Comparative approach of the antibiotics susceptibility of some bacterial strains concurrently isolated from raw milk and cattle feed (Water and Fodder) from some farms in the West Region of Cameroon (Central Africa)

ABSTRACT

Introduction: Foodborne resistant bacteria have become a challenge to food security. Milk and milk products are easy vectors of transmission of foodborne pathogens, these being the main sources of human infection by antimicrobial resistant pathogens. The present study aimed at making a comparative approach of the antibiotic sensitivity/resistance of 3 bacterial strains (*Escherichia coli, Salmonella spp. and Brucella spp.*) isolated from milk, drinking water and green fodder consumed by cows in the West Cameroon region (Central Africa).

Methodology: A total of 48 raw milk samples, 48 water samples and 48 green fodder samples were collected during the year 2020 and subjected to culture and identification of *Escherichia coli, Salmonella spp.* and *Brucella spp.* Antibiotic susceptibility testing was performed using the antibiotic disc diffusion method.

Results: *Escherichia coli* isolates showed high resistance (56-100%) to ampicillin, amoxicillin/clavulanic acid, cefotaxime, ceftazidime and ceftriaxone in all three samples. *Salmonella spp.* isolates showed resistance to ampicillin only (62, 67 and 67%). *Brucella spp.* strains isolated from raw milk and drinking water showed high sensitivity (78-100%) to azithromycin, doxycycline, ciprofloxacin, levofloxacin, gentamicin, rifampicin and trimethoprim/sulfamethoxazole, streptomycin and tetracycline. Antibiotic sensitivity/resistance to *Escherichia coli* and *Salmonella spp.* strains largely did not differ between samples (P>0.05). No difference in sensitivity/resistance (P>0.05) of *Brucella spp.* strains isolated from milk and water was observed with respect to the 10 antibiotics tested.

Conclusions: The emergence of resistance to various antibiotics commonly used in medical and veterinary practices has important implications for public health. It

seems necessary to strengthen of the regulations covering the sale and prescription of antibiotics.

Keywords: *Escherichia coli, Salmonella spp, Brucella spp, raw milk, drinking water, green fodder, antibiotic susceptibility*

1. INTRODUCTION

Currently, milk and milk products occupy a predominant place in the diet of Africans [1]. However, milk is an excellent substrate for the proliferation of pathogens and is therefore an important source of bacterial infection that can lead to serious diseases when consumed unpasteurized [2]. In addition to the presence of potentially pathogenic bacteria, raw milk contains antibiotic-resistant microbes and thus its incorporation into the daily diet can facilitate the spread of antimicrobial resistance genes (ARGs) to the human gastrointestinal tract [3,4].

The diet of dairy cows plays a key role in determining better health and production. The quality of water and green fodder consumed by cows is extremely important for the animal and it is one of the most important biosecurity measures to control the health status and productivity of a herd. They are potential sources of microorganisms that can impact the digestive microbiota and contaminate the milk [5]. Bacteria in milk are thought to originate from contamination of the external environment, the skin of the mammary gland or the oral cavity of the offspring [6]. However, several studies suggested that bacteria in milk do not originate solely from external colonization and an endogenous route of bacterial transmission has been proposed. Therefore, microorganisms from different anatomical locations in the udder may somehow enter the mammary gland. Thus, in the entero-

mammary pathway, it has been hypothesized that bacteria can leave the intestinal lumen, travel through the mesenteric lymph nodes to the mammary gland, probably *via* immune cells such as dendritic cells [6]. The transfer of intestinal bacteria to the mammary gland of cows has been reported [7].

Cow milk production in Cameroon faces many management problems. In the West region, milk production systems consist mainly of small rural dairies where animals are fed on grass, crop residues and cultivated fodder, or they roam the land in search of pasture and water. Herders prefer to reserve land for crops and refrain from growing feed for their animals. They prefer to feed the breeding herd with collected fodder. Milk from the farms is sold in the region and consumed raw or processed into yoghurt or cheese. In addition, dairy farms in this region face difficulties with medicines, vaccination programs, veterinary services and disease knowledge: medicines and veterinary services are not available. These conditions can affect the microbiological quality of the milk produced [8].

Previous studies on the microbiological quality of raw milk in the region revealed that the milk produced was compromised by several pathogenic bacteria, including *Escherichia coli, Salmonella spp.* and *Brucella* spp. The abundance dynamics of microorganisms found in drinking water and green fodder consumed by cows is in most cases significantly and positively related to that of the raw milk produced [8]. Furthermore, Maïworé and collaborators, after isolating several strains of *Staphylococcus aureus* from raw milk sold in the northern region of Cameroon (in Maroua) observed a high level of antibiotic resistance [9]. Antibiotic susceptibility testing revealed 95% resistance to β-lactamases, 78% to Macrolides, 42% to Glycopeptides, 16% to Quinolones, 5% to Aminosides and Cotrimoxazole and 0% to Chloramphenicol [9]. Another study on the pathogenicity and antimicrobial resistance profile of *Staphylococcus aureus* in beef and milk in the North West and South West regions of Cameroon revealed that the majority of isolates were resistant to erythromycin (82%), vancomycin (80%), tetracycline (76%) and oxacillin (74%) [10]. The

increasing prevalence of antibiotic resistance (AR) is a global concern and the role of raw milk in the spread of AR is unclear [4]. Information on the antibiotic susceptibility of bacterial strains in cattle feed (drinking water and green fodder) could greatly contribute to the understanding of bacterial bioresistance in milk. The present study aims to make a comparative approach of the antibiotic susceptibility of 3 bacterial strains (*Escherichia coli*, *Salmonella spp.* and *Brucella spp.*) isolated from raw milk, drinking water, and green fodder consumed by cows in the West Cameroon region (Central Africa).

2. MATERIALS AND METHODS

2.1. The study area

The study was carried out in the Western Highlands region of Cameroon. The region covers an area of 13.892 km^2 and is home to over 1982100 inhabitants. It is the most populated region in Cameroon with a density of 124 inhabitants per km^2 [11]. The region is located between 5-7° North latitude and 9-11° East longitude. Its morphology is made up of a series of plateaus. An altitude varies between 800 and 2740 m and large volcanic edifices dominate the region. The region has a temperate climate with two main seasons: a dry season from November to March, and a rainy season from April to October. Temperatures vary between 15°C and 30°C on average with a strong daily variation. Annual rainfall is abundant (on average 1400 to 2500 mm/year) with peaks in July, August and October (the wettest period of the year). The hydrographic network is linked to the morphology of the region. The province is the third largest cattle production area (500000 cattle). The main vegetation is savannah. The West Cameroon Highlands is a tsetse fly free area and therefore well suited for cattle rearing [12].

