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# The contamination of local crumbled Kope cheeses distributed in Urmia-Iran with *Brucella* species and evaluation of the antibiotic-resistant pattern of isolates

## *Brucella* in Urmia local Kope cheeses and antibiotic resistance

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**ABSTRACT**

Brucellosis is a momentous zoonotic disease. Unpasteurized milk and milk products are the leading sources of *Brucella* transmission to humans. The present research was conducted to investigate the contamination of local crumbled Kope cheeses distributed in Urmia city with *Brucella* species and evaluate the antibiotic-resistance pattern of the isolates. Fifty samples of local cow's Kope cheese were randomly collected from traditional dairy retailers in different regions of Urmia by sterile conditions in 2022. The samples were cultured in *Brucella* enrichment broth and then in *Brucella* selective agar with a supplement. Molecular identification of *Brucella* spp. was done using specific primers by polymerase chain reaction (PCR). Antimicrobial susceptibility testing on the isolates was performed by the Kirby-Bauer disc diffusion method. Among the tested samples, two samples were contaminated with *Brucella* (4%). One was contaminated with *B. abortus* bv. 1, 2, and 4 (2%), and the other with *B. melitensis* (2%). The isolates were sensitive to azithromycin, imipenem, doxycycline, rifampin, and co-trimoxazole antibiotics and were resistant to ampicillin, amoxicillin-clavulanic acid, tetracycline, gentamicin, and ceftriaxone antibiotics. Also, the *B. abortus* strain was sensitive to ciprofloxacin, but the *B. melitensis* strain was resistant. The isolates showed multi-drug resistance (MDR) characteristics. From the findings, it can be concluded that *Brucella* contamination in local Kope cheeses distributed in Urmia city is low; However, due to the high pathogenicity of *B. melitensis* for humans, it is recommended to screen infected cows, vaccinate sheep and goat herds, and prevent the supply of unpasteurized milk and milk products. It is also recommended to evaluate azithromycin and imipenem antibiotics along with other common antibiotics in brucellosis.

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## 1- Introduction

Cheese jar[1] It is one of the well-known and popular traditional cheeses in Kurdistan and Azerbaijan regions of Iran. This cheese is ripe[2]traditionally made from raw sheep's milk or a mixture of raw sheep's and goat's milk, mainly with rennet[3] And without adding primer[4] is prepared Currently, due to the high demand for consumption, this cheese is also made from cow's milk. To prepare this cheese in traditional conditions, rennet is added to milk and kept at a temperature of 33-34 degrees Celsius for 45-60 minutes. clot[5] After forming, it is cut and poured into cloth bags and pressed for 14-15 hours to completely extract the whey. Then the cheeses are sprinkled with salt and crushed. At this stage, if needed, aromatic vegetables are added and mixed. Shredded cheeses are pressed and filled in clay, plastic or tin containers. The lid of the containers is tightly closed and they are kept in a suitable place underground, cellar or warehouses with controlled temperature and humidity conditions for a certain period (2-3 months) [1, 2 and 3]. Considering that traditional jar cheeses are made from raw milk; Therefore, the possibility of contamination by important pathogenic agents that can be transmitted to humans, for example*brucella*[6] There is in them.

Bacteria of the genus*brucella* Facultative intracellular coccobacilli are Gram-negative, without capsule, flagella and endospores. Although growth*Brucella*It occurs aerobically, but many species such as*Brucella abortus* For optimal growth, they need a concentration of 5-10% carbon dioxide. The optimal pH range for growth is 6.6-7.4, but species *brucella* They can survive at less than 0.5% lactate acidity level and urease enzyme production enables them to tolerate stomach acidity. Seven species*brucella* It is known by the origin of soil, which are:*Brucella melitensis*[7] (sheep and goat),*Brucella Switzerland*[8] (Pig),*Brucella abortus*[9] (cow),*Brucella ovis*[10] (sheep),*Brucella canis*[11] (Dog),*Brucella neotome*[12] (*Desert wood rat*[13]) And*Brucella microti*[14] (Rat). Among the species,*Brucella melitensis* It occurs mostly in the general population and is the most pathogenic and invasive species; Followed by,*Brucella Switzerland* ،*Brucella abortus* And*Brucella canis* They are in order.*Brucella abortus* In addition to the main host, it can infect buffaloes, camels, deer, dogs, horses, sheep and humans. also*Brucella melitensis* It can be transmitted to cattle and it is also very infectious to humans. Although*brucella* It is found less in fermented milk products, but it has been reported that acidic pH has only a small effect on this organism. Also, these bacteria survive well in cold conditions and deep freezing, but they quickly die at 60 degrees Celsius in 10 minutes. This organism survives in the cheese production process and can survive during cheese storage. In fact, cheese made from unpasteurized milk is one of the foods that often plays a role as a source of infection [4,5].

brucellosis[15] Basically, it is a contagious disease of domestic animals, but humans also get this disease in different ways. Infectious dose reported for*brucella* It varies between 10 and 100 organisms. This disease is transmitted to humans through direct contact with infected animals and their secretions. It may also spread through eating contaminated food products such as raw milk and unpasteurized milk products [4]. The virulence of the strain*brucella* And the safety of the host depends. Symptoms usually appear 2-8 weeks, but in acute cases, on average 10-14 days after infection. Human brucellosis appears with symptoms of headache, fever, joint pain, muscle pain, profuse sweating, chills, weakness, lethargy, insomnia, anorexia, constipation, nervousness and depression. The mortality rate in untreated cases is

