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EFFICACY EVALUATION OF SOME ANTIBIOTICS AGAINST SYRIAN *BRUCELLA* spp ISOLATES, *IN VITRO*

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ABSTRACT

Brucellosis is an endemic zoonosis in Syria, affecting large numbers of animals and there are an increasing number of cases in humans. The aim of this study is to investigate the in vitro efficacy of various traditional and new antibiotics against 89 *Brucella* isolates (isolated from domestic animals) collected from different Syrian regions. Minimum inhibitory concentrations (MICs) of seventeen antibiotics were determined. Ciprofloxacin and ofloxacin were the most effective antibiotics, whereas sparfloxacin, levofloxacin, doxycycline and tetracycline had a moderate activity. In contrast, moxifloxacin and rifampicin had a low activity, while streptomycin, spiramycin and cephalosporines were ineffective. As a result, we come to the conclusion that a combination between one effective quinolone and doxycycline has a good efficacy against *Brucella*. Further *in vivo* studies are necessary to support this suggestion.

Key words: Antibiotics, *Brucella*, quinolones

INTRODUCTION

Brucella is a facultative intracellular pathogen, a family of small, nonmotile, gram-negative coccobacilli, that causes abortion in domestic animals (sheep, cattle, and goats), and a febrile illness ("undulant fever") in humans (8, 9). In Syria, it affects a large number of animals and an increasing number of cases in humans. The last recommendation by the World Health Organization (WHO) for the treatment of human acute brucellosis in adults suggested 600 to 900 mg rifampicin and 200 mg doxycycline daily for a minimum of 6 weeks, or doxycycline for 6 weeks plus streptomycin for 2–3 weeks (4). However, the relapse rates associated with doxycycline/ rifampicin and doxycycline/streptomycin regimens are 4.6–24% and 5–8%, respectively (27).

The combination between the rifampicin and trimethoprim-sulfomethoxazole is the suggested regimen for children (3, 15, 16). Triple-antibiotic combinations were found to be of value in some cases of brucellar endocarditis, meningitis, and spondylitis (2, 21, 31). Fluoroquinolones are an alternative. Only *in vitro* observations exist for moxifloxacin and levofloxacin (19), whereas various combinations that incorporate ciprofloxacin and ofloxacin have been tried clinically, yielding similar efficacy to that of the classic regimens (13). Fluoroquinolones have good anti-brucellosis activity *in vitro* (1, 18, 25) and reach high intracellular concentrations.

Brucella isolates are generally considered susceptible to

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the antibiotics that are recommended by the WHO. Nevertheless, sporadic cases of a kind of antibiotic resistance have been reported (6, 19). The aim of this study is to determine the susceptibility of doxycycline, rifampicin, tetracycline, streptomycin, spiramycin, amoxicillin, flucloxacillin, cefixime, cefprozil, cefotaxime, ceftazidime, cefazolin, ciprofloxacin, levofloxacin, ofloxacin, moxifloxacin and sparfloxacin against the *Brucella* isolates identified in the Laboratory of Molecular Biology and Biotechnology, Atomic Energy Commission, Syria, collected from milk samples over different provinces.

MATERIALS AND METHODS

Microorganisms and growth conditions

Eighty nine brucella isolates were collected prospectively between 2004 and 2007 from bovine and ovine milk from different Syrian provinces. Syrian territories were divided into four regions as follows: Northern region (including: Al-Hasakah, Dir-Alzour, Al-Rakah and Aleppo provinces); Central region (including: Edleb, Hama and Homs provinces); Coastal region (including: Tartous and Lattakia provinces) and Southern region (including: Al-Quonaitra, Daraa, Al-Souaida, Damascus and Damascus rural provinces). Bacteria were isolated from milk cultures at the Immunology and Microbiology Laboratory, AECS (5). *Brucella* was grown under optimal conditions in 2YT agar {Difco, BD, USA (peptone [10 g], sodium chloride [5 g], meat extract [5 g], agar [20 g], distilled water [1 litre])} at 37°C in a water bath (Grant, Cambridge, UK) to ensure sufficient cell density. Following antibiotics (Oxoid, UK) were added to inhibit the growth of organisms other than *Brucella*, cycloheximide (100 mg), bacitracin (25000 units), polymyxin B sulphate (5000 units), vancomycin (20 mg), nalidixic acid (5 mg) and nystatin (100000 units). To prepare the solid selective media, basal medium was melted and then cooled to 56°C in a water bath and stock solutions of the antibiotics added with 5% of horse serum (PAN-Biotech, Gmbh, Germany). The biotyping of the bacteria used the following tests: CO_2 requirement, H_2S production, urease and oxidase positivity, growth in the presence of dyes (thionine and basic fuchsine), and reaction with monospecific anti-A and anti-M sera (Arcomex, Jordan). Strains identified as *B. melitensis* (81 isolates) or *B. abortus* (8 isolates) were stored in 2YT medium at -20°C until susceptibility testing. During the experiment, laboratory workers were wearing impermeable protective clothes, gloves, and face masks.