2.2. Sampling

This study was carried out on 12 dairy farms in the West Cameroon region. The working period took place during the months of February, April, June and August 2020. The samples that were taken at each of the 4 visits were: a sample of raw milk blend and a sample of drinking water and a sample of green fodder in each farm. In total, 48 raw milk samples, 48 water samples and 48 green fodder samples were collected.

Aseptic collection of milk samples at farm level was carried out according to the National Mastitis Council (NMC) guidelines [13]. All milk samples corresponded to the morning milking milks. At each arrival, approximately 250 ml of raw milk was collected in sterile glass bottles and transported at temperatures of 4-8°C in coolers for analysis within a maximum of 2-3 hours after sampling.

The water resource consisted of well water, river water and artificial lakes. The water samples were collected in 500 mL sterile glass vials. These vials were filled 3/4 full of water to allow homogenization before plating. In each farm, these vials were first rinsed in the field with the water to be analyzed, then filled to the brim and capped to limit outgassing [14]. All labeled samples were transported to the laboratory in a refrigerated chamber for analysis.

Green fodder harvested from fallow and natural or cultivated pastures was fed to the animals whole for some and cut into small pieces for others. Single samples were combined into a bulk sample. This was then mixed as homogeneously as possible and spread evenly over a clean surface. The fodder was shortened to a maximum stem length of 5 cm. The representative sample was taken from the bulk sample by re-sampling approximately 3 single samples evenly distributed. Each sample was placed in tightly sealed plastic bags, from which the air was evacuated beforehand to protect the sample from air, light, heat and moisture. The samples were then transported to the laboratory [15].

2.3. Culture, isolation and identification of bacteria

The isolation method used was according to Rodier and collaborators [14]. For each milk sample, 1ml was added to a sterile test tube containing 9ml of sterile physiological water (0.85% NaCl) and decimal dilutions ranging from 10^{-5} to 10^{-7} were made to determine the dilution factor resulting in a well-counted number of colonies. One ml of each dilution was taken and poured onto a Petri dish [14].

For each water sample, 1ml was added to a sterile test tube containing 9ml of sterile physiological water. Decimal dilutions ranging from 10^{-5} to 10^{-7} were performed. 100 µl of each dilution was taken with a micropipette and spread on selected agar culture medium in Petri dishes around the sterility diameter defined by the Bunsen burner flame [14].

In the laboratory, one gram of each green fodder sample was ground with a mortar and homogenized in 9mL of sterile physiological water using a blender for 60 seconds to achieve good homogenization. Decimal dilutions ranging from 10^{-2} to 10^{-8} were performed to determine the dilution factor resulting in a well-counted number of colonies. One ml of each dilution was taken and poured onto a Petri dish [14].

Escherichia coli (*E. coli*), *Salmonella spp.* and *Brucella spp.* were isolated. *E. coli* were counted by mass plating on Endo medium. Incubation was at 44°C for 24 hours. For the counting of *E. coli* colonies formed units (CFU), brick red colonies with a diameter of 0.5mm, and having a precipitation zone were considered. The identification of *E. coli* was done by the Mac Kenzie test. This test allows simplified detection of *E. coli* by the production of indole at 44°C.

Before attempting to isolate *Salmonella spp.*, we encouraged their multiplication by using an enrichment medium: Sodium Selenite medium distributed in tubes and inoculated at a rate of 1ml. The seeded broth was incubated for 24 hours at 37°C [12]. For the isolation of *Salmonella spp.*, the SS (Salmonella-Shigella) agar was used. This medium was streaked

with a platinum loop from the 24-hour culture on Sodium Selenite medium. The colonies with black centers were then considered. Identification of the two strains was carried out according to standard biochemical criteria [16].

Isolation and culture of *Brucella spp.* was performed on brucella agar made selective by addition of cycloheximide, bacitracin, polymyxin B, nalidixic acid, nystatin, vancomycin (brucella supplement) in an atmosphere containing 5-10% CO_2 at 37°C for 2 days of incubations [17]. All visible suspects *Brucella spp.* colonies were subcultured, typed and identified using standard microbiological procedures [18]. The different cultural characteristics were as follows: translucent, round colonies with regular edges of 2-3 mm in diameter and smooth colonies of 1-2 mm in diameter.

2.4. Antimicrobial susceptibility tests

Antimicrobial susceptibility testing was carried out on isolates using the agar disc diffusion method in accordance with Clinical and Laboratory Standards Institute (CLSI) guidelines [19]. The stored isolates (previously preserved in 20% glycerol) were heated to 37^{o}C (by incubation) to activate the microorganisms. The activated bacteria were inoculated onto nutrient agar (NA) plates and incubated at 37ºC for 24 h, after which antimicrobial susceptibility testing was performed on Muller-Hinton (MH) agar. The MH agar plates, which contained 4 mm thick agar, were warmed at room temperature in the incubator with the lids open for 10-15 min to allow excess moisture to be absorbed into the medium, according to Clinical and Laboratory Standards Institute (CLSI) guidelines [19]. Enterobacteriaceae (*E. coli* and *Salmonella spp.*) were tested for susceptibility to antibiotics commonly used in veterinary and human practice, namely: Ampicillin (AM: 10µg), Amoxicillin/clavulanic acid (AMC: 20/10µg), Cefotaxime (CTX: 30µg), Ceftazidime (CAZ: 30µg), Ceftriazone (CRO:

30µg), Ofloxacin (OFX: 5µg), Gentamicin (GM:10µg), Chloramphenicol (C: 30µg), Tetracycline (TE: 30µg).

Antimicrobial susceptibility testing of *Brucella spp.* strains was performed with 10 antimicrobial agents routinely used in the treatment of brucellosis in humans. These were azithromicin (AZM: 15µg), tetracycline (TE: 30µg), doxycycline (DO: 30 µg), ciprofloxacin (CIP: 5µg), levofloxacin (LVX: 5µg), gentamicin (GM-10 µg), Rifampicin (RA5: 5µg), Trimethoprim/Sulfamethoxazole (SXT: 1.25/23.75µg), Chloramphenicol (C: 30µg) and Streptomycin (S:10µg). As acceptable limits have not yet been established, some values were interpreted according to the Clinical and Laboratory Standards Institute guidelines for the fastidious bacterium *Haemophilus spp* [19].