1 . Crumbled Kope cheese
2 . Ripened cheese
3 . Ran
4 . Starter
5 . Curd
6 . *Brucella*
7 . *Brucella melitensis*
8 . *Brucella am*
9 . *Brucella abortion*
10 . *Brussels sprouts*
11 . *Brucella the dog*
12 . *Brucella neotomae*
13 . Desert wood rat
14 . *Brucella microti*
15 . Brucellosis

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less than 2%, but for infections*Brucella melitensis* more than. In severe cases of brucellosis, the skeletal system may be affected, causing spondylitis[16] and arthritis[17] to be Genitourinary tract may also be affected, leading to orchitis[18]Prostatitis[19] and epididymo-orchitis[20] become young in men. Rarely, neurological complications and cardiac complications may also occur. Infective endocarditis is responsible for the majority of brucellosis-related deaths [4, 5]. In animals, brucellosis causes abortion, fetal death and genital infections [6]. Currently, the diagnosis of brucellosis relies on serological, microbiological and molecular methods [7].

Antibiotics are commonly used to treat brucellosis and may suppress bacterial growth. One of the obstacles of antibiotic treatment is that*Brucella* They can survive in intracellular environments and multiply in macrophages and dendritic cells. For this reason, the antibiotics used must have intracellular activity [8]. Antibiotics commonly used in the treatment of brucellosis include: tetracyclines, trimethoprim-sulfamethoxazole (cotrimoxazole), aminoglycosides, rifampin (rifampicin) and fluoroquinolones. The low effectiveness and frequent relapses after monotherapy led to the transition to a combined treatment program in 1986 [9]. Currently, the treatment programs recommended by WHO[21] It includes the combination of doxycycline and rifampin for 6 weeks or the combination of doxycycline (for 45 days) and streptomycin (for 21 days) [9, 10]. Trimethoprim-sulfamethoxazole alone or together with rifampin or gentamicin for the treatment of pregnant women or patients who are related to Tetracycline intolerance is useful. Also, rifampin with cotrimoxazole has been recommended for the treatment of uncomplicated disease in children [4]. However, combined programs also have less than 100% success due to the development of resistance to antibiotics [11].

Various studies on the level of contamination of local cheeses with species*brucella* And the pattern of antibiotic resistance of isolates has been done in different regions of the world and Iran [12, 13, 14, 15, 16, 17, 18 and 19]. The present research aims to investigate the level of contamination of local jarred cheeses sold in Urmia city with species*brucella* And the antibiotic resistance pattern of the isolates was evaluated.

## 2- Materials and methods

### 2-1- Isolation and identification of species*brucella*

50 samples of local cow's jar cheese were randomly collected from different dairies in Urmia city in accordance with the sterile conditions in 1401 and were transferred to the food quality and hygiene control laboratory of the Faculty of Veterinary Medicine in the vicinity of the ice. Considering the zoonotic nature*brucella*The experiments were carried out in compliance with level two biosafety requirements [20]. First, 10 grams of cheese samples in 90 ml of broth*brucella* (Qulab, Canada) were homogenized in a Stomaker machine. Then, the homogenized samples were poured into sterile bottles and enriched at 37°C for 48 hours in aerobic and anaerobic greenhouses (with 10% carbon dioxide concentration). After the time spent in the greenhouse, the bottles were homogenized and placed on agar*brucella* (Qulab, Canada) with 5% defibrinated sheep blood and selected supplement*brucella*FD005 was supplemented with polymyxin B (2500 IU), bacitracin (12500 IU), nystatin (50000 IU), cycloheximide (50 mg), nalidixic acid (2.5 mg) and vancomycin (10 mg) (Himedia, Mumbai, India). ; Line cultures were given. The plates were kept at 37°C for 48 hours in aerobic and anaerobic greenhouse conditions. Plates with white to gray and non-mucoid plaques were considered as possible positive plates for biochemical tests. To identify the species*brucella*, Gram staining, catalase, oxidase, indole production, hydrogen sulfide production, urease and growth experiments were performed in the presence of 10% carbon dioxide [21 and 22].

[16] . Spondylitis
[17] . Arthritis
[18] . Orchitis
[19] . Prostatitis
[20] . Epididymo-orchitis
[21] . World health organization

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## 2-2- Identification of isolates with specific antisera

To perform this test of specific antisera*Brucella abortus* And*Brucella melitensis* Iran's Bahar Afshan Company was used. First, a drop of sterile saline was poured on a slide with a black background. Then, using a sterile ring anase, one colony was taken from the fresh culture of the isolates in blood agar and dispersed in saline. Next, a drop of specific antiserum was added to the bacterial suspension and the slide was gently shaken. The observation of agglutination was the proof of the positive reaction and the identification of the desired bacteria.