Antibiotics susceptibility testing

In order to estimate the antibiotics susceptibility, well broth microdilution method was used with 96-well plates, (TPP, Switzerland). Antibiotics were twofold diluted in Brucella broth® (acumedia®, Michigan, USA) and wells were inoculated with 10^6 CFU of bacteria (in a 0.2 ml final volume). The incubation period was 48 h at 37°C. MIC testing was performed according to the recommendations of the CLSI (22). Unsupplemented brucella broth media were used. The pH of the broth was adjusted to a range of 7.1 ± 0.1. The range of concentrations assayed for each antibiotic was 0.07 to 100 µg/ml. The lowest concentration that completely inhibited visual growth was recorded and interpreted as the MIC. The absorbance was determined at 590 nm (Thermo-lab Systems Reader, Finland). All tests were performed in triplicate and then averaged.

Investigated antibiotics were the following: doxycycline (Sigma, St. Louis, USA), rifampicin (Sigma), tetracycline (Sigma-Aldrich, USA), streptomycin (Sigma), spiramycin (Sigma), amoxicillin (Fluka, Sigma-Aldrich, USA), flucloxacillin (Square Pharma, Bangladish), cefixime (Fluka, Sigma-Aldrich, USA), cefprozil (Bristol-Myers Squibb, New-York, USA), cefotaxime (Sigma), ceftazidime (Sigma), cefazolin (Fluka, Sigma-Aldrich, USA), ciprofloxacin (Bayer, Istambul, Turkey), levofloxacin (Sigma), ofloxacin (Sigma), moxifloxacin (Bayer, Istambul, Turkey) and sparfloxacin (Sigma).

A well containing no antibiotics was also employed.

Plates were incubated in the conditions mentioned above.

RESULTS

The MIC_{range}, MIC_{50} and MIC_{90} values of the antibiotics are shown in Table 1. No activity was observed at all Syrian regions when the following antibiotics were used: streptomycin, spiramycin, amoxicillin, flucloxacillin, cefixime, cefprozil, cefotaxime, ceftazidime, and cefazoline. Almost all the isolates were resistant to these antibiotics. The MIC_{range} values of doxycycline and tetracycline were low against the isolates collected from central region (0.3-0.75 µg/ml and 0.15-0.75 µg/ml, respectively), and moderate to high against that collected from the other regions. Whereas, MIC_{range} values of rifampicin were very high against the isolates collected from all regions (from 1.5-6.25 µg/ml against isolates collected from southern and central regions, to 25-50 µg/ml against that collected from northern region. Among the quinolones, ciprofloxacin was the most effective against the isolates collected from all regions (MIC_{range}: 0.07-6.25 µg/ml; from 0.07-0.3 µg/ml against isolates collected from northern region to 0.75-6.25 µg/ml against that collected coastal region), and moxifloxacin had the highest MIC_{range}. Other than ciprofloxacin, the MIC_{range} of ofloxacin was the lowest, particularly against the isolates collected from northern region (0.07-0.75 µg/ml), but it was moderate to high against that collected from the other regions. Levofloxacin and sparfloxacin had moderate activities against the isolates collected from all regions.

Table 1. MICs values of antibiotics against Syrian isolates collected from different regions. Efficacy evaluation of some antibiotics against Syrian *Brucella* spp isolates, *in vitro*. (Safi and AL-Mariri)