Purified colonies were homogeneously suspended in tubes containing 2 ml of sterile physiological solution and turbidity was adjusted to an equivalent of a Mc Farland standard of 0.5 using a colorimeter. Sterile cotton-tipped swabs were dipped into the homogenized suspensions and excess fluid was removed by pressing and rotating the swab against the side of the tube above the level of the suspension. The swabs were then spread evenly over the entire surface of Mueller-Hinton (MH) agar (Biokar, France) to produce a confluent lawn of bacterial growth. The inoculated MH agar plates were left to dry for 5 minutes before placing the antibiotic discs on the surface using sterile forceps. The plates were inverted and incubated aerobically at 37°C for 18-24 hours. After the incubation period, the plates were examined for clear zones of inhibition around the discs. The diameter of each inhibition zone was measured in millimeters (mm) using a caliper, and the results were recorded. The size of the zones was classified as sensitive (S), intermediate (I) and resistant (R) according to Clinical and Laboratory Standards Institute guidelines [19].

2.5. Data collection and statistical analysis

All data were entered into Microsoft excel 2016 and presented in tables as percentages. Student's Z test was performed using XLSTAT-Pro software version 2014.5.03 to compare the percentages of antibiotic sensitive, intermediate and resistant strains.

.3. RESULTS AND DISCUSSION

3.1. Contamination of samples

Of the 144 samples collected from the different dairy farms, 18% samples were found to be positive for *E. coli*. Of these, 26.9% were from milk (n=7), 34.6% from drinking water (n=9) and 38.4% from green fodder (n=10). *Salmonella spp.* contamination in milk (13/48) was higher in drinking water (6/48) and in green fodder consumed by the cows (9/48). Seventeen (17) phenotypic strains of *Brucella spp.* were isolated from the milk samples (n =9) and the drinking water samples (n =8).

3.2. Antibiotic susceptibility of *Escherichia coli* strains

In the present study, the antibiotic susceptibility of *E. coli* strains varied according to sample type (Fig 1). The isolates showed 100% resistance to ampicillin in all three samples. We observed high resistance at the following percentages in raw milk, drinking water and green fodder respectively: 71, 78 and 60% for amoxicillin/clavulanic acid; 86, 67 and 70% for cefotaxime; 71, 56 and 60% for ceftazidime and 86, 56 and 60% for ceftriaxone.

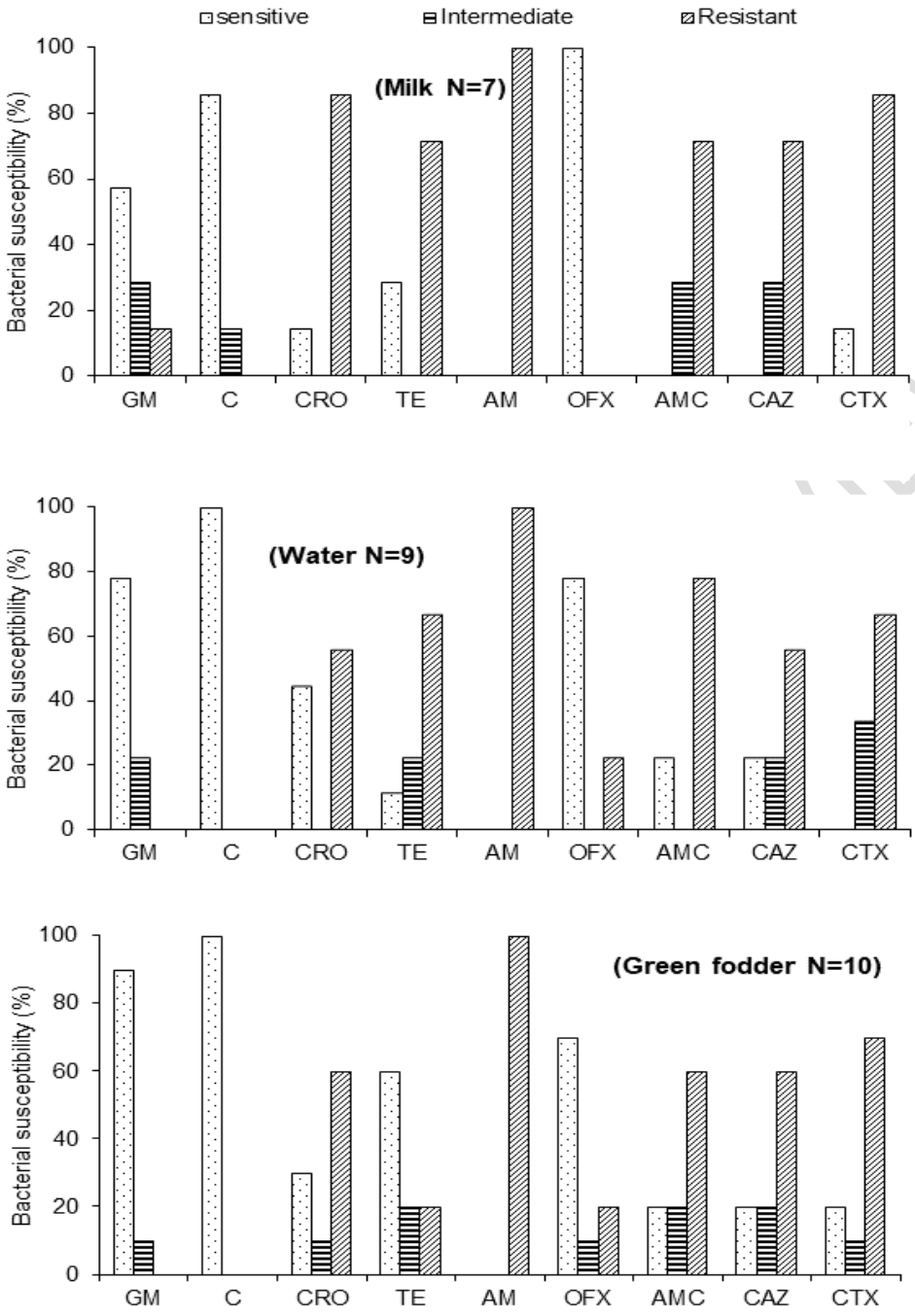

Fig 1. Susceptibility of *Escherichia coli* isolates to antibiotics **(**AM: Ampicillin; AMC: Amoxicillin+Clavulanic Acid ; CTX: Cefotaxime; CAZ: Ceftazidime; CRO: Ceftriaxone; OFX: Ofloxacin; GM: Gentamicin; C: Chloramphenicol; TE: Tetracycline)

All these 5 antibiotics belong to the β-lactam family. The indiscriminate use of these antimicrobial agents in animal husbandry and human medicine could justify the high rates of resistance among isolates. Indeed, the β-lactam antibiotics are the most widely used by farmers due to their wide availability on the informal market, their low cost and their ease of administration (not requiring the presence of veterinarian or health personnel) [9]. In human medicine, the low toxicity of β-lactams and the broad spectrum of action of some of them make β-lactams the most prescribed class of antibiotic drugs. Furthermore, the study revealed that 71% and 67% of *E. coli* were resistant to tetracycline in milk and drinking water respectively, while less resistance (20%) was observed in green fodder. Antibiotic resistance can be transferred to humans via the food chain through consumption of antimicrobial residues or contamination of resistant bacteria in animal products [10].