## 2-3- Molecular identification of isolates by polymerase chain reaction (PCR)

DNA extraction of the isolates was done using the DNA extraction kit by column method (FAST DNrich Bacteria Kit) (Kimiya Tab Gostar Company, Iran). Molecular identification*Brucella abortus* (Beauvars 1, 2 and 4) and*Brucella melitensis* It was done using the primers provided by Amoupour et al. [23]. The sequence of primers used is shown in Table 1. The primers were produced in Sinagen Iran. The final PCR mixture for each isolate consisted of 12.5 microliters of the original mixture (PCR buffer, Taq DNA polymerase enzyme, dNTPs and MgCl2), 2 microliters of primers, one microliter of extracted DNA, and 9.5 microliters of double-distilled distilled water. The microtubes containing the final mixture were placed in the thermocycler and the PCR reaction was performed according to the temperature and time schedule in Table 2. Then the PCR products were checked for amplification or non-amplification of the target gene by agarose gel electrophoresis. For this purpose, first, 1.5% agarose gel was prepared. In the first well of the gel, 3 microliters of 100 bp DNA Ladder and in the next wells, 5 microliters of positive control PCR products and isolates were mixed with 2 microliters of loading buffer and loaded. Electrophoresis was performed with a voltage of 100 volts and for one hour. Then, DNA bands were observed and recorded using a gel documentation device and ultraviolet (UV) radiation. In the last step, the size of the PCR product was estimated by comparing the position of the amplified DNA band with the size of the marker bands [7, 23].

Table 1. Specific primer sequence used in PCR test for identification of *Brucella* spp.

| *Brucella* spp | Primer name | Primer sequence | Product size | References |
|---|---|---|---|---|
| *B. abortion* (bv 1, 2 and 4) | Ba-sp | 5 - GAC GAA CGG AAT TTT TCC AAT CCC- 3 | 498 bp | 7, 23 |
| | IS711-sp | 5 - TGC CGA TCA CTT AAG GGC CTT CAT- 3 | | |
| *B. melitensis* | Bm-sp | 5 - AAA TCG CGT CCT TGC TGG TCT GA – 3 | 731 bp | 7, 23 |
| | IS711-sp | 5 - TGC CGA TCA CTT AAG GGC CTT CAT- 3 | | |

Table 2. Temperature and time program used for PCR test

| Stage | Temperature | Time | Cycle number |
|---|---|---|---|
| Initial denaturation | 95 ℃ | 5 min | 1 |
| Denaturation | 95 ℃ | 30 sec | 40 |

| | | | |
|---|---|---|---|
| Annealing | 54 ℃ | 30 sec | |
| Extension | 72 ℃ | 30 sec | |
| Final extension | 72 ℃ | 5 min | 1 |

**2-4- Antimicrobial sensitivity of isolates*brucella***

Antibiotic susceptibility of isolates*brucella* It was determined by Kirby-Bauer disc diffusion method. First, the isolates were cultured in blood agar. Then, a bacterial suspension was prepared from the cells in sterile saline. The turbidity of the suspension was adjusted visually with McFarland standard turbidity of 0.5. Then, the bacterial suspension was cultured linearly and uniformly on Mullerhinton agar plates supplemented with 5% defibrinated sheep blood using a sterile swab. Antibiotic discs of azithromycin (15 micrograms), ampicillin (10 micrograms), amoxicillin-clavulanic acid (10-20 micrograms), imipenem (10 micrograms), tetracycline (30 micrograms), trimethoprim-sulfamethoxazole (23.1-75.25 micrograms), Gentamicin (10 µg), doxycycline (30 µg), rifampin (5 µg), ceftriaxone (30 µg), ciprofloxacin (5 µg), and chloramphenicol (30 µg) (Antibody Medicine, Iran) were placed on the inoculated plates. Antibiotic discs were selected based on common antibiotics used in the treatment of brucellosis and antibiotics tested in different articles. The plates were incubated at 37°C for 48 hours under anaerobic conditions with 10% carbon dioxide. Then, the diameter of the growth inhibition zone of each antibiotic was measured and compared with the criteria for slow-growing bacteria (species*Haemophilus*) Clinical and Laboratory Standards Institute (CLSI)[22] Comparison and results sensitively[23]semi-sensitive[24] and resistant[25] were registered The antibiogram of the isolates was performed in three replicates. Also, isolates that were resistant to three or more types of antibiotics; Multi-drug resistant or MDR[26] were defined [24, 25 and 26].

[22] . Clinical and Laboratory Standards Inistitue (CLSI)
[23] . Susceptible
[24] . Intermediate
[25] . Resistant
[26] . Multi-Drug Resistant (MDR)

## 3. Results and Discussion

### 1-3- The degree of contamination of local jar cheeses of Urmia city with species*brucella*

The results of microbial culture and PCR tests showed that out of 50 tested samples, 2 samples were infected with*brucella* (4 percent) were. A sample to*Brucella abortus* (2 percent) and another example, to*Brucella melitensis* (2%) were infected (Chart 1 and Figure 1).