	MICs at Northern region (µg/ml)			**MICs at Costal region (µg/ml)**			**MICs at Central region (µg/ml)**			**MICs at Southern region (µg/ml)**		
	MIC_{range}	MIC_{50}	MIC_{90}	MIC_{range}	MIC_{50}	MIC_{90}	MIC_{range}	MIC_{50}	MIC_{90}	MIC_{range}	MIC_{50}	MIC_{90}
Doxycycline	0.3-6.25	0.75	3	6.25-12.5	6.25	12.5	0.3-0.75	0.75	0.75	0.75-3	0.75	3
Rifampicin	25-50	25	50	25-50	25	50	1.5-3	3	3	1.5-6.25	3	6.25
Tetracycline	1.5-12.5	6.25	12.5	6.25-12.5	6.25	12.5	0.15-0.75	0.3	0.75	0.75-6.25	1.5	6.25
Streptomycin	50->100	100	>100	>100	>100	>100	>100	>100	>100	50->100	100	>100
Spiramycin	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100
Amoxicillin	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100
Flucloxacillin	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100
Cefixime	>100	>100	>100	50->100	100	>100	50->100	100	>100	>100	>100	>100
Cefprozil	12.5->100	25	100	6.25->100	12.5	100	12.5->100	25	100	>100	>100	>100
Cefotaxime	12.5->100	25	100	6.25->100	12.5	100	12.5->100	25	>100	>100	>100	>100
Ceftazidime	25-100	50	100	6.25->100	12.5	100	6.25-100	25	100	>100	>100	>100
Cefazolin	12.5-50	12.5	25	12.5->100	25	>100	6.25-50	12.5	25	50->100	50	>100
Ciprofloxacin	0.07-0.3	0.07	0.3	0.75-6.25	1.5	3	0.3-0.75	0.75	0.75	0.15-1.5	0.75	1.5
Levofloxacin	0.75-12.5	1.5	6.25	0.15-25	0.75	25	0.75-6.25	1.5	3	0.3-6.25	0.75	6.25
Ofloxacin	0.07-0.75	0.15	0.75	0.15-50	0.75	25	0.15-12.5	0.75	6.25	0.3-6.25	0.75	3
Moxifloxacin	0.75-50	6.25	50	12.5-100	25	100	1.5-50	12.5	50	12.5-100	25	>100
Sparfloxacin	0.15-12.5	0.75	12.5	1.5-100	3	50	0.15-12.5	0.3	6.25	3-12.5	6.25	12.5

DISCUSSION

Treatment of human brucellosis needs antibiotics that can penetrate macrophages and can act in the acidic intracellular environment. *Brucella* is considered to be susceptible to the antibiotics recommended by the WHO for treatment of brucellosis. Relapses, at a rate of about 10 percent, usually occur in the first year after the infection, but they are caused in most cases by inadequate treatment (24). Strains resistant to the main antimicrobial agents may emerge and lead on to treatment inhibition (20).

The isolates included in this work were collected from different areas of Syrian cities. They were totally resistant against streptomycin, macrolide (spiramycin), amoxicillin, flucloxacillin, and against all cephalosporins used in this work (including: cefixime, cefprozil, cefotaxime, ceftazidime, and

cefazolin). This result did not concord, in part, with that found by Palenque *et al.* (23); where they found that cefotaxime was among the most effective agents tested, with MICs ranging from 0.25 to 2 µg/ml. However, it concords with the result of ceftazidime where the MICs was between 4 and 64 µg/ml.

All the isolates were susceptible to tetracycline, the MIC_{range} of this antibiotic was very low, particularly against the isolates collected from the central region (0.15-0.75 µg/ml). This finding agrees with previous reports (6, 7, 16, 30).

In addition, doxycyline had a very good activity against all isolates, with a MIC_{range} of 0.3-0.75 µg/ml in the coastal region; whereas, rifampicin showed a very low activity against almost all isolates. On the other hand, eleven isolates were totally resistant to rifampicin. This finding did not agree with that found by Turkmani *et al.* (29). However, Baykam *et al.* (6) and Dimitrov *et al.* (10) found that 9.6% and 8% of the isolates were resistant to rifampicin *in vitro*, respectively.

Most studies that examined the use of quinolones against *B. melitensis* showed, generally, low MICs values; where the MIC_{90} to the ofloxacin was 0.02-2.5 mg/l (11, 14, 17); to the levofloxacin 0.5 mg/l (17, 28); to the sparfloxacin 0.12-2 mg/l (11, 12, 26); to the ciprofloxacin 0.06-2 mg/l (7, 17, 19); and to the moxifloxacin 0.5-1 mg/l (19, 28). However, among the quinolones that are used in our study, ciprofloxacin revealed the lowest MIC_{range} against the isolates collected from all Syrian regions (MIC_{range} 0.07-6.25 µg/ml), and moxifloxacin showed the highest MIC_{range} (MIC_{range} 1.5-100 µg/ml).

In conclusion, among the antibiotics used in this study, quinolones were the most effective agents against Syrian brucella isolates. Ciprofloxacin and ofloxacin showed a very good activity; sparfloxacin and levofloxacin were quite active, whereas, moxifloxacin had limited effects. Further and more specific studies, *in vivo*, are much recommended to determine the efficacy of quinolones in the treatment of brucellosis infections.