The high resistance detected among *E. coli* strains has been reported in other studies in Africa. In the northern region of Ghana, isolates from milk and milk products showed greater resistance to chloramphenicol, gentamicin, trimethoprim-sulfamethoxazole and ceftriaxone, but were most susceptible to ciprofloxacin and ampicillin [20]. In Ethiopia, Tadesse and collaborators, showed that *E. coli* strains isolated from dairy, fruit juice and cow's milk were highly resistant to gentamicin (100%), ciprofloxacin (90%), ampicillin (70%), tetracycline (60%) and chloramphenicol (50%) [21]. In Nigeria, Reuben and Owuna, detected 100% resistance of *E. coli* isolated from raw milk to tetracycline [22]. Similarly, Bonyadian and collaborators, reported that *E. coli* isolates from raw cow's milk and unpasteurized cheese showed resistance to ampicillin (66%), gentamicin (69.6%) and ciprofloxacin (56.7%) [23]. Resistance rates in our study are lower than those reported in Nigeria, but far higher than those reported in the northern region of Ghana [24, 20]. In this study we also detected high sensitivity of *E. coli* to ofloxacin (100, 78 and 70%), chloramphenicol (57, 78 and 90%) and gentamicin (86, 100 and 100%) (Fig 1). Studies conducted in the Northern Region of Ghana by Frederick and collaborators, reported low resistance of *E. coli* isolates from milk and

hands of milkers in Nyankpala community to Gentamicin (25.93%) [25]. *E. coli* isolates from raw cow's milk, yoghurt and cheese were also reported to have low resistance to gentamicin (6.81%) [26].

3.3. Antibiotic susceptibility of *Salmonella spp.*

In this study, all *Salmonella spp*. isolates were susceptible to ofloxacin (100%). For strains isolated from milk, drinking water and green fodder, the sensitivity was respectively 92, 100 and 89% for gentamicin and chloramphenicol; 85, 83 and 89% for ceftazidime and 69, 83 and 89% for tetracycline in (Fig 2).

Salmonella spp. is one of the main causes of foodborne infections in humans and a large number of animals. It is a pathogen involved in the spread of antimicrobial resistance as it can accumulate antibiotic resistance genes. We suggest that ofloxacin in combination with gentamicin and chloramphenicol may be the most promising drug to treat *Salmonella spp.* infections in the region. These results are similar to those of Gargano and collaborators, who showed that all *Salmonella spp.* isolates collected from pets, livestock, wildlife and food in Sicily (Italy) were susceptible to chloramphenicol, ciprofloxacin, cefotaxime, ofloxacin, levofloxacin and ceftriaxone [27].

About 62, 67 and 67% of *Salmonella spp* strains isolated from milk, water and green fodder respectively, showed resistance to ampicillin (Fig 2). Ampicillin must be the most widely used antibiotic in the region to treat various diseases affecting the dairy sector. Its use does not require prescription by veterinarians. This antibiotic is also widely used in human medical practice to treat *Salmonella spp.* [28].

Different patterns of resistance in *Salmonella spp.* have been reported in various studies. *Salmonella spp.* isolated from raw milk and dairy products in the northern region of Ghana

showed high resistance to chloramphenicol (100.0%) and ampicillin (90-100.0%) respectively, but low resistance to ciprofloxacin (0-10%) and gentamicin (20.0%) [20]. Teshome and collaborators, reported that 95.0% of *Salmonella spp.* isolated from raw camel and goat milk from Somali region of Ethiopia were resistant to ciprofloxacin and 75.0% to gentamicin and chloramphenicol [29]. Tadesse and Dabassa, also reported that *Salmonella spp.* isolated from raw milk in Kersa district, southwestern Ethiopia, showed low resistance to tetracycline (12.0%), which is consistent with the results of this study [30].

3.4. Antibiotic susceptibility of *Brucella spp.* strains

Antibiotic susceptibility testing of *Brucella spp.* isolated from raw milk and drinking water showed high susceptibility to azithromycin (89 and 88%), doxycycline (89 and 100%), ciprofloxacin (78 and 88%), levofloxacin (89 and 100%), gentamicin (100 and 88%), rifampicin (89 and 100%) and trimethoprim/sulfamethoxazole (100 and 88%). All isolates were susceptible to streptomycin (100%) and tetracycline (100%). A lesser pattern of susceptibility was observed only for chloramphenicol (33 and 38%) (Fig. 3).

Brucellosis remains an important public health problem, and the most important aspect of the One-Health approach is the close link with humans, food and livestock. Brucellosis is a common zoonosis in Cameroon, and this creates a public health problem [31]. The most important aspect of One-Health is the close link between humans, food and livestock. Accurate diagnosis and species identification of *Brucella spp.* isolated from food and livestock is highly necessary for rapid treatment. Cow's milk is the main source of human infection, and the shedding of *Brucella spp.* in milk represents an increasing threat to consumers [32]. Treatment of brucellosis in cattle is not routinely practiced due to its high cost in developing countries. In humans, doxycycline plus rifampicin or fluoroquinolones plus rifampicin are the most common antibiotic combinations recommended by the World Health Organization for the treatment of brucellosis [33].

