



Antimicrobial Susceptibility Testing and Molecular Genotyping of *Brucella melitensis* Isolates at the Human Animal Interface in Upper Egypt and Egyptian Boundaries



Nour H. Abdel-Hamid¹, Mai R. Zaffan¹, Mahmoud E. R. Hamdy¹, Maha A. Sabry², Mahmoud H. Abdel-Haleem¹, Ahmed Y. M. Elshiekh³, Walid Elmonir⁴ and Mahmoud E. Hashad⁵

¹WOAH Reference Laboratory for Brucellosis, Department of Brucellosis Research, Agricultural Research Center, Animal Health Research Institute, P.O. Box 264-Giza, Cairo 12618, Egypt.

²Department of zoonosis, Faculty of Veterinary Medicine, Cairo University, Cairo, Egypt.

³Department of Critical Care, Faculty of Medicine, Cairo University, Cairo, Egypt.

⁴Department of Hygiene and Preventive Medicine (Zoonoses), Faculty of Veterinary Medicine, Kafrelsheikh University, Kafrelsheikh, Egypt.

⁵Department of Microbiology, Faculty of Veterinary Medicine, Cairo University, Cairo, Egypt.

Abstract

THIS study aimed to assess the antimicrobial susceptibility testing (AST) using the disc diffusion method of *Brucella melitensis* isolates (n = 42) recovered from humans and slaughtered seropositive animals to 10 antibiotics commonly prescribed for the treatment of human brucellosis. Additionally, we used ERIC-PCR to evaluate the genetic diversity of *Brucella* isolates recovered from animals and humans in Upper Egypt and the Egyptian borders. The *Brucella* isolates from small ruminants (n = 29) and humans (n = 13) with fevers of unknown cause were identified as *B. melitensis* biovar 3 by both bacteriological and molecular methods. Concerning susceptibility to antibacterial therapeutics, three human isolates (3/13; 23%) and 23 animal isolates (23/29; 79.3%) showed resistance to rifampicin. All *B. melitensis* strains recovered from small ruminants and six (46%) strains of human origin conferred resistance to sulfamethoxazole-trimethoprim, marking a first record in Egypt. All the isolates were resistant to ciprofloxacin. ERIC-PCR fingerprinted the *B. melitensis* selected strains into 18 (M1-M18) ERIC types (ET), of which 2 ERIC types consist of 2 identical strains: one ET (M1_Human_Fayoum) for human isolates (2 isolates) and another ET (M8_Goat_Wadi Jadid) for goat isolates (2 isolates). In conclusion, most Egyptian *B. melitensis* isolates recovered in our study were susceptible to most antibiotics commonly prescribed for human brucellosis treatment. The *in vitro* resistance of Egyptian *B. melitensis* strains to sulfamethoxazole-trimethoprim, ciprofloxacin, and rifampicin highlights the necessity of regular antibiotic susceptibility testing and breakpoint updating to minimize human brucellosis relapse cases. The emergence of resistant *Brucella* strains to various antibiotics recommended by the WHO (first and alternative therapies) will reduce the efficacy of treatment options, potentially leading to further complications. The high genetic diversity of *B. melitensis* bv3 based on ERIC-PCR fingerprinting patterns demonstrates the simplicity, reliability, and cost-effective genotyping approach for distinguishing between *Brucella* strains, particularly in developing countries.

Keywords: *Brucella*, ERIC-PCR, AST, genotypes, Egyptian borders.

Introduction

Brucellosis is a highly contagious and zoonotic disease affecting terrestrial mammals. and is considered a neglected zoonosis, especially in

developing countries [1]. Four *Brucella* species, *B. melitensis*, *B. abortus*, *B. suis*, and *B. canis*, have been documented and identified in Egypt using either traditional bacteriology or molecular methods [2–6].

*Corresponding author: Nour H. Abdel-Hamid, E-mail: nour78_78@yahoo.com, Tel.: +201117959509 (Received 22 July 2024, accepted 16 September 2024)

DOI: 10.21608/EJVS.2024.306357.2270

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Each *Brucella* species has a preferred host, but they can also infect other animal species, including domestic livestock, wild animals, and humans. *Brucella* species are highly infectious and can be transmitted to humans through direct contact with infected animals, ingestion of contaminated milk and milk products, inhalation of aerosols, or laboratory exposure. In animals, brucellosis can cause reproductive disorders, including abortion, infertility, and decreased milk production, with significant economic implications for the livestock industry [7].

Specific biovars are more common in specific geographic areas. In Egypt, *B. melitensis* biovar 3 is enzootic in both native hosts (goats and sheep) and atypical hosts such as cattle, buffaloes, and dogs [4,8–10].

Brucellosis in humans is often characterized by fever, night sweats, fatigue, headaches, and muscle and joint pain. In certain situations, it might result in more serious complications, including endocarditis, neurobrucellosis, or osteoarticular involvement. The condition might persist and recur if not treated adequately [11]. Unfortunately, human brucellosis symptoms are broad, non-specific, and comparable to those of other febrile diseases, resulting in inaccurate diagnosis and inadequate medical treatment [12].