On the other hand, doxycycline and tetracycline had a quite good activity. Since rifampicin is commonly used for prevalent diseases such as tuberculosis, the regional susceptibility pattern of rifampicin should be assessed periodically.

Spiramycin, amoxicillin and cephalosporins were included in this study for research purposes only, as those agents are ineffective *in vivo* for brucellosis treatment.

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REFERENCES

1. Akalin, H.; Unal, S.; Gur, D.; Baykai, M. (1990). Ofloxacin in the treatment of brucellosis. *Eur. J. Clin. Microbiol. Dis.* 3, 326-328.
2. Akdeniz, H.; Irmark, H.; Anlar, O.; Demiroz, A.P. (1998). Central nervous system brucellosis:presentation, diagnosis and treatment. *J. Infect.* 36, 297-301.
3. Al Eissa, Y.A.; Kambal, A.M.; al Nasser, M.N.; al Habib, S.A.; al Fawaz, I.M.; al Zamil, F.A. (1990). Childhood brucellosis:a study of 102 cases. *Pediatr Infect. Dis. J.* 9, 74-79.
4. Anonymous. (1986). Joint FAO/WHO expert committee on brucellosis. *WHO Tech. Rep. Ser.* 740, 1-132.
5. Almariri, A. (2008). Ultraviolet C lethal effect on *Brucella melitensis*. *New Microbiologica.* 31, 47-55.
6. Baykam, N.; Esener, H.; Ergonul, O.; Eren, S.; Celikbas, A.K.; Dokuzoguz, B. (2004). *In vitro* antimicrobial susceptibility of *Brucella* species. *Intern. J. Antimicrob. Agents*. 23, 405-407.
7. Bodur, H.; Balaban, N.; Aksaray, S.; Yetener, V.; Akinci, E.; Colpan, A.; Erbay, A. (2003). Biotypes and antimicrobial susceptibilities of *Brucella* isolates. *Scand. J. Infect. Dis.* 35, 337-338.
8. Corbel, M.J. (1997). Brucellosis: an overview. *Emerg. Infect. Dis*. 3, 213-321.
9. DelVecchio, V.G.; Kapatral, V.; Redkar, R.J.; Patra, G.; Mujer, C.; Los, T.; Ivanova, N.; Anderson, I.; Bhattacharyya, A.; Lykidis, A.; Reznik, G.; Jablonski, L.; Larsen, N.; D'Souza, M.; Bernal, A.; Mazur, M.; Goltsman, E.; Selkov, E.; Elzer, P.H.; Hagius, S.; O'Callaghan, D.; Letesson, J.J.; Haselkorn, R.; Kyrpides, N.; Overbeek, R. (2002). The genome sequence of the facultative intracellular pathogen *Brucella melitensis*. *Proc. Natl. Acad. Sci. USA*. 99, 443-8.
10. Dimitrov, T.; Panigrahi, D.; Emara, M.; Awni, F.; Passadilla, R. (2004). Seroepidemiological and microbiological study of brucellosis in Kuwait. *Med. Princ. Pract.*13, 215-219.