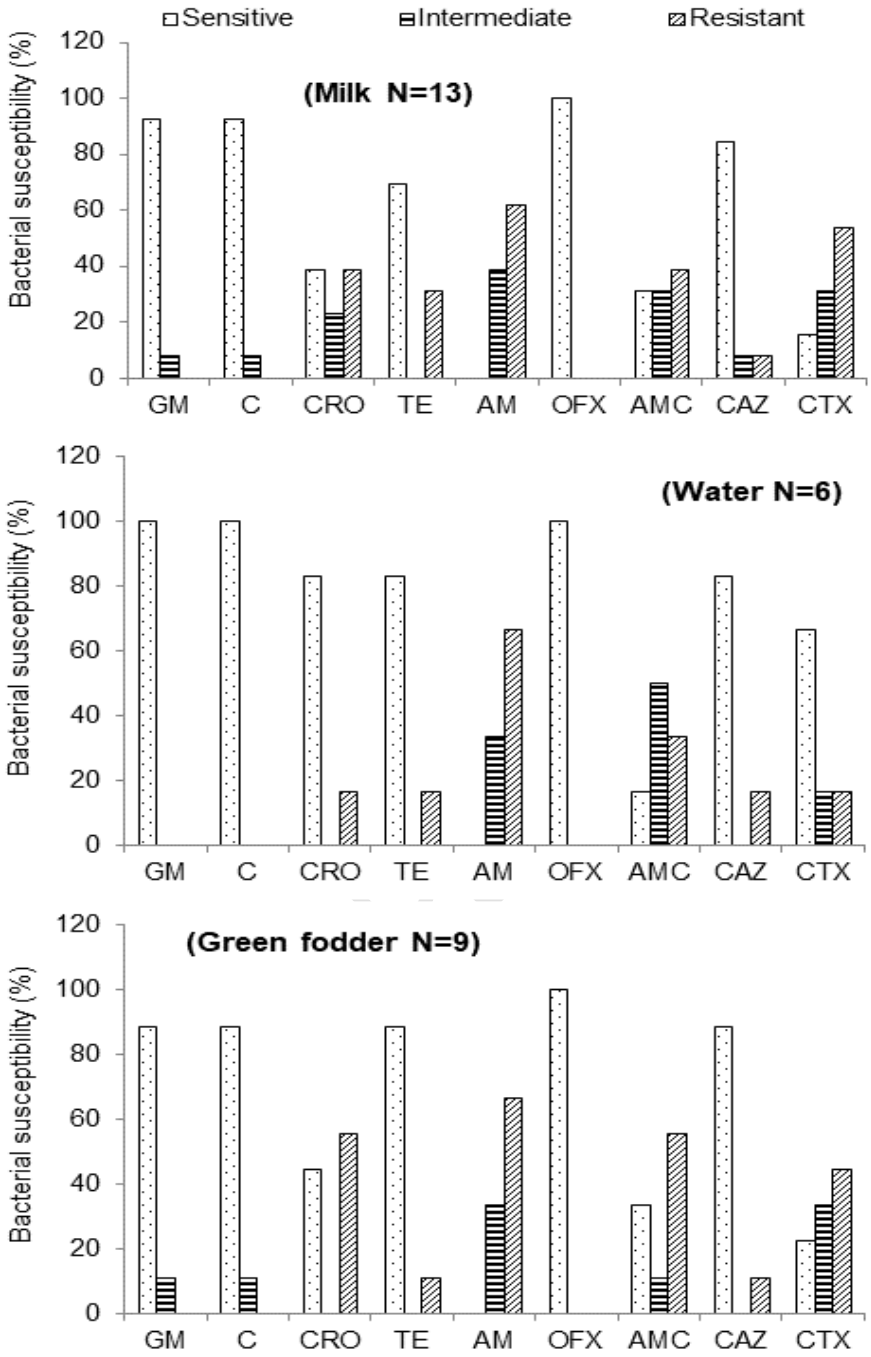

Fig 2. Susceptibility of *Salmonella spp*. isolates to antibiotics (AM: Ampicillin; AMC: Amoxicillin/Clavulanic Acid ; CTX: Cefotaxime; CAZ: Ceftazidime; CRO: Ceftriaxone; OFX: Ofloxacin; GM: Gentamicin; C: Chloramphenicol; TE: Tetracycline)

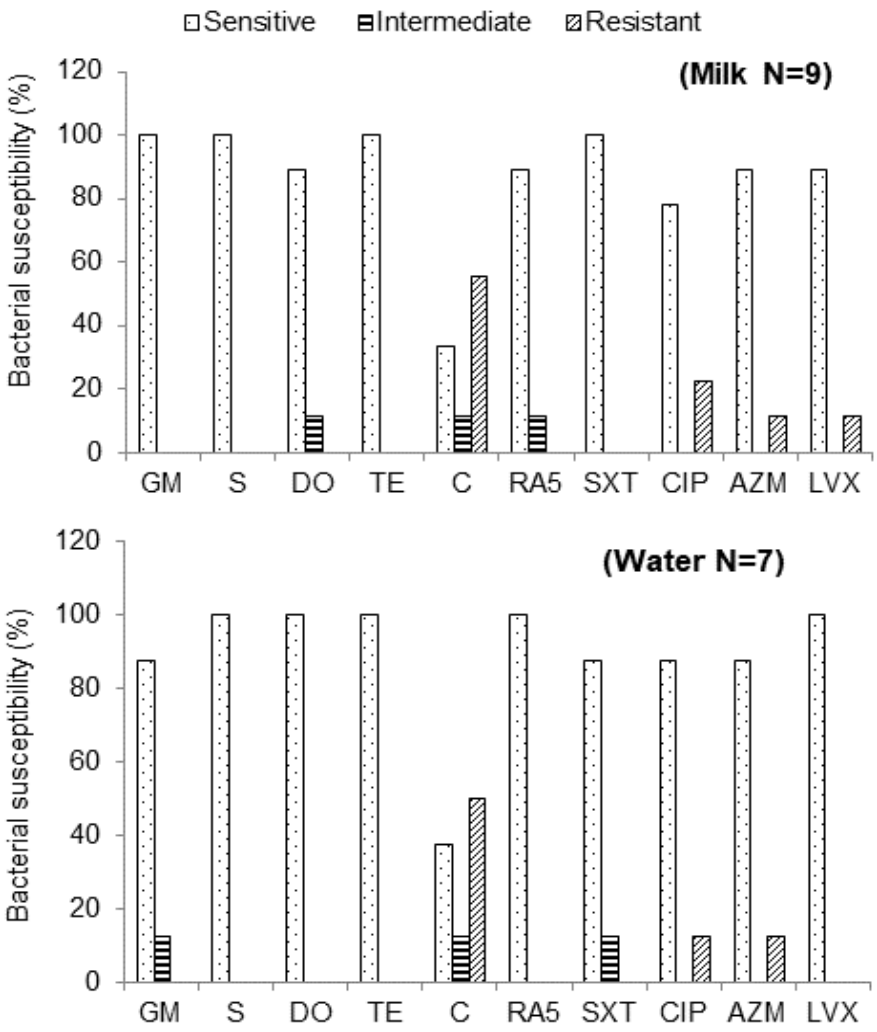

Fig 3. Susceptibility of *Brucella spp.* isolates to antibiotics (AZM : Azithromycin; TE : Tetracycline; DO : Doxycycline; CIP : Ciprofloxacin; LVX : Levofloxacin; GM : Gentamicin; RA5 :Rifampicin; SXT : Trimethoprim/Sulfamethoxazole; C : Chloramphenicol; S : Streptomycin)

Brucella spp. isolates from humans, milk and animals in Egypt have been reported to have high susceptibility to chloramphenicol, ciprofloxacin, doxycycline, gentamicin, levofloxacin, streptomycin, tetracycline, trimethoprim/sulfamethoxazole and tigecycline [33]. A 100% sensitivity of *Brucella spp.* strains to doxycycline, tetracycline, ciprofloxacin, streptomycin, gentamicin, trimethoprim/sulfamethoxazole and levofloxacin has also been reported in isolates from Turkey, China and Norway [34,35,36].