Several studies in different parts of the world on the level of contamination of local cheeses with species*brucella* There have been; Kara and Akkaya [27] attributed the contamination of the cheeses of the city of Afyonkarahisar, Türkiye to*Brucella abortus* And*Brucella tenensis* checked Pollution species*brucella* in fresh cheeses as much as 9% (2%).*Brucella abortus* and 7 percent*Brucella melitensis)* and in Afiontolom cheeses as much as 6% (2%).*Brucella melitensis* and 4 percent*Brucella abortus)* Recognized. Fadil and Khalil [14] to investigate the species*brucella* In the local cheeses of Jibeen al-Arab city of Baquba, Iraq, they dealt. Conventional culture method was used for isolation. pollution to*brucella* As much as 12 percent (8 percent*Brucella melitensis* and 4 percent*Brucella abortus* ) Recognized.

In different regions of Iran, investigations on the level of contamination of local cheeses with species*brucella* done. Shakarian [28] from 50 samples of local cheese tested in Isfahan and Chaharmahal Bakhtiari provinces by molecular method, contamination with*Brucella abortus* And*Brucella melitensis*particle for direct object reported 2.5 percent. In a survey by microbial culture method, Akbar Mehr [15] species contamination*brucella* He reported 2.2% in local cheeses sold in Sarab and Homah city, of which 7 samples (0.7%)*Brucella melitensis* and 15 samples (1.5 percent)*Brucella abortus* They were. In another study conducted by Abdoli et al. [19] on unpasteurized milk products in Shiraz province by PCR method; In samples of traditional cheese and ice cream contamination with*brucella* was not




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observed. Batani and Samadzadeh [29] showed that out of 140 samples of traditional cheese tested in Zanjan city by microbial culture method, 2 samples (1.4%) were infected with*brucella* They were. A sample infected with*brucella national Tennessee* and another sample infected with*Brucella abortus* They were. In another study conducted by Shakrian et al. [30] on 200 samples of unpasteurized white sheep cheese in Kurd city and suburbs by microbial culture method, only one sample was infected with*Brucella melitensis* (0.5 percent) was diagnosed. In another study by Yousefi Mashauf [31], the level of contamination with *brucella* In the fresh local cheeses of Hamedan city, it was determined by microbial culture to be 2.4%. Muslimi et al. [18] reported the contamination of unpasteurized cheeses supplied in Tehran province by real-time PCR method as 39.1%. In another study, Maruf et al. [17] showed contamination of traditional cheeses sold in East Azarbaijan province with species*brucella* reported 22.93% by real-time PCR method.

In the present research, pollution to*brucella*In the local jar cheeses of Urmia city, which is a type of ripened cheese; Recognized. The results of the present study with the findings of researchers in Iran and other countries in the field of pollution*Brucella in*Local and unpasteurized cheeses are suitable. Isolation and diagnosis*brucella*In local jar cheeses in Urmia city, it indicates the presence of this bacterium in livestock populations of these areas. It can also be concluded that bacteria*brucella* It can survive in the stages of preparation and storage of traditional jar cheese and due to the low infectious dose*brucella*There is a possibility of developing brucellosis through consumption of these types of cheeses in the region. On the other hand, in the present research of cow cheeses,*Brucella melitensis* It was isolated that this finding was also reported in the results of Shafii et al. [7] and probably due to the close breeding of sheep and goat herds with cattle herds in the region, contamination with*Brucella melitensis* It happened on a non-preferred host.

As seen, the amount of pollution to*brucella* It is different in local and unpasteurized cheeses in different studies. In studies that used molecular methods such as real-time PCR [20, 21]; High levels of contamination have been obtained, the reason of which is related to the fact that in molecular methods, the DNA of dead and live bacteria is identified together, while in the microbial culture method, only live bacteria are isolated. Also pollution to*brucella* In both fresh local cheeses and ripened local cheeses, it was reported that the level of contamination was higher in fresh local cheeses [27]. Therefore, it can be concluded that the consumption of fresh pasteurized local cheeses can have a high risk of brucellosis. On the other hand, other factors such as sample size, hosting factors, geographic location, screening of infected animals, inoculation against brucellosis in animals, the way animals are raised, the sensitivity of the studied tests and the way traditional cheeses are produced can affect the level of contamination.*brucella* have a role [28].

The main cause of milk contamination*brucella*, the occurrence of brucellosis in cattle herds. bacteria*brucella* In the supramammary lymph nodes, it replaces infected animals and causes milk contamination. If the milk of infected animals is consumed without heat treatment or if it is used in the preparation of milk products; They will cause consumers to suffer from malt fever. Therefore, the most important method of preventing the occurrence of brucellosis in humans is to screen infected cows, to vaccinate sheep and goat herds, to educate the nomads and villagers of the region about the dangers and ways of disease transmission, and to prevent the supply and consumption of unpasteurized milk and milk products, especially in infected areas.

