The Sudan J.Vet. Res.(2001), ***17:*** *65-71*
With 3 tables in the text *www.sudanjvr.net*

Brucella Organisms isolated in the Sudan *Part II:* Antibiotic Sensitivity Test Results and Resistogram Profiles

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ملخص البحث

لقد تم اختبار حساسية عترات الصنف الحيوى 6 للبروسيلا المجهضه والصنف الحيوي 3 لبروسيلا الحمي المالطيه التي عزلت في السودان في فترات وأماكن مختلفه ، لثلاثه وعشرين نوعا من المضادات الحيويه .كما تم تصنيف عترات نوعي البروسيلا الي دون الصنف اعتمادا علي درجة حساسيتها للمضادات الحيوية المستخدمة.لقد وجدت عترات البروسيلا المجهضة المختبره حساسه لإحدى عشر من المضادات الحيويه ، ومقاومه لسبع.ولكن نتائجها اختلفت في خمس منها . عترات الصنف الحيوي 6 تشابهت في درجة الحساسيه لمعظم المضادات الحيويه واختلفت في القليل منها وهذه النتائج ميزت العترات حسب مواقعها الجغرافيه التي عزلت منها .أما بالنسبة لعترات الصنف الحيوي3 لبروسيلا الحمي المالطيه فان درجة حساسيتها لمعظم المضادات الحيويه اختلفت بعضها عن بعض وهذه ميزة فرقت بينها إلى مجموعتين(subtypes) مختلفتين .

Summary

***Brucella abortus* (*B. abortus*) biovar 6 and *B. melitensis* biovar 3 strains isolated from animals at different times and localities in the Sudan were tested for antibiotic sensitivity and subtyped using antibiotic resistogram profiles.The organisms were found sensitive to carbenicillin, cefoxitin,cinoxacin, cephardine, fusidic acid, kanamycin, latamoxef, norfloxacin, streptomycin, spectinomycin and tobramycin. They were resistant to erythromycin, furazolidone, linocmcyin, mecillinam, oxacillin, oxolinic acid and penicillin G.Their sensitivity or resistance varied with ampicillin, cephazolin, nalidixic acid, neomycin and novobiocin.**

The *B. abortus* biovar 6 strains showed similar antibiotic resistogram profiles, but in a few instances a limited number of profiles was found each representing a certain geographic locality. In case of *B. melitensis* biovar 3 strains, the profiles varied for most antibiotics distinguishing the isolates into different subtypes.

Introduction

B. abortus biovar 6 and *B. melitensis* biovar 3 were found to be the major causes of brucellosis in indigenous animals in the Sudan (Musa *et al.,* 2000). The predominance of a small number of biovars, often only one within a country, makes the value of biovar identification of little epidemiological significance. Hence there is a need for extension of identification of brucella strains beyond biovar level, using different methods including antibiotic resistogram patterns (Corbel, 1990).

The aim of this study was to examine antibiotic sensitivity of *Brucella* organisms isolated in the Sudan and study their resistogram profiles to subtype the predominant *B. abortus* and *B. melitensis* biovars into possible epidemiological entities.

Materials and Methods

Twenty-one *B. abortus* biovar 6 and two *B. melitensis* biovar 3 strains isolated in the Sudan between 1953 and 1993 and typed to biovar levels (Musa *et. al*., 2000), were used in this study. The *B. abortus* strains were isolated from cattle; Seven from Darfur region, 13 from South Kordofan State and one from Blue Nile State. The seven were identified by the numbers 93/3 to 93/7,93/9 and 93/10, the 13 by 93/33, 93/35, 93/39, 93/41to 93/43,93/45B, 93/48 to 93/51 and 93/54 and the one from the Blue Nile State by 93/57.

The two *B. melitensis* strains were isolated from an ovine and bovine milk in Gezira State, Central Sudan, and were identified by numbers 93/55 and 93/56, respectively.

Antibiotic sensitivity tests:

Twenty-three (Oxoid) antibiotic sensitivity discs were used in the study. These antibiotics and their concentrations in micrograms/ml (μg/ml) were as follows:

Ampicillin$_2$ (AMP_2) carbenicillin$_{10}$ (CAR_{10}), cefoxitin$_{30}$ (FOX_{30}), cephazoline 30(Kz_{30}), cephardine$_{30}$ (CE_{30}), cinoxacin$_{10}$ (CIN_{100}) erythomycin$_5$ (E_5), furazolidone$_{25}$ (FR_{25}), fusidic acid$_5$ (FD_5), kanamycin$_5$ (K_5), latamoxef$_{30}$ (Mox_{30}), lincomycin$_5$ (My_{15}), mecillinam$_{10}$ (MEL_{10}), nalidixic acid$_{30}$ (NA_{30}), neomycin$_{10}$ (N_{10}), norfloxacin$_{10}$ (NOR_{10}), novobiocin$_{30}$ (NV_{30}), oxacilin$_5$ (OX_5), oxolinic acid$_2$ (OA_2), penecillinG_{1-5}(P_{1-5}), streptomycin$_{10}$ (S_{10}), spectino-mycin$_{25}$ (SH_{25}) and tobramycin$_{10}$ (TOB_{10}).

A milky suspension of each *Brucella* test strain was prepared by emulsifying a loopful of its culture on serum dextrose agar (SDA) slope in 1ml sterile distilled water in a bijoux bottle. Using a Pasteur pipette each suspension was used to inoculate three SDA plates, 5-7 drops per plate. The inocula were evenly spreaded and left to dry.