Antimicrobial susceptibility in *Brucella spp.* can be explained by the absence of classical antimicrobial resistance genes in the *Brucella spp.* genome [32]. In addition, the intracellular lifestyle of brucella, which impedes the penetration of various antimicrobials, may prevent the rapid development of resistance. Furthermore, it is now well established that *Brucella spp.* is an intracellular bacterium that escapes destruction by macrophages and causes severe mitochondrial fragmentation after 48 hours of bacterial entry into different cell types [37, 38]. Therefore, antibiotics for the treatment of brucellosis must have the ability to kill the bacterium by entering macrophages [39]. In our study, a lower sensitivity pattern was observed only for chloramphenicol (33 and 38%). Many studies have shown that inappropriate and widespread use of antibiotics can lead to antibiotic resistance among *Brucella spp.* [40,41,42].

3.5. Comparison of antibiotic susceptibility of strains tested

The Student's Z test for two proportions was performed to compare the antibiotic susceptibility of bacterial strains isolated from produced milk, drinking water and green fodder consumed in pairs. No significant difference ($P>0.05$) was noted for the susceptibility of *E. coli* isolated from raw milk and drinking water on the one hand and milk and green fodder on the other. The same observation was also made for resistance for this bacterium

when isolated from raw milk and green fodder. A difference in resistance of *E. coli* strains isolated from raw milk and green fodder was observed towards tetracycline. Apart from this difference in resistance, no other differences in resistance were observed (Table 1).

For *Salmonella spp.* strains the only significant difference ($P<0.05$) observed was with regard to susceptibility to cefotaxime. The sensitivity/resistance of *Salmonella spp.* strains did not differ between samples for most antibiotics (Table 2). No differences in sensitivity/resistance ($P>0.05$) of *Brucella spp.* strains isolated from milk and water were observed for the 10 antibiotics tested (Table 3). The similarity of antibiotic resistance was observed between bacterial strains isolated from raw milk and drinking water on the one hand and raw milk and green fodder on the other.

Table 1. *P*-values of the Z-test for proportions comparing sensitive (S), intermediate (I) and resistant (R) strains of *Escherichia coli* isolated from raw milk and drinking water on the one hand and from milk and green fodder on the other hand to antibiotics

Antibio-tics	Milk/Water			Milk/Fodder		
	Sensitive strains	Intermediate strains	Resistant strains	Sensitive strains	Intermediate strains	Resistant strains
AM	/	/	1,000	/	/	1.000
AMC	0.185	0.084	0.749	0.208	0.668	0.641
CTX	0.247	0.092	0.382	0.749	0.388	0.443
CAZ	0.185	0.749	0.539	0.208	0.668	0.641
CRO	0.198	/	0.198	0.443	0.388	0.246
OFX	0.185	/	0.185	0.110	0.388	0.208
GM	0.368	0.749	0.247	0.115	0.314	0.223
C	0.247	0.247	/	0.223	0.223	/
TE	0.361	0.185	0.864	0.208	0.208	**0.035***

*: $P<0.05$; AM: Ampicillin; AMC: Amoxicillin+Clavulanic Acid ; CTX: Cefotaxime; CAZ: Ceftazidime; CRO: Ceftriaxone; OFX: Ofloxacin; GM: Gentamicin; C: Chloramphenicol; TE: Tetracycline

This could be the result of the subsequent transfer of resistance genes and bacteria into the gut flora. Indeed, according to Al Muhairi and collaborators, there is a possibility of the spread of resistance to pathogenic and commensal bacteria in the gut flora through horizontal gene transfer mechanisms following the consumption of contaminated food. Resistance genes are commonly associated with mobile genetic elements (MGEs) [43]. These are called the mobilome. Bacteria have genetic material that allows the flow of resistance genes via MGEs, which are: plasmids, transposons and integrons. These flows can take place not only between bacteria of the same species and genus but also between several bacterial genera. MGEs are variably present in the bacterial population; therefore they do not carry elements essential for bacterial function. In principle, resistance genes can be acquired from any source, but in practice gene flow is likely to be structured by ecology, and by species that share similar ecological niches and sources of resistance genes [44,4].

Table 2. *P*-values of the Z-test for two proportions comparing sensitive (S), intermediate (I) and resistant (R) strains of *Salmonella spp.* isolated from raw milk and drinking water on the one hand and from milk and green fodder on the other hand to antibiotics

Antibio-tics	Milk/Water			Milk/Fodder		
	Sensitive strains	Intermediate strains	Resistant strains	Sensitive strains	Intermediate strains	Resistant strains
AM	/	0.833	0.833	/	0.810	0.810
AMC	0.521	0.425	0.833	0.921	0.271	0.404
CTX	**0.023***	0.521	0.129	0.673	0.921	0.645
CAZ	0.911	0.476	0.558	0.786	0.385	0.811
CRO	0.068	0.201	0.359	0.778	0.122	0.404
OFX	1.000	/	/	1.000	/	/
GM	0.476	0.476	/	0.811	0.811	/
C	0.476	0.476	/	0.811	0.811	/
TE	0.521	/	0.521	0.272	/	0.271

*: P<0.05; AM: Ampicillin; AMC: Amoxicillin+Clavulanic Acid ; CTX: Cefotaxime; CAZ: Ceftazidime; CRO: Ceftriaxone; OFX: Ofloxacin; GM: Gentamicin; C: Chloramphenicol; TE: Tetracycline

Table 3. *P*-values of the Z-test for two proportions comparing Sensitive (S), intermediate (I) and resistant (R) strains of *Brucella spp.* isolated from raw milk and drinking water of cows to antibiotics

Antibiotics	Milk/Water		
	Sensitive strains	Intermediate strains	Resistant strains
AZM	0.951	/	0.899
TE	1.000	/	/
DO	0.308	1.000	/
CIP	0.586	/	0.628
LVX	0.334	/	1.000
GM	0.343	1.000	/
RA5	0.308	1.000	/
SXT	0.343	1.000	/
C	0.830	0.899	0,.805
S	1.000	/	/