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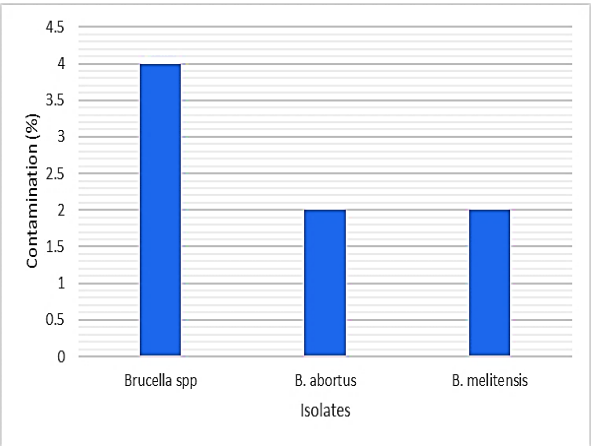


Fig 1. Contamination rate of *Brucella* isolates in local Kope cheeses of Urmia

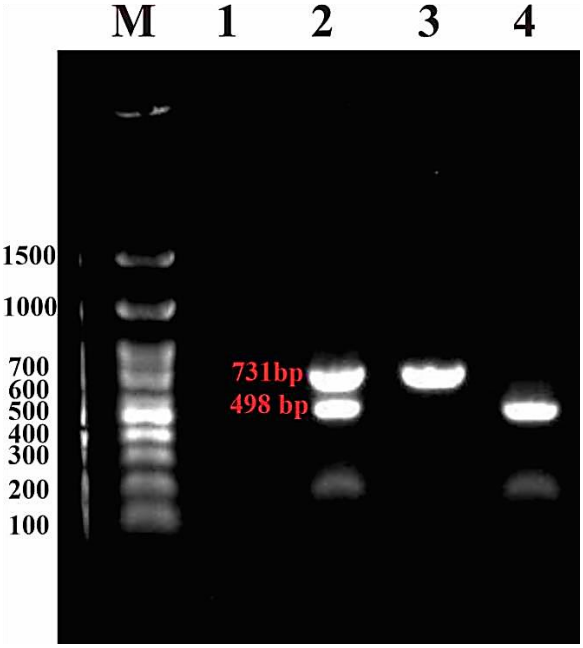


Fig 2. Electrophoresis of PCR products: lane M, DNA ladder 100 pb, lane 1: negative control, lane 2: positive control (band 731 bp for *Brucella melitensis* and band 498 bp for *Brucella abortion*), lane 3: positive sample of *B. melitensis*, lane 4: positive samples of *B. abortion.*

## 2-3- Antibiotic sensitivity of isolates*brucella* From the local jar cheeses of Urmia city

The antibiotic sensitivity results showed that the isolates*brucella* They are sensitive to azithromycin, imipenem, doxycycline, rifampin, trimethoprim-sulfamethoxazole antibiotics (Table 3). Also isolates*brucella* They were resistant to ampicillin, amoxicillin-clavulanic acid, tetracycline, gentamicin and ceftriaxone antibiotics (Table 3 and Diagram 2). separate*Brucella abortus* against ciprofloxacin, sensitive but isolated*Brucella melitensis* was resistant to it (Table 3 and Chart 2). On the other hand, the isolates showed multi-drug resistance or MDR (Table 4 and Chart 2).

Recently, the increase of microbial resistance to common antibiotics has attracted much attention to select new groups of antibiotics for the treatment of specific infectious diseases. According to the World Health Organization (WHO), only some limited antibiotics with clinical efficacy and good intracellular penetration are used in the treatment of brucellosis. Antibiotics of choice in this field are doxycycline, rifampin, trimethoprim-sulfamethoxazole and streptomycin. Single-drug treatments are associated with the risk of disease recurrence; Therefore, combined treatments are recommended.

Doxycycline is a semisynthetic derivative of oxytetracycline and is highly lipid soluble. As a result, it has higher intracellular penetration and better tissue distribution compared to other tetracyclines [26]. Doxycycline is ranked as a gold standard drug by the World Health Organization (WHO) and is the most commonly prescribed tetracycline derivative in the treatment of infections due to its superior pharmacokinetic profile.*brucella* It has been converted [32]. Synergistic effect of combining tetracyclines with streptomycin or rifampin on intracellular organisms*brucella*It has been seen [26]. Studies conducted in different countries have shown that isolates *brucella* have maintained their sensitivity to doxycycline [26, 32, 33, 34 and 35]. In the present research, the isolates*brucella* In line with these findings, they were sensitive to doxycycline.

Rifampin, also known as rifampicin. It is an antibacterial drug that can kill intracellular bacteria by inhibiting RNA synthesis. Rifampin is an essential and effective antibiotic in the treatment of brucellosis and is widely recommended for first-line treatment. It has bacteriostatic or bactericidal effects with ideal intracellular penetration and obvious synergy with other antibiotics. Therefore, such compounds have been suggested by WHO for the management of brucellosis [26]. A combination of rifampin and doxycycline is currently the best oral treatment for