Previous studies showed the therapeutic challenges of brucellosis in the Middle East and North African countries. Several Middle Eastern countries, including Iran, Saudi Arabia, Iraq, Egypt, and Turkey, are endemic to both animal and human brucellosis. Many relapse and antibiotic-resistant cases were reported from these regions. This relapse may be due to sequestration within infected sites or the development of acquired or intrinsic resistance against antimicrobial therapies [13].

Timely and appropriate antibiotic therapy is crucial for the successful management of human brucellosis. Doxycycline, rifampicin, streptomycin, gentamicin, and trimethoprim-sulfamethoxazole have shown consistent efficacy against *Brucella* species. However, the emergence of antibiotic resistance highlights the importance of ongoing antimicrobial resistance (AMR) and the development of novel treatment strategies. Even though few studies have been carried out to investigate the antimicrobial resistance of *Brucellae* worldwide and in Egypt [12,14,15]. However, further studies are needed to assess the extent and impact of AMR *Brucella* on livestock and humans in Egypt.

In the Fayoum governorate of Upper Egypt, researchers conducted a study to identify risk factors for brucellosis and molecular genotyping of *B. melitensis* isolates from humans and livestock. Such a study regarded combined REP-PCR/virulence genotyping as a quick tool for tracing the source of infection, particularly in developing endemic countries like Egypt [10].

The Enterobacterial Repetitive Intergenic Consensus (ERIC) sequences are short DNA repeats found throughout the genomes of most bacteria. These sequences are homologous but polymorphic. ERIC-PCR targets and amplifies these sequences in between repeats using semi-specific primers. The size and number of amplicons produced will vary depending on how many elements are clustered together, their orientation, and their distance apart [16].

Repetitive extragenic palindromic sequences are utilized in the ERIC-PCR technique, which is widely used to genotype and distinguish between *B. melitensis* strains. It is considered a favorable approach because it is a sensitive, rapid, and simple technique for the epidemiological investigation of brucellosis if performed under certain established conditions [17].

This study aimed to evaluate the antimicrobial susceptibility of *Brucella* isolates from humans and slaughtered seropositive animals in Egyptian borders and Upper Egypt to 10 antibiotics commonly prescribed for human brucellosis treatment using the disc diffusion method. Also, to seek genetic diversity and genotyping of *Brucella* isolates recovered from the study area.

Material and Methods

Study design and sampling

A total of 125 supramammary lymph nodes, 80 retropharyngeal lymph nodes, 15 aborted fetuses, and 85 milk samples were collected from seropositive sheep and goats raised in private farms along the Egyptian borders (Wadi Jadid, Red Sea, and Mersa Matruh). Furthermore, samples were also obtained from sheep and goats raised in private farms and households in Upper Egypt (Giza, Fayoum, Assiut, Beni Suef, Sohag, Aswan, and Qena). We targeted private farms and households in response to notifications of seropositive animals and late-stage abortions. Animals confirmed positive for brucellosis using the complement fixation test (CFT) were sent to government abattoirs to be slaughtered as part of the emergency slaughter for brucellosis, as stated in Ministerial Decree No. 1067 in 1988 issued in Egypt. This was done to comply with the current control strategy for brucellosis, which involves testing and slaughtering adult animals that test positive for the disease, providing compensation, and vaccinating young animals. Blood samples (n = 109) were collected from humans suffering from fevers of unknown origin. Blood samples were collected from human cases recruited to the Regional Fever Hospitals in Giza City and Fayoum to confirm the diagnosis and receive treatments.

Isolation and full typing of *Brucella* isolates at the genus and biovar levels

Isolation and identification of *Brucella* isolates were done following the methods described by Alton et al. [18] and WOA (1). A lysis-concentration technique was performed on blood samples to isolate *Brucella*, as described by Etemadi et al. [19].

Colonial morphology, biochemical tests (catalase, oxidase, and urease), and the acriflavine test were performed to type *Brucella* isolates at the genus level. *Brucella* biovar-specific tests were carried out, viz., CO₂ requirement, H₂S production, growth in the presence of thionin and basic fuchsin dyes, and agglutination with mono-specific anti-sera (A, M, and R).

Molecular typing of Brucella isolates at the species level using AMOS-PCR:

DNA extraction from *Brucella* cultures was done using the QIAamp DNA Mini kit (Qiagen, Germany) based on the manufacturer's instructions. For *Brucella* speciation, the AMOS PCR method was used, as described by Bricker and Halling [20].

Antimicrobial susceptibility testing of B. melitensis isolates:

For each isolate, a bacterial suspension was prepared using pure *Brucella* colonies. The tube turbidity was adjusted to match the 0.5 McFarland turbidity standard. Swabs of the suspensions were spread onto Muller-Hinton agar plates containing 5% defibrinated sheep blood and incubated at 37 °C. Antimicrobial susceptibility testing using the disc diffusion method was conducted to determine the susceptibility of *Brucella* isolates against ten antibiotics regularly used in human brucellosis treatments. The selection of these ten antibiotics was determined according to the treatment guidelines for human brucellosis provided by the World Health Organization [21].