Subsequently, the 23 antibiotics were dispensed on the three plates, eight discs each where applicable, using Oxoid antibiotics dispenser. The plates were incubated at 37°C in air + 10% Co_2 for 72 hours. The cultures were then read for growth inhibition or no inhibition with the aid of a colony counter and a magnifying glass (Gallenkamp). In case of no growth the zones of inhibition were measured from the edge of the antibiotic disc in millimeters (mm). The results for sensitivity or resistance were interpreted according to Collee *et al.* (1989) by considering the organisms examined sensitive if the diameter of their inhibition zone was ≥ 2.5. mm.

Antibiotic resistogram typing:

The results of antibiotic sensitivity tests were used for resistogram typing according to Chimera (1986) by suggesting an arbitrary cut off point for the diameter of inhibition zone of each antibiotic to the test strains as follows:

For E_5, FR_{15}, FD_5, MY_{15}, and MEL_{10}, = 4mm.

For AMP_2,KZ_{30},FOX_{30},CE_{30},K_5,NA_{30},OA_2,$OX_5$and $P_{1.5}$ = 5mm.

For CAR_{10}, CIN_{100}, MOX_{30}, NV_{30}, N_{10},NOR_{10}, S_{10}, SH_{25}, and Tob_{10}=7mm

Results

The *B. abortus* and *B. melitensis* strains examined were found to be similarly sensitive or resistant to 18(73.3%) of the antibiotics used (Table 1) but, their sensitivity varied with the remaining 5 (21.7%) antibiotics (Table 2). The latter table also shows 5 (23.8%) of the *B. abortus* biovar 6 strains reacted differently from the remaining 16(76.2%) strains: Thus strains 93/4 and 93/10 from Darfur were resistant to two different antibiotics each. Strains 93/3 and 93/4 from Darfur and 93/35 and 93/39 from South Kordofan were, unlike others sensitive to amplicillin. The two *B. melitensis* strains also showed different reactions to the 5 antibiotics (Table 2). The bovine strain 93/56, however, was identical to ampicillin sensitive strains in its pattern of sensitivity (Table 2). The brucella test strains. As a whole, showed five patterns of antiboitic sensitivity reaction (Table 2)

***Antibiotic Resistogram*:**

The results of antibiotic resistogram profiles were presented in Table 3. As shown in the table, the *B. abortus* stains from different localities in the country exhibited similar resistogram profiles for most antibiotics examined but the majority of Darfur isolates were resistant to cephazolin and nalidixic acid, while most South Kordofan and Blue Nile strains were sensitive to the two antibiotics. The *B. melitensis* strains showed different resistogram profiles and were distinguishable from each other (Table 3).

Table 1*:* Sensitivity and resistance of the brucellae examined to 18 antibiotics.

Brucella species biovar examined	**Antibiotics used**
B. abortus biovar 6 (21 strains)	+ CAR10 + CIN100 + FOX30 + CE30 + FD5 + K5 + MOX30 + NOR10 + S10 + SH25 + TOB10 - E5 - FR15 - MY15 - MEL10 - OX5 - OA2 - P1.5
B. melitensis biovar 3 (two strains)	+ + + + + + + - - - - - - - - -

+ = *sensitive* - = *resistant*

Table 2: Antibiotics sensitivity test results of 5 antibiotics on the brucellae examined.

Biovar examined	Antibiotics used				
	AMP_2	KZ_{30}	NA_{30}	N_{10}	NV_5
Biovar 6* strains 93/3, 93/85, 93/35 and biovar 3** strain 93/56	+	+	+	+	+
Biovar 6*: 16 strains	-	+	+	+	+
Biovar6* strains 93/4	+	+	+	-	-
Biovar 6*strain 93/10	-	-	+	+	+
Biovar 3** strain 93/55	-	-	-	+	-

* = *B. abortus* ** = *B. melitensis*

+ = Sensitive; - = resistant; AMP= Ampicillin; KZ = Cephazolin; NA = Nalisixics acid; N = Neomycin; NV = Novobiocin.

Discussion

In the United Kingdom. *B. abortus* biovar 1 was the only causative agent of brucellosis in cattle during the closing period of Brucellosis Eradication Scheme (Corbel, 1990). Antibiotic resistance Patterns were used to subtype strains of the organism for epidemiological reasons (Chimera, 1986). This investigator used 24 antibiotics including the 23 used in this study. He found a limited number of profiles each representing a certain geographical locality, which in turn suggested a common source of infection. In this study, the antibiotic sensitivity tests showed a similarity between 78.3% of the brucella examined, which was probably due to the fact that members of the genus *Brucella* are very homogeneous group (Vergor *et al.,* 1987). The differences found in the few strains (Table 2) could be attributed to individual characteristics especially that no natural plasmids were identified in members of the genus *Brucella* (Rigby and Fraster, 1989). The antibiotic resistance patterns of the *B. abortus* biovar 6 strains almost identified isolates from different localities, but in a limited scale probably due to extensive cattle movements throughout the country. The two distinct *B. melitensis* strains were isolated from an area where the organism had previously been isolated from goats (Dafalla, 1962). Presumptively, the bovine infection could have had resulted from goats or another source different from that of the ovine.

Acknowledgements

The authors wish to thank Dr. S. Broughton of the FAO/WHO Collaborating Centre for Reference and Research on Brucellosis, Central Veterinary Laboratory, New How, Addlestone, Surrey KYT_{15} 3NB U.K., for his kind permission to work in the laboratory and supplying facilities for the study. This paper is published by kind permission of the Director General, Animal Resources Research Corporation.

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