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brucellosis [32]. In the present study, the isolates*brucella* They were sensitive to rifampin. However, in different studies, percentages of resistance isolates*Brucella ratio*It has been reported to rifampin [26, 32, 33, 34, 35 and 36]. The emergence of resistance to rifampin can cause concern in the treatment of brucellosis. Rifampin resistance can be explained by similar treatment plans for brucellosis and tuberculosis in the Middle East region. Few molecular-based studies have evaluated the genetic basis of reduced susceptibility or resistance to specific antibiotics. Mutations in the rpoB gene*Brucella melitensis* Rifampin resistance has been reported in several studies. On the other hand, the combination of rifampin and doxycycline may cause problems in the treatment of tuberculosis in many developing countries, including Middle Eastern countries [26]. Aminoglycosides are bactericidal compounds that interfere with bacterial protein synthesis. Only three of these antibiotics have been used in the treatment of brucellosis, namely: streptomycin, gentamicin, and netilmacin. Streptomycin is known as one of the most effective compounds in the treatment of human brucellosis, but it is ineffective in the treatment of brucellosis alone. However, the synergistic effect of its combination with tetracyclines is well known. Studies on animal and human isolates*brucella* have been done, they reported the emergence of streptomycin-resistant isolates. Gentamicin has a similar activity to streptomycin, however, nephrotoxicity is often reported with gentamicin. Gentamicin is regularly used in patients with Brucella endocarditis. Reports of resistance of human and animal isolates*brucella* to gentamicin [26, 34 and 36]. In the present research, the isolates *brucella* In line with these reports, they were resistant to gentamicin. This finding may be due to the widespread use of aminoglycoside antibiotics alone or in combination with beta-lactam antibiotics in veterinary medicine. Cotrimoxazole is a combination of trimethoprim and sulfamethoxazole in a ratio of 1:5. Both compounds work by stopping bacterial purine synthesis, but at different levels. Trimethoprim is an antibacterial drug, but when combined with sulfamethoxazole; It is more effective. Therefore, the combination of trimethoprim-sulfamethoxazole shows synergistic effects on intracellular bacteria and is recommended for the treatment of brucellosis. This drug should be used in combination with rifampin in children under 8 years of age and pregnant women and together with doxycycline and rifampin in the treatment of endocarditis caused by*brucella* be used [26]. In the present research, according to the findings of Parlak et al. [32] and Elmian et al. [33] isolates*brucella*They were sensitive to trimethoprim-sulfamethoxazole. However, there are reports on human isolates that showed reduced sensitivity to trimethoprim-sulfamethoxazole [26, 35 and 36]. Ciprofloxacin and ofloxacin are the main quinolones used in the treatment of brucellosis [32]. Fluoroquinolones are a group of broad-spectrum bactericidal antibiotics that interfere with bacterial DNA synthesis. Their activity against*Brucella melitensis* reported in laboratory conditions. However, they are not effective as monotherapy against active brucellosis. Fluoroquinolones are associated with many unacceptable treatment failures and relapses, development of resistance and lack of synergism in laboratory conditions with other antibiotics. The effectiveness of ciprofloxacin in the treatment of brucellosis is discussed. In a number of studies, resistance*Brucella melitensis* compared to ciprofloxacin has been reported [26 and 36]. In the present research, Jadayeh*Brucella melitensis* In line with these reports, it was resistant to ciprofloxacin; But separate*Brucella abortus* He was sensitive to it. Cephalosporins, especially the third generation group, have a broad effectiveness against Gram-negative organisms by inhibiting the synthesis of bacterial cell wall mucopeptide. Although ceftriaxone has been reported in vitro against*brucella* It is effective [33], but there is a high prevalence of treatment failure in patients with active brucellosis. Also, a decrease in sensitivity to ceftriaxone in cases of human brucellosis has been reported from different countries [26 and 36]. In the present study, the isolates*brucella*They were resistant to ceftriaxone. Also, resistance to ampicillin-sulbactam and penicillin from human isolates*brucella* It has been reported [26 and 35]. In the current research, the isolates*brucella* They were resistant to ampicillin and amoxicillin-clavulanic acid. This finding can be attributed to the widespread use of beta-lactam antibiotics and cephalosporins in the treatment of mastitis in dairy herds.

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Carbapenems are a group of antibiotics belonging to the beta-lactam class. They have a wide range of activities. Carbapenems bind with high affinity to high molecular weight penicillin-binding proteins and cause bacterial lysis. Their effectiveness is complemented by low toxicity and low incidence of resistance. Imipenem was the first carbapenem to be widely available, licensed for use in the United Kingdom in 1988 [37]. A report on the occurrence of resistance in animal isolates*brucella* compared to Emi Panem from Egypt [38]. Also, in a survey in India, animal isolates*brucella* They were sensitive to imipenem [39]. In the present research, the isolates*brucella* They were sensitive to imipenem. Therefore, regional differences in antibiotic resistance of isolates*brucella* can be seen

Azithromycin is one of the macrolides that is known for its rapid distribution after oral consumption and with higher concentrations inside cells, especially phagocytes [26]. This antibiotic can be used in combination with rifampin in cases of brucellosis, especially during pregnancy. In many studies, azithromycin as an effective antibiotic against strains*brucella*It is known [32]. A study in Spain showed a slight difference in sensitivity*Brucella melitensis* showed azithromycin and tetracycline, indicating the promising therapeutic role of azithromycin in human brucellosis. However, in studies on human and animal isolates*brucella* Resistance to azithromycin has also been reported [26]. In the present research, the isolates*brucella*They were sensitive to azithromycin. This finding can be attributed to the less use of macrolide antibiotics in veterinary medicine, especially in dairy herds.