The inhibition zone diameter breakpoints were determined according to the manufacturer's instructions and the instructions adopted for *B. melitensis* by the European Committee on Antimicrobial Susceptibility Testing [22] for ciprofloxacin (5µg), ceftriaxone (30µg), streptomycin (10µg), doxycycline, sulfamethoxazole-trimethoprim, tetracycline (30µg), gentamycin (10µg), and rifampicin (5µg) (HiMedia, India). The inhibition zone diameter (mm) interpretation for florfenicol and azithromycin was carried out according to EUCAST guidelines [22] and CLSI guidelines [23] for the fastidious bacterium *Haemophilus influenza*.

Enterobacterial Repetitive Intergenic Consensus-Polymerase Chain Reaction (ERIC-PCR) technique-based Brucella melitensis genotyping

Among the 42 *Brucella melitensis* isolates recovered in this investigation, twenty (n = 20) were selected for fingerprinting by the ERIC-PCR technique, four (n = 4) were obtained from humans, and the remaining strains (n = 16) were recovered from small ruminants. Along with the reference strain Ether ATCC 23458, *Brucella* isolates were fingerprinted by the ERIC-PCR technique using the ERIC1R primer (5'-ATG TAA GCT CCT GGG GAT TCA C-3') and the ERIC2 primer (5'-AAGTAAGTGAC TGGGGTGAGCG-3').

The ERIC-PCR was done according to Abdel-Hamid et al. [17]. A dendrogram based on ERIC-PCR polymorphic band patterns was generated using GelJ software v.2.3, Jaccard coefficients, and unweighted pair group techniques [24]. Simpson's diversity index of ERIC-PCR was calculated through the online website

<https://www.omnicalculator.com/statistics/simpsons-diversity-index>.

Results

Bacteriological typing of Brucella isolates from humans and small ruminants:

The phenotypic characterization revealed that all the *Brucella* isolates grew as smooth colonies, and they were all positive for catalase, oxidase, and urease tests. All isolates were CO₂-independent with no H₂S production, and they were able to grow on media containing thionin and fuchsin dyes at a concentration of 1/50000. Positive agglutination was recorded by all isolates with anti-*Brucella* monospecific, anti-A, and anti-M monospecific sera. These characteristics are all typical for *B. melitensis* biovar 3.

For species identification by AMOS PCR, all *Brucella* isolates (n = 42) recovered from humans and small ruminants illustrated DNA bands at 731 bp specific to *B. melitensis*.

The 29 *B. melitensis* strains of animal origin were isolated from milk samples (n = 8), supramammary lymph nodes (n = 15), and retropharyngeal lymph nodes (n = 4) of seropositive sheep and goats, as well as from private goat farms, after notification of late-stage abortions. The remaining two *Brucella* strains were recovered from the aborted fetuses of a seropositive sheep and a seropositive goat, one each.

Four *Brucella* strains of human origin (n = 13) were obtained from human blood samples of farmers in Fayoum (n = 3) and Sohag (n = 1), who kept small and large ruminants inside their houses, from a man who reported no contact with animals at all, and from two butchers in Giza and Assuit governorates (Table 1). Another four *Brucella* strains of human origin were recovered from shepherds in Giza, Beni-Suef,

Assuit, and Sohag, who kept seropositive sheep in their herds. Two *Brucella* isolates were retrieved from field veterinarians in the Giza and Fayoum governorates (Table 1).

Antimicrobial susceptibility of B. melitensis isolates:

Antibiotic susceptibility testing of *B. melitensis* strains showed that all strains were susceptible to florfenicol, doxycycline, gentamicin, streptomycin, tetracycline, and azithromycin. Ten *B. melitensis* isolates of human origin (10/13; 76.9%) and six from animals (6/29; 20.6%) showed susceptibility to rifampicin, while three human isolates (3/13; 23%) and 23 animal isolates (23/29; 79%) showed resistance to rifampicin. *Brucella* isolates of animal origin showed resistance to sulfamethoxazole-trimethoprim, while seven human isolates (7/13; 53.8%) showed susceptibility, and six showed resistance (6/13; 46.2%). All the *Brucella* isolates of human and animal origins (n = 42) showed resistance to ciprofloxacin (Table 2).

Brucella genotyping and genetic diversity based on ERIC-PCR fingerprinting patterns:

The ERIC-PCR fingerprinting approach differentiated the *B. melitensis* isolates, selected as resistant to sulfamethoxazole-trimethoprim, ciprofloxacin, and rifampicin, into two distinct phylogenetic branches (Cluster 1 and Cluster 2) with 67% similarity (Figure 1). The reference strain Ether, *B. melitensis* bv3, was grouped with nine *B. melitensis* strains isolated from goats in Wadi Jadid (Egypt's West Border Governorate) and Assuit (Upper Egyptian Governorate) to form a single cluster (cluster 2). *B. melitensis* biovar 3 strains isolated from Egyptian local goat breeds of cluster (2) have a genetic similarity of 69%. *B. melitensis* isolates obtained from humans (n = 4) showed a 77% genetic similarity with strains isolated from goats (n = 6) and sheep in five Upper Egyptian governorates (Fayoum, Beni-Suef, Assuit, Sohag, and Aswan), as well as a border governorate (Wadi Jadid).