AZM : Azithromycin; TE : Tetracycline; DO : Doxycycline; CIP : Ciprofloxacin; LVX : Levofloxacin; GM : Gentamicin; RA5 :Rifampicin; SXT : Trimethoprim/Sulfamethoxazole; C : Chloramphenicol; S : Streptomycin

4. CONCLUSION

The study revealed high levels of resistance in *Escherichia coli* to commonly prescribed antibiotics in veterinary medicine. Less resistance was observed for *Salmonella spp.* isolates in the different samples. Most of the antibiotics tested on *Brucella spp.* strains in this study, with the exception of chloramphenicol, showed effective inhibitory activity. This suggests the effectiveness of antibiotics commonly used for the treatment of brucellosis. Similarities in antibiotic resistance were observed between bacterial strains isolated from raw milk, drinking water and green fodder. The emergence of resistance to various antibiotics commonly used in medical and veterinary practices has important implications for public health. This situation calls for a strengthening of the regulations covering the sale and prescription of antibiotics. Indeed, foodborne bacteria resistant to antibiotics can cause complicated, untreatable and prolonged infections in humans, resulting in higher health costs and sometimes death.

COMPETING INTERESTS

The authors declare that there is no conflict of interest regarding the publication of this paper.

REFERENCES

1. Ndahetuye JB, Artursson K, Båge R, Ingabire A, Karege C, Djangwani J and al. Milk Symposium review: Microbiological quality and safety of milk from farm to milk collection centers in Rwanda. J. Dairy Sci. 2020; (103): 9730-9739.

2. Fusco V, Chieff D, Fanelli F, Antonio FL, Cho GS, Kabisch J and al. Microbial quality and safety of milk and milk products in the 21st century. Compr. Rev. Food Sci. Food Saf. 2020;19:2013-2049.

3. Alexa EA. Dairy products and dairy-processing environments as a reservoir of antibiotic resistance and quorum-quenching determinants as revealed through functional metagenomics. mSystems 2020.

4. Liu J, Zhu Y, Jay-Russell M, Lemay DG. Mills DA. Reservoirs of antimicrobial resistance genes in retail raw milk. Microbiome. 2020; 8:99. https://doi.org/10.1186/s40168-020-00861-6.

5. Malmuthuge N, Guan L. Understanding host-microbial interactions in rumen: searching the best opportunity for microbiota manipulation. J. Anim. Sci. Biotechnol. 2017; 8.

6. Addis MF, Tanca A, Uzzau S, Oikonomou G, Bicalho RC, Moroni P. The bovine milk microbiota: Insights and perspectives from-omics studies. Mol. Biosyst. 2016;12: 2359-2372. https://doi.org/10.1039/C6MB00217J.

7. Young W, Hine BC, Wallace OA, Callaghan M, Bibiloni R. Transfer of intestinal bacterial components to mammary secretions in the cow. Peer J. 2015;3. Article e888. https://doi.org/10.7717/ peerj.888.

8. Ngo Bahebeck P, Nougang Ebiane M, Nana PA, Metsopkeng CS, Tamsa Arfao A, Noah Ewoti O et al. Relative importance of the bacteria *Brucella, Salmonella, Staphylococcus* and other indicator bacteria in some mountain farm waters in West-Cameroon (Central Africa) and the potential role of some environmental factors. World J. Adv. Res. Rev. 2020; 08(02): 081-094.

9. Maïwore J, Youssouf D, Amadou TA, Baane MP, Ngoune TL, Montet D. *Staphylococcus* in raw cow's milk from Maroua (Cameroon): determination of their resistance level to antibiotics. Acta Sci. Microbiol. 2020; 3(4): 44-52.

10. Bissong AE, Tahnteng BF, Ateba C, Akoachere JF. Pathogenic potential and antimicrobial resistance profile of *Staphylococcus aureus* in milk and beef from the Northwest and Southwest regions of Cameroon. Biomed Res. Int. 2020 Article ID 6015283, 12 pages https://doi.org/10.1155/2020/6015283.

11. BUCREP (Central Bureau of Census and Population Studies of Cameroon). Report on the presentation of the final results. 2010. www.statistics-cameroon.org. French.

12. Bayemi PH, Mah GD, Ndamukong KV, Nsongka M, Leinyuy I, Unger H and al. Bovine brucellosis in cattle production systems in the western highlands of Cameroon. Int. J. Anim. Biol. 2015;1(2): 38-44.

13. NMC (National Mastitis Council) Laboratory Handbook on Bovine Mastitis. Rev. ed. National Mastitis Council Inc., New Prague, MN. 2017.

14. Rodier J, Legube B, Merlet N. The analysis of water: Natural water, wastewater, sea water, Technique and engineering, Paris, Dunod, 10 ed. 2016: 1824p. French

15. Glauser W. Food sampling on farms. Fact sheet for the current ALP practice. 2007 : 30. French

16. Holt JG, Krieg NR, Sneath PH, Staley JT, Williams ST. Bergey's manual of determinative bacteriology. 9th edition. Lippincott Williams and Wilkins (Ed.), Philadelphia. 2000:787p.

17. Scholz HC, Banai M, Cloeckaert A, Kämpfer P, Whatmore AM. *Brucella spp*. bergey's manual of systematics of Archaea and Bacteria. 2018: 1-38.

18. OIE. Brucellosis (infections with *B. abortus, B. melitensis* and *B. suis*). Chapter 2.1.4', in Manual of diagnostic tests and vaccines for terrestrial animals, World Health Organization, Paris, viewed May 2016, from https://www.oie.int.

19. CLIS Clinical and Laboratory Standards Institute. Performance Standards for Antimicrobial Susceptibility Testing. 3st ed. CLSI Supplement M100, Wayne, PA, USA. 2021.

20. Vicar KE, Feglo KP, Acquah KES, Williams W, Saba SKC, Mensah GI. Antibiotic resistant bacteria in raw cow milk and milk products retailed in the Northern Region of Ghana; a food safety challenge. J. Food Safe Hyg. 2019; 5(4): 206-213.

21. Tadesse HA, Gidey NB, Workelule K, Hailu H, Gidey S, Bsrat A and al. Antimicrobial resistance profile of *E. coli* isolated from raw cow milk and fresh fruit juice in Mekelle,

Tigray, Ethiopia. Vet. Med. Int. 2018, Article ID 8903142, 7 p. https://doi.org/10.1155/2018/8903142.

