to (Clinical and Laboratory Standard Insiute, 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 ciprofolxacin and gentamicin share tetracycline its rate, because they also record (66.66%) as well this provide suitable alternatives for treatment. Rifampicin and cephalexin 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 refampicin. 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.

References

- **Abbas, B. and Aldeewan, B. (2010).** Isolation, identification and biotyping of Brucella spp. from milk product at Basrah province. Basrah J. of Vet. Res. (9): p. 152-162. https://doi.org/10.33762/bvetr.2010.55133

- **Al-Bayatti, S.A.; Al-Thwani, A.N. and Haddi, A.A. (2004).** Epidemiological study on brucellosis in sheep in Iraq. Al-Qadisia J. Vet. Med. Sci. Vol. 3 No. 1 p. 70-76.

- **Al-Bayatti, S.A.; Makkawi, T.A.; Mohammad Ali, N.K. and Alawi, E.A. (2005).** Histopathological changes in aborted fetuses infected with *Brucella* organism. Iraqi J. Agric. Vol. 10 No. 2 p. 158-165. https://doi.org/10.30539/iraqijvm.v36i0a.370

- **Al-Bayatti, S.A.; Al-Thwani, A.N.; Al-Sammaraie, S.A. and Haddi, A.A. (2006).** Bacteriological and serological study of brucellosis in Buffalo in Baghdad. The Iraqi J. Vet. Med. Vol. 30(2) p.23-30. https://doi.org/10.30539/iraqijvm.v30i2.813

- **Al-Bayatti, S.A. and Al-Thwani, A.N.(2009).** Evaluation of PCR, ELISA and Culture Methods for the Diagnosis of Animal Bbrucellosis.Diyala Agri. Sci. J. 1(1)p: 1-17.

- **Alton, G.G.; Jones, L.M.; Angus, R.D. and Verger, J.M. (1988).** Techniques for the brucellosis laboratory. 1st ed. INRA. Paris. Ch.2 p. 114.

- **Ariza, J.; Bosikovski, M.;Cascio, A.; Colmenero, J.; Corbel, M.; Falagas, M., *et al*. (2007).** Perspectives for the treatment of brucellosis in the 21 st century: the Ioannina recommendations 4(12): e317. https://doi.org/10.1371/journal.pmed.0040317

- **Baily, G.G.; Krahn, J.B.; Drasar, B.S. and Stoker, N.G. (1992).** Detection of *Brucella melitensis* and *Brucella abortus* by DNA amplification. J. Tropical Med. Hyg. Vol. 95(4): 271-275.

- **Bloland, P. B. (2001).** Drug resistance in malaria. World Health Organization. WHO/CDS/CSR/DRS/2001,4. https://doi.org/10.18356/f96d078a-en

- **Clinical and Laboratory Standards Institute**, Performance Standard for Antimicrobial Disk Susceptibility Tests, 2009.

- **Elbehiry, A.; Aldubaib, M.; Al Rugaie, O.; Marzouk, E.; Abaalkhail, M.; Moussa, I.; El-Husseiny, M.; Abalkhail, A. and Rawway, M. (2022).** Proteomics-based screening and antibiotic assessment of clinical and subclinical *Brucella* species: An evolution of brucellosis infection control. PLoS ONE17(1): e0262551.http://doi.org/10.1371/journal.pone. 0262551.

- **Hsueh,** P. R., Ko w. C., Wu, J. J., Wang, F. D., Wu, H. Y., *et al.* Consensus statement on the adherence to Clinical and Laboratory Standards Institute (CLSI) Antimicrobial Susceptibility Testing Guidelines (CLSI-2010 and CLSI-2010-update) for Enterobacteriaceae in clinical microbiology laboratories in Taiwan. 2010; 43(5) 452- 455. https://doi.org/10.1016/s1684-1182(10)60070-9

- **Hussain, M.; Galvin, H.; Haw, T.; Nutsford, A. and Husain, M. (2022).** Drug resistance in influenza A virus: the epidemiology and management. Open Access Volume 10, 2017-issue P: 121-134. https://doi.org/10.2147/idr.s105473

- **Johansen, T.; Scheffer, L.; Jensen, V.; Bohlin, J. and Feruglio, S. (2018).** Whole-genome sequencing and antimicrobial resistance in *Brucella melitensis* from Norwegian prespective. 8: 8538. Scientific Reports. https://doi.org/10.1038/s41598-018-26906-3

- **Joint FAO/ WHO (1986)**: Expert Committee on Brucellosis 6th Report, WHO, Tech. Rep. Ser. No. 740, WHO, Geneva.

- **Khan, A.; Shell, W.; Melzer, F.; Syour, A.; Ramadan, E.; Elschner, M. *et al*. (2019).** Identification, genotyping and antimicrobial susceptibility testing for *Brucella* spp. Isolated from livestock in Eygpt.7(12) 603. https://doi.org/10.3390/microorganisms7120603

- **Lounes, N.,** Bouyoucef, A., Lazri, M., Benamrouche, N., Maamar, H. T., & Rahal, K. (2018). Antimicrobial resistance of *Brucella* isolated from seropositives cattle in the department of Tizi Ouzou, Algeria. 47th World Congress on Microbiology, September 10-11, London, UK.