ERIC-PCR genotyping differentiates *B. melitensis* selected strains of both clusters into 18 ERIC types (M1-M18), excluding the reference strain, 16 are singleton (representing single strain) ERIC types (Figures 1 and 2). The remaining two ERIC types include two identical strains each, M1_Human_Fayoum and M8_Goat_Wadi Jadid. Both shared genotypes circulate in humans and goats in Fayoum and Wadi Jadid.

Simpson's diversity index of ERIC-PCR was calculated to be 0.88, showing the high discriminatory power of ERIC-PCR as a DNA-based fingerprinting tool in the context of this investigation.

Discussion

Antibiotics have played a pivotal role in mitigating the impact of brucellosis by providing effective therapeutic options. However, the emergence of antibiotic resistance in *Brucella* species poses a critical challenge, necessitating a comprehensive understanding of the antibiotic susceptibility patterns in different regions.

This is particularly true for countries like Egypt, where the prevalence of *Brucella* infections in livestock and the possibility of transmission to humans are areas of considerable concern. Despite the lack of routine susceptibility testing for *Brucella* spp. due to ineffective farm animal treatment, human medicine urgently follows strict therapy regimes instead of periodic *in vitro* susceptibility testing.

Despite several studies that have been carried out to investigate the antimicrobial resistance of *Brucellae* worldwide, only three studies were done in Egypt on *Brucella* isolates recovered from humans, animals, or both [12,14].

In vitro, a variety of antimicrobial medicines are effective against *Brucella* species; however, routine susceptibility testing may not always correlate with clinical effectiveness [25].

A new phenotypic resistance of *Brucella* isolates to trimethoprim-sulfamethoxazole is observed in this study, which is the first record in Egypt, as previous studies have found *B. melitensis* to be susceptible to sulfamethoxazole-trimethoprim, which was applied on 355 *Brucella* spp. isolates recovered from acute febrile ill patients [14], 34 *Brucella* isolates from animals [15], and 35 *B. melitensis* and *B. abortus* strains isolated from animals and humans [12]. Sulfamethoxazole-trimethoprim resistance has also been also conferred by *Brucella* isolates in other countries, including Saudi Arabia. Elbehiry et al. [26] reported a 35.36% resistance rate to sulfamethoxazole-trimethoprim among ten *B. melitensis* isolates using the E-test and disc diffusion methods. Intermediate resistance was observed in Iran. E-test was used on 149 human isolates [27].

Resistance to ciprofloxacin was reported in this study, which is in line with the findings of Khan et al. [15], unlike previous studies that showed *in vitro* susceptibility to ciprofloxacin [12,14]. *Brucella* isolates from this study showed diversity in susceptibility to rifampicin. The rest of the antibiotics showed no differences from previous studies.

According to the WHO, the first treatment for early *Brucella* infection in humans is rifampicin and doxycycline for 6 weeks; and doxycycline for 45 days plus streptomycin for two to three weeks [28].

Other studies recommended the doxycycline-aminoglycoside combination as the first drug of choice for the treatment of uncomplicated brucellosis. While doxycycline-rifampin or doxycycline-sulfamethoxazole-trimethoprim should be alternatives, quinolones can be considered as an alternative regimen.

A 6-week regimen of sulfamethoxazole, trimethoprim, and rifampin may be the most appropriate treatment for children younger than eight years of age. Alavi et al. [29] suggest using a combination of sulfamethoxazole-trimethoprim for 6 weeks and gentamicin for 5 days. Additionally, Maves et al. [30] used a combination of sulfamethoxazole-trimethoprim, doxycycline, and rifampin to treat *Brucella*-induced endocarditis.

Different authors reported that sulfamethoxazole-trimethoprim is an effective antibiotic with low MIC levels [14,31,32]. Sulfamethoxazole-trimethoprim was found to be the most effective antimicrobial agent in treating human brucellosis [33]. However, a high resistance rate to sulfamethoxazole-trimethoprim has been noticed in Saudi Arabia [34,35]. Yet, no relapse was noticed when sulfamethoxazole-trimethoprim was used in combination with rifampicin with or without streptomycin [36].

Phenotypic resistance strains of *B. melitensis* to both rifampicin and sulfamethoxazole-trimethoprim as recorded in our study may lead to repetitive relapse and consequently to adverse complications as reported in previous studies [12,13].

The increase in phenotypic-resistant *Brucella* strains, as reported in Egyptian-published articles [12,14,15,37] and elsewhere [38,39], may be attributed to the dependence on strict medical regimens without performing regular antimicrobial susceptibility testing and, lack of standards for antibiotics' inhibition zone diameter breakpoint interpretation for *Brucellae* either in the CLSI or EUCAST protocols; instead, the interpretation relies upon zone diameter interpretation criteria of other fastidious micro-organisms like *Hemophilus influenza*.