22. Reuben RC, Owuna G. Antimicrobial resistance patterns of *Escherichia coli* O157:H7 from Nigerian fermented milk samples in Nasarawa State, Nigeria. Int. J. Pharma Sci. Invent. 2013; 2: 38-44.

23. Bonyadian M, Moshtaghi H, Taheri MA. Molecular characterization and antibiotic resistance of enterotoxigenic and entero-aggregative *Escherichia coli* isolated from raw milk and unpasteurized cheeses. Vet. Res. Forum. 2014; 5: 29-34.

24. Abike TO, Olufunke OA, Oriade KD. Prevalence of multiple antibiotic resistant *Escherichia coli* serotypes in cow raw milk samples and traditional dairy products in Osun State, Nigeria. British Microbiol. Res. J. 2015; 5: 117-25.

25. Frederick A, Courage KSS, Gabriel AT. Antibiotic susceptibility of *Escherichia coli* isolated from milk and hands of milkers in Nyankpala community of Ghana. Curr. Res. Dairy Sci. 2016; 6-11. DOI: 10.3923/crds.2016.6.11.

26. Lues J, De Beer H, Jacoby A, Jansen KE, Shale K. Microbial quality of milk, produced by small scale farmers in a peri-urban area in South Africa. Afric J. Microbiol Res. 2010; 4: 1823-30.

27. Gargano V, Sciortino S, Gambino D, Costa A, Agozzino V, Reale S and al. Antibiotic susceptibility profile and tetracycline resistance genes detection in *Salmonella spp.* strains isolated from animals and food. J. Antibiot. 2021 ; 10, 809. https:// doi.org/10.3390/antibiotics10070809.

28. Santé Canada (2009). Categorization of antimicrobial drugs based on their importance in human medicine. http://www.hc-sc.gc.ca/dhp-mps/vet/antimicrob/amr_ram_hum-med-rev-fra.php. French.

29. Teshome B, Tefera G, Belete B. Prevalence and antimicrobial susceptibility pattern of S*taphylococcus aureus* from raw camel and goat milk from Somali region of Ethiopia. Afric. J. Microbiol. Res. 2016; 10: 1066-71.

30. Tadesse T, Dabassa A. Prevalence and antimicrobial resistance of *Salmonella* isolatedFrom raw milk samples collected from Kersa District, Jimma Zone, Southwest Ethiopia. J. Med. Sci. 2012; 12: 224-28.

31. Awah-Ndukum J, Mouiche MM, Kouonmo-Ngnoyum L, Bayang HN, Manchang TK, Poueme RS and al. Seroprevalence and risk factors of brucellosis among slaughtered indigenous cattle, abattoir personnel and pregnant women in Ngaoundere, Cameroon. BMC Infect. Dis. 2018; 18:611:13. https://doi.org/10.1186/s12879-018-3522-x

32. Wareth G, El-Diasty M, Abdel-Hamid NH, Holzer K, Hamdy MER, Moustafa S and al. Molecular characterization and antimicrobial susceptibility testing of clinical and non-clinical *Brucella melitensis* and *Brucella abortus* isolates from Egypt. Int. J. One Health. 2021;13:100255.

33. WHO World Health Organization. Brucellosis in humans and animals. http://www.who.int/csr/resources/publications/deliberate/WHO_CDS_EPR_2006_7/en/ , 2006.

34. Dal T, Durmaz R, Ceylan A, Bacalan F, Karagoz A, Celebi B and al. Molecular investigation of the transmission dynamics of brucellosis observed among children in the province of South - East Anatolia, Turkey, Jundishapur. J. Microbiol. 2018: 11, e58857, https://doi.org/10.55812/ jjm.58857.

35. Liu ZG, Cao XA, Wang M, Piao DR, Zhao HY, Cui BY and al. Whole-genome sequencing of a *Brucella melitensis* strain (BMWS93) isolated from a bank clerk and exhibiting complete resistance to rifampin. Microbiol. Resour. Announc. 2019:8, e01645-01618. https://doi.org/10.01128/MRA.01645-01618.

36. Johansen TB, Scheffer L, Jensen VK, Bohlin J, Feruglio SL. Whole-genome sequencing and antimicrobial resistance in Brucella melitensis from a Norwegian perspective. Sci. Rep. 2018:8, 8538. https://doi.org/10.1038/s41598-018-26906-3.

37. Palanduz A, Palanduz S, Güler K, Güler N. Brucellosis in a mother and her young infant: probable transmission by breast milk. Int. J. Infect. Dis. 2000 ; 4: 55-56. https://doi.org/10.1016/s1201-9712(00)90068-7.

38. Giannacopoulos I, Eliopoulou MI, Ziambaras T, Papanastasiou DA. Transplacentally transmitted congenital brucellosis due to *Brucella abortus*, J. Infect. 2002;45:209-210. https://doi.org/10.1016/s0163-4453(02)91043-1.

39. Ackelsberg J, Liddicoat A, Burke T, Szymczak WA, Levi MH, Ostrowsky B and al. *Brucella* exposure risk events in 10 clinical laboratories, New York City, USA, 2015 to 2017. J. Clin. Microbiol. 2020; 58. https://doi.org/10.1128/jcm.01096-19.

40. Zhang N, Huang D, Wu W, Liu J, Liang F, Zhou B, Guan P. Animal brucellosis control or eradication programs worldwide: a systematic review of experiences and lessons learned. Prevent. Vet. Med. 2018;160: 105-115. https://doi.org/10.1016/ j.prevetmed.2018.10.002.

41. Bagheri Nejad R, Krecek RC, Khalaf OH, Hailat H, Arenas-Gamboa AM. Brucellosis in the Middle East: current situation and a pathway forward. PLoS Negl. Trop. Dis. 2020 ;14, e0008071. https://doi.org/10.1371/journal.pntd.0008071.

42. Hayoun MA, Muco E, Shorman M. Brucellosis, in: Stat Pearls, Publishing LLC, Treasure Island (FL). 2020.

43. Al Muhairi FM, Bulshawareb AM, Al Nuaimi AM, Al Hemeiri MK, Al Aiyan A, Mohteshamuddin K. The role of antimicrobials in food animals in the emergence of resistant human pathogens. Int. J. Med. Rev. Case Rep. 2019; 3(9): 598-608.

44. Wellington EM, Boxall AB, Cross P, Feil EJ, Gaze WH, Hawkey PM. The role of the natural environment in the emergence of antibiotic resistance in gram-negative

bacteria. Lancet Infect. Dis. 2013; 13(2) : 155-165. doi: 10.1016/S1473-3099(12)70317-1.