- **Marques, O.; Alves, C. and Prestes, L. (2001).** Detection of *Brucella* DNA from aborted bovine foetuses by polymerase chain reaction. Aust. Vet. J. Vol. 79 No. 7 p. 500-501. https://doi.org/10.1111/j.1751-0813.2001.tb13025.x

- **Oluranti, O.; Majolagbe, O.; Elutade, O.; Obisesan, I.; Omotayo, O.; Ajayi-Odoko, O.; Olaogun, T.; Akinsola, O.; Oladapo, C.; Ojo, P. and Ramatu, S. (2023).** Molecular Characterization of

Multi-Drug Resistant Fungal Isolates Obtained From Raw Cow Milk in Bowen University, Nigeria. IOP Conf. Series: Earth and Environment Science 1219 (2023) 012001. https://doi.org/10.1088/1755-1315/1219/1/012001

- **Pasmatzi, E.; Schwartz, R.; Kaneko, F. and Guarneri, F. (2012).** Prevalence and Antibiotic Susceptibility of Community-Associated Methicillin- Resistant *Staphylococcus aureus* in a Rural Area of India: Is MRSA Replacing Methicillin-Susceptible *Staphylococcus aureus* in the Community. Open Access Volume 2012| Article ID 248951| https:// doi.org/ 10.5402/ 2012/248951.

- **Saeed, A.; Maryam, D.; Afshar, E.; Davoud, A. and Mohammad, A. (2019).** Antimicrobial susceptibility of Brucella spp. isolated from Iranian patients during 2016-2018. Iranian J. of Microbiology, 11(15) p. 363-367. https://doi.org/10.18502/ijm.v11i5.1953

- **Scarcelli, E.; Piatti, R.; Cardoso, M.; Miyashiro, S.; Campos, F.; Teixeira, S.; Castro, V. and Genovez, M. (2004).** Detection of bacterial agents by isolation and identification and multiplex PCR in aborted bovine fetuses. Revista Brasileira de Reproducao Animal 28: 23-27. https://doi.org/10.1590/s1517-83822009000400029

- **Showman, A.** (1986). The Biological Statistic. Al-Mosul Univ. Press. https://doi.org/10.31826/9781463217075-002

- **Wareth, G.; El-Diasty, M.; Abdel-Hamid, N.; Holzer, K.; Hamdy, M.; Mustafa, S.; *et al*. (2021).** Molecular characterization and antimicrobial susceptibility testing of clinical and non-clinical *Brucella melitensis* and *Brucella abortus* isolates from Egypt. 13: 100255. https://doi.org/10.1016/j.onehlt.2021.100255

- **Wareth, G.; Dadar, M.; Ali, H.; Mahmoud, E.; Hamdy, R.; Al-Talhy, A.; Elkharsawi, A.; Abd Eltwab and Neubauer, H. (2022).** The perspective of antibiotic therapeutic challenges of brucellosis in the Middle East and North Africa countries: Current situation and therapeutic management. Open Access. https://doi.org/10.1111/tbed.14502.

- **WHO Technical Report Series. (1978).** Surveillance for the prevention and control of health hazards due to antibiotic-resistant enterobacteria. N0. 624 (Report of WHO meeting). https://doi.org/10.1111/tbed.14502

- **World Health Organization. (1997).** Fact sheet N173, July 1997. World Health Organization, Geneva Swizerland.

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 البروسيلات والذي كان ولايزال يعالج بنفس المجموعة من المضادات ولعقود من الزمن, مما يثير التساؤل فيما اذا اكتسبت جراثيم البروسيلا مقاومة لهذه المضادات . لدراسة حساسية جراثيم البروسيلا للمضادات تم استخدام (٢٠) جنين مجهض وبواقع ١٢ من الاغنام و٨ من الجاموس . خضعت هذه الاجنة لفحص الـ PCR والزرع الجرثومي وتم استخدام سبعة انواع من المضادات الحيوية لفحص المقاومة والحساسية . تم عزل جراثيم البروسيلا من (١٥) جنين مجهض وبواقع (٩) من الاغنام و (٦) من الجاموس . نتائج اختبارات التنميط الحيوي اظهرت كونها تنتمي للبروسيلا المالطية والمجهضة ولكلا النوعين من الحيوانات على التوالي . النسبة المئوية للتوافق بين اختباري الـ PCR والزرع الجرثومي بلغ ٩٥% مع تفوق الـ PCR على الزرع الجرثومي . فحص الحساسية والمقاومة اظهر بانه لازال كل من التتراسايكلين والسايبروفلوكساسين والجنتامايسين كفوءة في علاج البروسيلا وبنفس المستوى فيما اظهر كل من الريفامبسين والسيفالكسين نتائج معتدلة ولم يظهر اي من النيومايسين او الأريثرومايسين اي كفاءة ضد جراثيم البروسيلا . نستنتج مما سبق بأن جراثيم البروسيلا لم تكتسب مناعة ضد المضادات الحيوية التي لطالما استخدمت في علاجها.