Fortunately, EUCAST [22] provides zone diameter breakpoints (mm) for some drugs frequently used to treat human brucellosis caused by *B. melitensis* infection. Depending on the interpretation of these antibiotics, the resistance pattern of *B. melitensis* in the same report [22] differs dramatically from that of *Hemophilus influenza*.

In *B. melitensis*, resistance patterns to ciprofloxacin, sulfamethoxazole-trimethoprim, and rifampicin are less than 27 mm, 29 mm, and 20 mm, respectively. The analogous resistance pattern for

Hemophilus influenza is less than 30 mm, 20 mm, and 18 mm, respectively. The sulfamethoxazole-trimethoprim zone diameter breakpoints (mm) for the *B. melitensis* isolates in this study were between 24 and 27 mm. Based on the interpretation of zone diameter breakpoints (mm) for *B. melitensis* [22], we considered the isolates to be resistant to sulfamethoxazole-trimethoprim.

In contrast, *B. melitensis* isolates with the same figures of zone diameter breakpoints (mm) for sulfamethoxazole-trimethoprim will be considered susceptible if we depend upon the criteria provided for *Hemophilus influenza* in the same report of EUCAST [22]. Thus, depending on the zone diameter breakpoints (mm) interpretation for sulfamethoxazole-trimethoprim using the criteria provided for *Hemophilus influenza* may explain the increased rate of relapse cases of brucellosis in the last years when sulfamethoxazole-trimethoprim was prescribed for human brucellosis treatment as a secondary alternative therapy, and the same was true for other antibiotics. As suggested by Liu et al. [40,41], and Wareth et al. [12], this hypothesis could be one of the alternative explanations for why *B. melitensis*, which phenotypically resists rifampicin based on zone diameter breakpoints (mm) interpretation for *Hemophilus influenza*, exhibits a lack of *rpoB* gene mutations. The second hypothesis for increasing *Brucellae* phenotypic resistance to antibiotics is the use of sub-antibiotic dosages or insufficient duration of treatment. Finally, the random and uncontrolled sale (over-the-counter) of antibiotics, particularly sulfamethoxazole-trimethoprim, ciprofloxacin, and others, in Egyptian veterinary and human pharmacies expatriates drug resistance to numerous bacterial diseases, including *Brucella*.

The illegal introduction or smuggling of animals infected with brucellosis from the Egyptian western and southern borders [42] supports another theory that suggests the spread of new genotypes, or *B. melitensis* strains, to the border governorates and, consequently, the spread of infection to other governorates with those strains of *Brucella* that may phenotypically resist ciprofloxacin, rifampicin, and sulfamethoxazole-trimethoprim.

ERIC sequences are found in a wide range of bacterial genomes, including those of Enterobacteriaceae and *Brucellae* [43]. ERIC is often used in gram-negative enteric bacteria as a repetitive extragenic palindromic PCR (rep-PCR) test using primers that target highly conserved repetitive sequence elements [44].

ERIC-PCR was used in this investigation to distinguish between the selected 20 isolates of *B. melitensis* bv3. By using ERIC-PCR, *B. melitensis*

isolates were categorized into two clusters (1 and 2) and 18 *B. melitensis* Eric types (M1–M18). Given a discrimination score of 0.89, our genotyping tool demonstrated an acceptable level of discriminatory power. According to prior reports, the ERIC-PCR can effectively differentiate *Brucella* isolates to the strain level, with the possibility of intra-species discrimination between individual strains [17,45,46].

Variations in the genotypes found could indicate that multiple outbreak strains were brought into the research area before the bilateral certificate denoting animals free of brucellosis was put into place, during the open importation policy period of the 1970s through the 1990s.

For *B. melitensis* bv3, which we identified in our investigation, as well as *B. melitensis* isolated from ruminants outside the study area in the Nile Delta and Upper Egyptian governorates, ERIC-PCR showed sufficient intra-species discrimination. This could make it possible to use ERIC-PCR as an epidemiological tool for effectively distinguishing between different *Brucella* isolates and in routine epidemiological surveillance. These results support the use of ERIC-PCR as a rapid, low-cost method for *B. melitensis* traceability in low-income countries endemic to brucellosis.

The Simpson diversity index score has been calculated for the ERIC PCR. It ranges from 0 to 1 [47]. A high score indicates greater diversity, whereas a low score indicates less diversity. The diversity index was estimated to be 0.88, showing the high discriminatory power of ERIC-PCR in the context of this investigation and matching the results reported by Abdel-Hamid *et al.* [17] and Mustafa *et al.* [46].

Conclusion

Understanding the antibiotic susceptibility profiles of *Brucella* species in Egypt is of paramount importance. This knowledge not only informs

treatment decisions but also contributes to the broader understanding of antimicrobial resistance trends in zoonotic pathogens. New resistant strains to sulfamethoxazole-trimethoprim appeared in Egypt. Most Egyptian *B. melitensis* isolates are still susceptible to antibiotics commonly used for human brucellosis treatment. The *in vitro* resistance of Egyptian *B. melitensis* strains to sulfamethoxazole-trimethoprim, ciprofloxacin, and rifampicin highlights the necessity of regular antibiotic susceptibility testing instead of applying strict medical regimens to decrease the increase in human brucellosis relapse cases. The cost-discrimination effectiveness of repeatable ERIC-PCR is demonstrated by the high genetic diversity of *B. melitensis* bv3 fingerprinted in this study and elsewhere.