The emergence of antibiotic resistance in bacteria is a global public health issue and compromises treatment options regarding the effectiveness of antibiotics and the control of bacterial infections. The widespread spread of antibiotic resistance in bacteria is due to the inappropriate and uncontrolled use of antibiotics in veterinary medicine and medicine in developing countries [38]. Reports of the emergence of strains*brucella* with resistance to several drugs [35, 36, 38 and 39]. In the present study, the isolates*brucella* They showed resistance to several drugs. Emergence and spread of strains*brucella* With the resistance of several drugs, it can be a serious threat to humans. Because it may make hospital care inappropriate and limit treatment options in public health settings.

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Table 3. Antibiotic susceptibility profile of *Brucella* isolates in local Kpoe cheeses of Urmia

| Antibiotic | ***Brucella* spp (N=2)** | | | ***B. abortion* (N=1)** | | | ***B. melitensis* (N=1)** | | |
|---|---|---|---|---|---|---|---|---|---|
| | S | I | R | S | I | R | S | I | R |
| Ampicillin (AM) | 00 (00%) | 00 (00%) | 02 (100%) | 00 (00%) | 00 (00%) | 01 (100%) | 00 (00%) | 00 (00%) | 01 (100%) |
| Amoxicillin-clavulanic acid (AMC) | 00 (00%) | 00 (00%) | 02 (100%) | 00 (00%) | 00 (00%) | 01 (100%) | 00 (00%) | 00 (00%) | 01 (100%) |
| Azithromycin (AZM) | 02 (100%) | 00 (00%) | 00 (00%) | 01 (100%) | 00 (00%) | 00 (00%) | 01 (100%) | 00 (00%) | 00 (00%) |
| Ceftriaxone (CRO) | 00 (00%) | 00 (00%) | 02 (100%) | 00 (00%) | 00 (00%) | 01 (100%) | 00 (00%) | 00 (00%) | 01 (100%) |
| Ciprofloxacin (CP) | 01 (50%) | 00 (00%) | 01 (50%) | 01 (100%) | 00 (00%) | 00 (00%) | 00 (00%) | 00 (00%) | 01 (100%) |
| Chloramphenicol (C) | 00 (00%) | 00 (00%) | 02 (100%) | 00 (00%) | 00 (00%) | 01 (100%) | 00 (00%) | 00 (00%) | 01 (100%) |
| Co-trimoxazole (SXT) | 02 (100%) | 00 (00%) | 00 (00%) | 01 (100%) | 00 (00%) | 00 (00%) | 01 (100%) | 00 (00%) | 00 (00%) |
| Doxycycline (D) | 02 (100%) | 00 (00%) | 00 (00%) | 01 (100%) | 00 (00%) | 00 (00%) | 01 (100%) | 00 (00%) | 00 (00%) |
| Gentamycin (G) | 00 (00%) | 00 (00%) | 02 (100%) | 00 (00%) | 00 (00%) | 01 (100%) | 00 (00%) | 00 (00%) | 01 (100%) |
| Imipenem (IPM) | 02 (100%) | 00 (00%) | 00 (00%) | 01 (100%) | 00 (00%) | 00 (00%) | 01 (100%) | 00 (00%) | 00 (00%) |
| Rifampin (RA) | 02 (100%) | 00 (00%) | 00 (00%) | 01 (100%) | 00 (00%) | 00 (00%) | 01 (100%) | 00 (00%) | 00 (00%) |
| Tetracycline (TE) | 00 (00%) | 00 (00%) | 02 (100%) | 00 (00%) | 00 (00%) | 01 (100%) | 00 (00%) | 00 (00%) | 01 (100%) |

N: number; S: susceptible; I: intermediate; R: resistant

Table 4. Antibiotic resistance pattern and frequency of multi-drug resistant strains in *Brucella* isolates of Urmia local Kope cheeses

| Isolates | Antibiotic resistance | | | | | | | MDR |
|---|---|---|---|---|---|---|---|---|
| | R1 | R2 | R3 | R4 | R5 | R6 | R7 | |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| *Brucella* spp (N=2) | 00 (00%) | 00 (00%) | 00 (00%) | 00 (00%) | 00 (00%) | 00 (00%) | 02 (100%) | 02 (100%) |
| *B. abortion*(N=1) | 00 (00%) | 00 (00%) | 00 (00%) | 00 (00%) | 00 (00%) | 01 (100%) | 00 (00%) | 01 (100%) |
| *B. melitensis* (N=1) | 00 (00%) | 00 (00%) | 00 (00%) | 00 (00%) | 00 (00%) | 00 (00%) | 01 (00%) | 01 (100%) |

N: number; R1: resistance to one; R2: resistance to two; R3: resistance to three; R4: resistance to four; R5: resistance to five; R6: resistance to six; R7: resistance to seven; MDR: multi-drug resistance (resistance to three or more antibiotics)