11. Garcia-Rodriguez, J.A.; Garcia-Sanchez, J.E.; Trujillano, I. (1991). Lack of effective bactericidal activity of new quinolones against *Brucella* spp**.** *Antimicrob. Agents Chemother.* 35, 756–759**.**
12. Garcia-Rodriguez, J.A.; Garcia-Sanchez, J.E.; Trujillano-Martin, I.; Garcia-Sanchez, E.; Garcia-Garcia, M.I.; Fresnadillo, M.J. (1994). Activity of BAY y 3118, a novel 4-quinolone, against *Brucella melitensis*. *J. Chemother.* 6, 102–106.
13. Karabay, O.; Sencan, I.; Kayas, D.; Sahin, I. (2004). Ofloxacin plus rifampicin versus doxycycline plus rifampicin in the treatment of brucellosis: arandomized clinical trail [ISRCTN11871179]. *BMC Infct. Dis.* 4, 18.
14. Khan, M.Y.; Dizon, M.; Kiel, F.W. (1989). Comparative *in vitro* activities of ofloxacin, difloxacin, ciprofloxacin, and other selected antimicrobial agents against *Brucella melitensis*. *Antimicrob. Agents Chemother*. 33, 1409–1410.
15. Khuri-Bulos, N.A.; Daoud, A.H.; Azab, S.M. (1993). Treatment of childhood brucellosis:results of a prospective trail on 113 children. *Pediatr Infect. Dis. J.* 12, 377-381.
16. Kilic, S.; Dizbay, M.; Hizel, K.; Arman, D. (2008). *In vitro* synergistic activity of antibiotic combinations against *Brucella melitensis* using E-test methodology. *Braz. J. Microbiol.,* 39, 1-7.
17. Kocagoz, S.; Akova, M.; Altun, B.; Gur, D.; Hascelik, G. (2002). *In vitro* activities of new quinolones against *Brucella melitensis* isolated in a tertiarycare hospital in Turkey. *Clin. Microbiol. Infect.* 8, 240–242.
18. Lang, R.; Rubinistein, E. (1992). Quinolones for the treatment of brucellosis. *J. Antimicrob. Chemother.* 29, 357-363.
19. Lopez-Merino, A.; Contreras-Rodriguez, A.; Megranas-Ortiz, R.; Orranita-Gradin, G.M.; Hernandez-Oliva, A.; Gutierrez-Rubio, T.; Cardeñosa, O. (2004). Susceptibility of Mexican *brucella* isolates to moxifloxacin, ciprofloxacin and other antimicrobials used in the treatment of human brucellosis. *Scand. J. Infect. Dis.* 36, 636-638.
20. Marianelli, C.; Ciuchini, F.; Tarantino, M.; Pasquali, P.; Adone, R. (2004). Genetic bases of the rifampin resistance phenotype in *Brucella* spp. *J. Clin. Microbiol.* 42, 5439-5443.
21. Mert, A.; Kocak, F.; Ozaras, R.; Tabak, F.; Bilir, M.; Kucukugulu, S.; Ozturk, R,; Aktuglu, Y. (2002). The role of antibiotic treatment alone for the management of *Brucella* endocarditis in adults:a case report and literature review. *Ann. Thorac. Cardiovascu. Surg.* 8, 381-385.
22. National Committee for Clinical Laboratory Standards. (2003). Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. Approved standard M7-A6. *National Committee for Clinical Laboratory Standards*, Wayne, Pa.
23. Palenque, E., Otero, J.R.; Noregia, A.R. (1986). *In vitro* Susceptibility of *Brucella melitensis* to New Cephalosporins Crossing the Blood-Brain Barrier. *J. Antimicrob. Chemother.* P, 182-183.
24. Pappas, G.; Akritidis, N.; Bosilkovski, M.; Tsianos, E. (2005). Brucellosis. *N. Engl. J. Med.* 352, 2325-2336.
25. Qadri, S.M.H.; Akhter, J.; Ueno, Y.; Saldin, H. (1993). *In vitro* activity of 8 fluoroquinolones against clinical isolates of *Brucella melitensis*. *Ann. Saudi. Med.* 13, 37–40.
26. Qadri, S.M.; Halim, A.; Uneo, Y.; Abumustafa, M.; Postle, A.G. (1995). Antibacterial activity of azithromycin against *Brucella melitensis*. *Chemotherapy* (Basel). 41, 253-256.
27. Roushan, M.R.H.; Mohraz, M.; Hajlahmadi, M.; Ramzani, A.; Valayati, A.A. (2006). Efficacy of gentamicin plus doxycycline versus streptomycin plus doxycycline in the treatment of brucellosis in humans. *Clin. Infct. Dis.* 42, 1075-1080.
28. Trujillano-Martin, I.; Garcia-Sanchez, E.; Martinez, I.M.; Fresnadillo, M.J; Garcia-Sanchez, J.E.; Garcia-Rodriguez, J.A. (1999). *In vitro* activities of six new fluoroquinolones against *Brucella melitensis*. *Antimicrob. Agents Chemother.* 43, 194–195.
29. Turkmani, A.; Ioannidis, A.; Christidou, A.; Psaroulaki, A.; Loukaides, F.; Tselentis, Y. (2006). *In vitro* susceptibilities of *Brucella melitensis* isolates to eleven antibiotics. *Ann. Clin. Microbiol. Antimicrob*. 5, 24-29.
30. Yamazham, T.; Aydemir, S.; Tunger, A.; Serter, D.; Gokengin, D. (2005). *In vitro* activities of various antimicrobials against *Brucella melitensis* strains in the Aegean region in Turkey. *Med. Princ. Pract*. 14, 413-416.
31. Yilmaz, E.; Parlak, M.; Akalin, H.; Heper, Y.; Ozakin, C.; Mistik, R.; Oral, B.; Helvaci, S.; Töre, O. (2004). Brucellar spondylitis. Review of 25 cases. *J. Clin. Rheumatol.* 10, 300-307.