Conflicts of interest

The authors declared no competing interests

Funding sources

This research receives no external fund.

Acknowledgment

The authors would like to express their gratitude to the director of the Animal Health Research Institute and the research committee for permitting them to perform the study

Ethical approval

Agricultural Research Center, the Institutional Animal Care and Use Committee (ARC-IACUC; 105/24) approved the study protocol, which adheres to the World Organization guidelines for Animal Health (WOAH) and guides for the care and use of laboratory animals' 8th edition 2011. The scientific research ethics committee, Faculty of Medicine, Fayoum University, Egypt (No. 195/79) approved the study design. We obtained written informed consent from all the participants.

TABLE 1. Susceptibility of *B. melitensis* strains recovered from humans and small ruminants in Upper Egypt and Egypt's border governorates to sulfamethoxazole-trimethoprim, rifampicin, and ciprofloxacin

S N	Specimen	Month / Year	Location	Species	Description	Disc diffusion method		
						Sulfamethoxazole-trimethoprim (30µg)	Rifampicin (5µg)	ciprofloxacin (5µg)
1	Blood	4/23	Fayoum	Human	Male, farmer	R	S	R
2	Blood	9/23	Fayoum	Human	Male, farmer	R	S	R
3	Blood	10/23	Fayoum	Human	Female, farmer	R	S	R
4	Blood	8/23	Fayoum	Human	Male, veterinarian	R	S	R
5	Blood	11/22	Assuit	Human	Male, butcher	S	R	R
6	Blood	12/22	Sohag	Human	Male, shepherd	R	S	R
7	Blood	3/23	Giza	Human	Male, veterinarian	S	R	R
8	Blood	10/22	Giza	Human	Male, butcher	R	R	R
9	Blood	1/23	Giza	Human	Male, no history of animal contact	S	S	R
10	Blood	1/23	Giza	Human	Male, shepherd	S	S	R
11	Blood	2/23	Beni-Suef	Human	Male, shepherd	S	S	R
12	Blood	2/23	Assuit	Human	Male, shepherd	S	S	R
13	Blood	1/23	Sohag	Human	Male, farmer	S	S	R
14	S.L.N.	2/23	Wadi Jadid	Goat	Late-stage abortion	R	R	R
15	S.L.N.	2/23	Wadi Jadid	Goat	Late-stage abortion	R	R	R
16	S.L.N.	2/23	Wadi Jadid	Goat	Late-stage abortion	R	R	R
17	S.L.N.	2/23	Wadi Jadid	Goat	Late-stage abortion	R	S	R
18	S.L.N.	2/23	Wadi Jadid	Goat	Late-stage abortion	R	S	R
19	S.L.N.	2/23	Wadi Jadid	Goat	Late-stage abortion	R	R	R
20	R.L.N.	2/23	Wadi Jadid	Goat	Late-stage abortion	R	R	R
21	R.L.N.	3/23	Wadi Jadid	Goat	Late-stage abortion	R	S	R
22	Aborted fetus	3/23	Assuit	Goat	Seropositive animal	R	R	R
23	Milk	7/23	Assuit	Goat	Seropositive animal	R	S	R
24	Milk	7/23	Assuit	Goat	Seropositive animal	R	R	R
25	Milk	3/23	Assuit	Goat	Seropositive animal	R	S	R
26	S.L.N.	3/23	Assuit	Goat	Seropositive animal	R	R	R
27	S.L.N.	3/23	Assuit	Goat	Seropositive animal	R	S	R
28	Aborted fetus	6/23	Aswan	Sheep	Seropositive animal	R	R	R
29	Milk	7/23	Beni-Suef	Goat	Seropositive animal	R	R	R
30	Milk	7/23	Beni-Suef	Goat	Seropositive animal	R	R	R
31	R.L.N.	8/23	Beni-Suef	Goat	Seropositive animal	R	R	R
32	S.L.N.	8/23	Beni-Suef	Goat	Seropositive animal	R	R	R
33	Milk	7/23	Beni-Suef	Goat	Seropositive animal	R	R	R
34	Milk	9/23	Red Sea	Goat	Seropositive animal	R	R	R
35	Milk	9/23	Red Sea	Goat	Seropositive animals	R	R	R
36	S.L.N.	9/23	Mersa Matruh	Goat	Seropositive animal	R	R	R
37	S.L.N.	9/23	Mersa Matruh	Goat	Seropositive animal	R	R	R
38	S.L.N.	5/23	Qena	Goat	Late-stage abortion	R	R	R
39	S.L.N.	5/23	Sohag	Goat	Late-stage abortion	R	R	R
40	S.L.N.	3/23	Beni-Suef	Goat	Seropositive animal	R	R	R
41	S.L.N.	4/23	Giza	Goat	Seropositive animal	R	R	R
42	S.L.N.	6/23	Aswan	Goat	Seropositive animal	R	R	R

SN: sample number, S.L.N: supramammary lymph node, R.L.N.: retropharyngeal lymph node, R: resistant, S: susceptible

TABLE 2. Inhibition zone (mm) diameters and interpretation of antimicrobial sensitivity test results for *Brucella melitensis* isolates recovered from humans and small ruminants in Upper Egypt and Egypt's border governorates.

