In Vitro Susceptibilities of Brucella Abortus Isolates to Eight Antibiotics

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ABSTRACT

Background and Objectives: Brucellosis is a global zoonotic disease, endemic in Sudan. Transmitted to man from infected domestic animals. There are an increasing number of cases in humans. The aim of this study was to investigate of the antibiotic susceptibility profile of brucella isolates. A total of 7 brucella strains (all Brucella abortus) were isolated from cattle and camels in Northern State, Sudan, between March 2010 to September 2011. Material and Methods: Seven Brucella abortus isolates were tested to eight antibiotics sensitivity according to the recommendations of the Notional Committee for Clinical Laboratory Standards (NCCLS), 1997, the disc method was performed. Result: The seven of isolates tested were sensitive to Ampicilhin, Doxycycline, Cefriaxone, Cortimoxazole and Chlormphenicol. However, one of tested isolates, showed in vitro resistance to Rifampicin. Conclusion: This is the second report showing, reduced susceptibility to rifampicin, in Sudan. However, Brucella isolates remain susceptible in vitro to most antibiotics used for treatment of brucellosis.

Keywords: wards Susceptibilities, Antibiotics

1. INTRODUCTION

Brucellae are Gram negative, partially acid fast, aerobic facultative, non motile, non sporting bacteria. Human brucellosis constitutes a serious risk to public health and economy (Moreno 2002). The disease causes mortality, acute and chronic debilitating disease in man (Poester et al. 2000). Treatment recommended by World Health Organization for acute brucellosis in adults is rifampicin 600 to 900 mg and doxycycline 100 mg twice daily for minimum of six weeks (FAO/ WHO 1986). Some still claim that, the combination of intramuscular streptomycin (19/day for 2-3 week) with an oral tetracycline (29/day for 6 weeks) gives fewer relapses (Ariza et al., 1985, Mantur et al., 2006). Karabay et al., (2004) reported that trimethoprim sulfamethoxazole (TMP/ SMX) was a popular compound in many areas, usually used in triple regimens. Various combinations that incorporated ciprofloxacin and a floxacin have been tried clinically, yielding similar efficacy to that of classic regimens. The present investigation was carried out to study the susceptibility of antibiotics of brucella isolates from cattle and camels in Northern State, Sudan.

2. MATERIALS AND METHODS

2.1 Source of Microorganism

Seven Bruella abortus isolates recovered from cattle and camels in Northern State, Sudan were investigated and tested to the antibiotics sensitivity during 2011. According to the recommendations of the Notional Committee for Clinical Laboratory Standards (NCCLS), 1997, the disc method was performed.

2.2 Preparation of inoculums

The isolates to be tested were emulsified in saline and the turbidity of each was adjusted to 0.5 McFarland standards.

2.3 Inoculation of the test plates

a. After adjusting the turbidity of each isolate to be tested within 15 minutes, a sterile cotton swab was dipped into the adjusted suspension.

b. The swab was rotated several times and pressed firmly in the inside wall of the tube above the fluid level.

c. The surface of a Muller Hinton agar plate was inoculated by streaking the swab over the entire sterile agar surface. This procedure was repeated by streaking two more times, rotating the plate approximately 60º each time to ensure an even distribution of the inoculums and finally the rim of the agar were swabbed.

2.4 Application of the disc

a. Commercially prepared discs 6mm in diameter were used. Discs were stored in sealed containers with a desiccant at 4-8º C. before being opened for use.

b. The containers were allowed to warm up solely at room temperature to minimize condensation of moisture, which might lead to hydrolysis of the antibiotics.

c. The drugs to be tested against Brucella sp. were applied in sets of four discs per plate.

d. The antibiotics used were: Ampicilllin (A10)

E. Doxycycline (DO30)

F. Azithromycin (AZI100)

G. Cipramycin (CIP5)

H. Rifampicin (RD5)

I. Cefriaxone (CRO30)

J. Cortimoxazole (SXT25)

K. Chloramphenicol (C30).

The concentrations in µg were shown in subscript of each antibiotic abbreviation.

e. The predetermined battery of antimicrobial discs was dispensed into the surface of the Brucella-inoculated agar plates.

f. Each disc was pressed down to ensure complete contact with the agar surface.

g. The plates were incubated at 35-37º C for 24 - 48 hours.
2.5 Quality control

Staphylococcus aureus ATCC (25923) and Escherichia coli ATCC (25922) were inoculated in separate plates and tested against same antibiotics used for Brucella spp.as controls. Plates were incubated at 35-37º C for 48 – 72 hours before reading.

2.6 Readings of the plates

According to Jorgenson (1997), the inhibition zones were determined by measuring the distance of the zone in millimeters. The sizes of the inhibition zones were measured, interpreted and reported as; Sensitive, Intermediate or Resistant.

3. RESULTS AND DISCUSSION

The seven Brucella isolates were Susceptible to the antibiotics tested are shown in Table 1: Antibiotic sensitivity tests.

<table>
<thead>
<tr>
<th>Antibiotic Strain</th>
<th>A10</th>
<th>DO30</th>
<th>S300</th>
<th>CIP5</th>
<th>RD5</th>
<th>CRD30</th>
<th>SXT15</th>
<th>C30</th>
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<tr>
<td>Staph. aureus ATCC 25923</td>
<td></td>
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<td>30</td>
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<td>E. coli ATCC 25922</td>
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<td>Milk.2</td>
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</table>

Key:

A= Ampicillin
DO= Doxycycline
S= Streptomycin
CIP= Ciprofloxacin
RD= Rifampicin
CRO= Ceftriaxone
SXT= Cotrimoxazole
C= Chloramphenicol
* =Diameter of the inhibition zone

Treatment of brucellosis with a single antibiotic is not recommended due to high rates of relapses; however, a clear optimal dual therapy has not been agreed upon (A cocella et al. 1989). The World Health Organization recommends treatment with doxycycline for 6 weeks and streptomycin for 14-21 days or gentamycine for 7-10 days. However, streptomycin and gentamycine must be administered parenterally making outpatient treatment difficult. Consequently, doxycycline in combination with rifampicin (which both can be taken orally) is considered to be the principal alternative therapy for treatment of human brucellosis and must be taken for at least 6 weeks (Young, 2000). Fluoroquinolones e.g ciprofloxacin or trimethoprim/ sulfamethoxazole in combination with doxycycline or rifampicin are recommended as secondary alternative therapies .Complicated brucellosis, where the patient develops meningitis or endocarditic, necessitates triple antibiotic therapy (doxycycline, co-trimoxazole and rifampicin). In children, a 3 week treatment of doxycycline or trimethoprim/ sulfamethoxazole is recommended a long with a 5 day course of gentamycine. The present study showed that, the seven of isolates tested were sensitive to ampicillin, doxycycline, streptomycin, ciprofloxacin, rifampicin, ceftriaxone, cotrimoxazole and Chloromphenicol. The isolates were susceptible to streptomycin, in agreement with, Loprz- Merino et al. (2004), Turkmoni et al., (2006) and Mohamed et al. (2011). However, one of the tested isolates, showed in vitro resistance to rifampicin .Similar finding were reported by Bagkom et al. (2004) in Turkey, Lopez et al. (2004) in Mexico, , Ali (2007) in Sudan and Mohamed et al. (2011) in Egypt. Suggesting the emergence of isolates with variable degrees of resistance to this drug. To our knowledge, this is the second report showing, reduced susceptibility to rifampicin, in Sudan. However, Brucella isolates remain susceptible in vitro to most antibiotics used for treatment of brucellosis.

4. CONCLUSION

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