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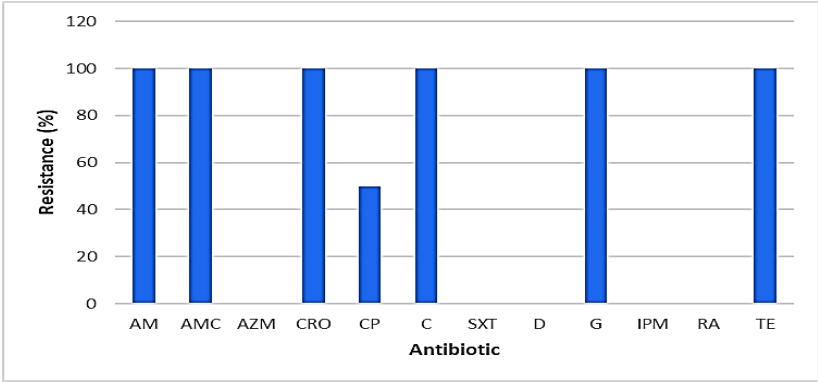


Fig 2. Antibiotic reistance frequency of *Brucella* isolates in local Kope cheeses of Urmia

## 4 - Conclusion

This study showed that although the local jar cheese samples of Urmia city were less contaminated with*Brucella abortus* And*Brucella melitensis* have; But due to the high pathogenicity of the species*brucella* For humans, preventive measures in the region should be carried out by competent authorities. It is suggested to carry out brucellosis eradication programs such as screening of infected cattle, complete inoculation of sheep and goat flocks in the region. Small workshops producing traditional dairy products in the region should be equipped with pasteurizing devices. Also, the nomads and villagers of the region should be given the necessary training on the dangers and ways of transmission of Maltese fever. On the other hand, considering the sensitivity of the isolates to the antibiotics azithromycin and imipenem, it is suggested that these antibiotics be evaluated in the treatment programs of brucellosis along with common antibiotics.

## 5- Gratitude

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مقاله علمی_پژوهشی

# آلودگی پنیرهای کوزه‌ای محلی عرضه‌شده در شهرستان ارومیه به گونه‌های *بروسلا* و ارزیابی الگوی مقاومت آنتی‌بیوتیکی جدایه‌ها

## *بروسلا* در پنیرهای کوزه‌ای محلی ارومیه و مقاومت آنتی‌بیوتیکی

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**چکیده**

بروسلوز یک بیماری زئونوز مهم می‌باشد. شیر و فرآورده‌های شیر غیرپاستوریزه از منابع اصلی انتقال *بروسلا* به انسان هستند. تحقیق حاضر با هدف بررسی میزان آلودگی پنیرهای کوزه‌ای محلی عرضه‌شده در شهرستان ارومیه به گونه‌های *بروسلا* و ارزیابی الگوی مقاومت آنتی‌بیوتیکی جدایه‌ها انجام شد. ۵۰ نمونه پنیر کوزه‌ای گاوی محلی از خرده‌فروشی‌های لبنیات سنتی مناطق مختلف شهرستان ارومیه به‌صورت تصادفی و با شرایط سترون در سال ۱۴۰۱ جمع‌آوری شدند. نمونه‌ها، ابتدا در آب‌گوشت غنی‌سازی *بروسلا* سپس در آگار انتخابی *بروسلا* با مکمل کشت داده شدند. شناسایی مولکولی گونه‌های *بروسلا* با استفاده از پرایمرهای اختصاصی و با واکنش زنجیره‌ای پلیمراز (PCR) انجام شد. آزمایش حساسیت ضدمیکروبی روی جدایه‌ها به‌روش انتشار دیسک کربی-بائر انجام شد. از میان نمونه‌های آزمایش‌شده، ۲ نمونه آلوده به *بروسلا* (۴ درصد) تشخیص‌داده شدند. یک نمونه به *بروسلا آبورتوس* بیووار ۱، ۲ و ۴ (۲ درصد) و نمونه دیگر به *بروسلا ملی‌تنسیس* (۲ درصد) آلوده بودند. جدایه‌ها در برابر آنتی‌بیوتیک‌های آزیترومایسین، ایمی‌پنم، داکسی‌سایکلین، ریفامپین، کوتریموکسازول، حساس و در برابر آمپی‌سیلین، آموکسی‌سیلین-کلاوولانیک اسید، تتراسایکلین، جنتامایسین و سفتریاکسون مقاوم بودند. هم‌چنین سویه *بروسلا آبورتوس* در برابر سیپروفلوکساسین، حساس ولی سویه *بروسلا ملی‌تنسیس* در برابر آن مقاوم بودند. از طرفی جدایه‌ها ویژگی مقاومت به چند دارو (MDR) نشان دادند. از یافته‌ها می‌توان نتیجه‌گیری نمود که آلودگی به *بروسلا* در پنیرهای کوزه‌ای محلی عرضه‌شده در شهرستان ارومیه پایین می‌باشد؛ ولی نظر به بیماری‌زایی بالای *بروسلا ملی‌تنسیس* برای انسان، غربال‌گری گاوهای آلوده، مایه‌کوبی گله‌های گوسفند و بز و جلوگیری از عرضه شیر و فرآورده‌های شیر غیرپاستوریزه پیشنهاد می‌گردد. هم‌چنین توصیه می‌گردد آنتی‌بیوتیک‌های آزیترومایسین و ایمی‌پنم توام با سایر آنتی‌بیوتیک‌های رایج در درمان بروسلوز ارزیابی گردند.