Antibiotics	Disk content (µg)	Inhibition zone diameter breakpoints (mm)		Inhibition zone diameter breakpoints (mm)			
				S ≥		R <	
		S	R	H	A	H	A
Florfenicol	30	≥ 28	< 28	13	29	-	-
Sulfamethoxazole-trimethoprim	25	≥ 29	< 29	7	0	6	29
zithromycin	15	≥ 12	-	13	29	-	-
Doxycycline	30	≥ 42	< 42	13	29	-	-
Tetracycline	30	≥ 42	< 42	13	29	-	-
Ciprofloxacin	5	≥ 50	< 27	-	-	13	29
Ceftriaxone	30	≥ 30	< 30	13	29	-	-
Rifampicin	5	≥ 20	< 20	10	6	3	23
Streptomycin	10	≥ 15	< 15	13	29	-	-
Gentamicin	10	≥ 23	< 23	13	29	-	-

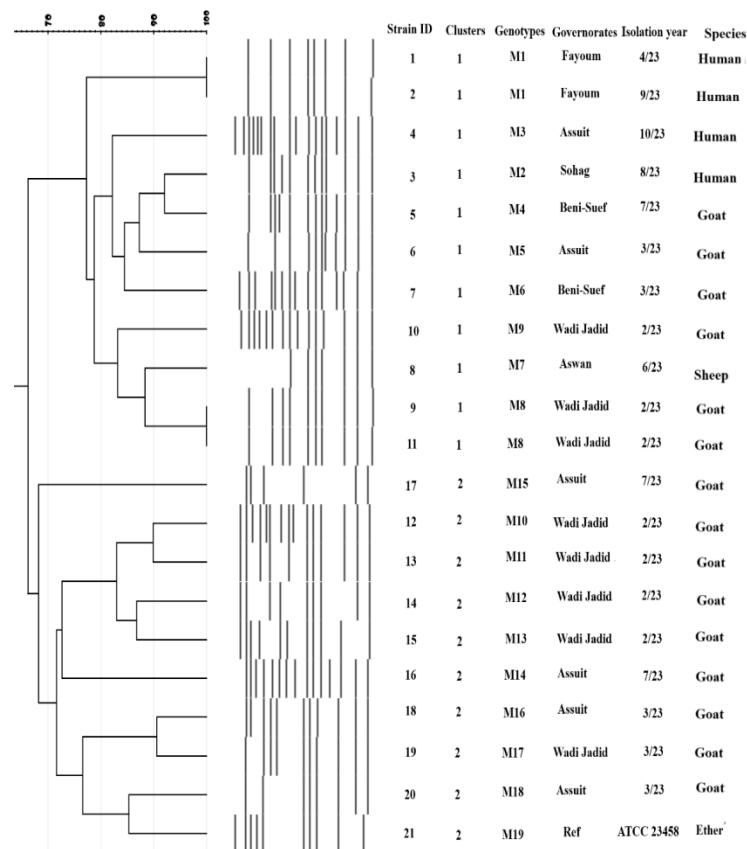


Fig. 1. Cluster and genotypic analysis of ERIC-PCR fingerprints of 21 *B. melitensis* field strains recovered in Egypt, including the reference strain Ether. The band profiles of each strain match the lines of the dendrogram. Columns reflect ERIC genotypes, clusters, governorates, isolation year, species, and strain serial numbers.

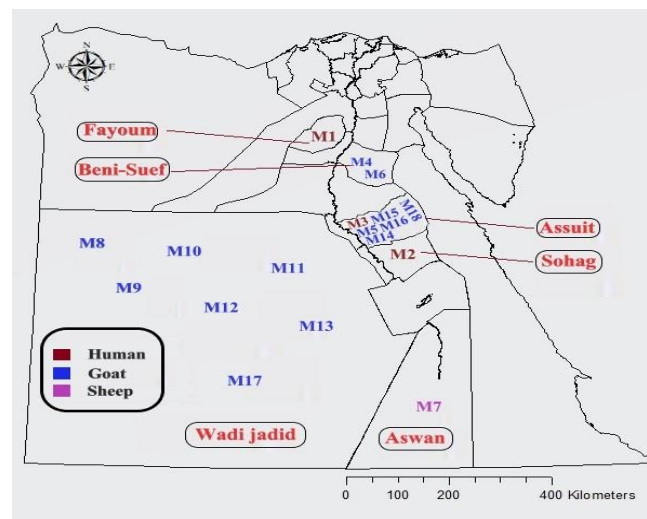


Fig. 2. Local *B. melitensis* genotypes recovered from humans and small ruminants' distribution in some Upper Egyptian and boundary governorates.

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اختبار الحساسية للمضادات الحيوية والتنميط الجيني لمعزولات البروسيلات الماطية (ميليتنسيز) المعزولة من الإنسان والحيوان في صعيد مصر والحدود المصرية

نور الدين حسني عبد الحميد¹ ، مي رمضان زعفان¹ ، محمود عصام رشاد¹ ، مها صبري² ، محمود حسن عبد الحليم¹ ، أحمد يحيى الشيخ³ ، وليد ماجد المنير⁴ و محمود حشاد⁵

¹ قسم بحوث البروسيلات، المختبر المرجعي للبروسيلات للمنظمة العالمية للصحة الحيوانية – معهد بحوث الصحة الحيوانية، مركز البحوث الزراعية، القاهرة، مصر.

² قسم الامراض المشتركة، كلية الطب البيطري، جامعة القاهرة، القاهرة، مصر.

³ قسم طب الحالات الحرجة، جامعة القاهرة، القاهرة، مصر.

⁴ قسم الصحة والطب الوقائي، كلية الطب البيطري، جامعة كفر الشيخ، كفر الشيخ، مصر.

⁵ قسم الميكروبيولوجيا، كلية الطب البيطري، جامعة القاهرة، القاهرة، مصر.

الملخص

هدفت هذه الدراسة إلى تقييم اختبار الحساسية للمضادات الحيوية باستخدام طريقة الانتشار القرصي لمعزولات البروسيلات ميليتنسيز (العدد = 42) المعزولة من الحالات البشرية المصابة والحيوانات المذبوحة والإيجابية للفحص السيرولوجي للبروسيلات وذلك باستخدام 10 أنواع من المضادات الحيوية التي توصف عادة لعلاج داء البروسيلات في الإنسان. بالإضافة إلى ذلك، استخدمنا اختبار ERIC-PCR لتقييم التنوع الجيني لمعزولات البروسيلات المعزولة من الحيوانات والإنسان في صعيد مصر والحدود المصرية. وقد تم تصنيف البروسيلات المعزولة من المجترات الصغيرة (ن = 29) والحالات البشرية (ن = 13) المصابة بحمى غير معلومة المصدر على أنها بروسيلات ميليتنسيز النوع الحيوي 3 باستخدام الطرق البكتريولوجية والجزيئية. وفيما يتعلق بالحساسية للعلاج بالمضادات الحيوية، أظهرت ثلاث معزولات معزولة من الحالات البشرية المصابة (13/3؛ 23%) و 23 معزولة حيوانية (29/23؛ 79.3%) مقاومة للريفامبيسين. كما أظهرت جميع سلالات البروسيلات ميليتنسيز المعزولة من المجترات الصغيرة وستة عترات من البروسيلات (46%) معزولة من الحالات البشرية المصابة مقاومة لسلفاميثوكسازول-تريميثوبريم، مما يمثل أول تقرير يرصد ذلك في مصر. وأخيراً أظهرت جميع المعزولات مقاومة للسبيروفلوكساسين. استطاع اختبار ERIC-PCR تمييز سلالات البروسيلات ميليتنسيز المختارة إلى 18 نمط جيني (M1-M18)، منهم نمطان يتكون كلا منهما من سلالتين متطابقتين: واحدة (M1_Human_Fayoum) ET من معزولات بشرية (معزولتان) وأخرى (ET (M8_Goat_Wadi New) لمعزولات من الماعز (2 معزولة). في الختام، فإن معظم معزولات البروسيلات ميليتنسيز المصرية المعزولة في دراستنا أظهرت حساسية لمعظم المضادات الحيوية الموصوفة عادة لعلاج داء البروسيلات في الإنسان. تسلط المقاومة المخبرية لسلالات البروسيلات ميليتنسيز المصرية لسلفاميثوكسازول-تريميثوبريم، وسبيروفلوكساسين، وريفامبيسين الضوء على ضرورة إجراء اختبار حساسية للمضادات الحيوية بشكل منتظم وتحديث تفسير قطر التثبيط لأقراص المضادات الحيوية وذلك لتقليل حالات انتكاسات داء البروسيلات في الحالات البشرية المصابة. إن ظهور سلالات البروسيلات المقاومة لمختلف المضادات الحيوية التي أوصت بها منظمة الصحة العالمية (العلاجات الأولى والبدلية) سوف يقلل من فعالية الخيارات العلاجية، مما قد يؤدي إلى مزيد من مضاعفات المرض. يوضح التنوع الجيني العالي للبروسيلات ميليتنسيز استناداً إلى التنميط الجيني بواسطة اختبار ERIC-PCR البساطة والموثوقية ونهج التنميط الجيني الفعال من حيث التكلفة للتمييز بين سلالات البروسيلات، خاصة في البلدان النامية.

الكلمات الدالة: البروسيلات والإيريك بي سي ار واختبار الحساسية للمضادات الحيوية والانماط الجينية والحدود المصرية.