Multidrug-resistant *Escherichia coli* in Raw Milk: Molecular Characterization and the potential impact of camel’s Urine as an Antibacterial Agent

Ayman Elbehiry, Eman Marzouk, Ihab M. Moussa, Afrah Alenzi, Khalid S. Al-Maary, Ayman S. Mubarak, Hanan D. Alshammari, Dalia Al-Sarar, Roua A. Alsubki, Hassan A. Hemeg, Saleh A. Kabib, Osama A. Attala

**Abstract**

Raw milk is one of the most important vehicles for transmitting various pathogens, especially *Escherichia coli* (*E. coli*). Multidrug-resistant pathogens are highly prevalent among mastitic cows in various dairy farms worldwide. Therefore, our current study is based on the identification of *E. coli* from mastitic cow’s milk and their resistance to various antibacterial agents. As well, the impact of camel’s urine on multi-drug resistant *E. coli* were also evaluated. Thirty-three *E. coli* isolates were recovered from 254 milk samples. All strains were initially identified phenotypically by culturing on specific media and Vitek 2 Compact System. The protein fingerprinting technique was used as a confirmatory method. The *Stx1*, *Stx2* and *eae* genes were also verified by polymerase chain reaction (PCR). The antimicrobial resistance of *E. coli* strains was tested by the Vitek 2 AST-GN69 cards. Thirty multi-drug resistant *E. coli* strains (20 from mastitic milk and 10 from clinical samples) were laboratory tested with different concentrations (100%, 75%, 50% and 25%) of virgin and breeding camel’s urine, using the paper disc diffusion method. Our findings showed that 93.94% of *E. coli* strains were recognized by the Vitek™2 system. The results of proteomic investigation illustrated that 100% of *E. coli* strains were identified at log values ≥2.00. The genotypic identification of the three virulence genes illustrated that 90.1%, 63.64%, and 30.55% of *E. coli* strains were able to carry the *Stx1*, *eae*, and *Stx2* genes, respectively. Most strains of *E. coli* showed strong resistance against cefazolin (78.79%), ceftazidime (66.67%), cefotaxime (60.61%), ceftriaxone (54.55%), and cefepime (39.40%). The results of the antibacterial effect of camel’s urine revealed that the mean inhibitory zones of virgin camel’s urine were 28 mm, 17 mm, and 14 mm, for the concentrations of 100%, 75%, and 50%, respectively. Whereas; the inhibitory zones for the breeding camel’s urine were 18 mm, 0 mm, and 0 mm, for the concentrations of 100%, 75%, and 50%, respectively. We concluded that the majority of *E. coli* strains were able to harbor some virulence genes and resist many antibiotics. Our study also provided a robust evidence that the camel’s urine, particularly from the virgin camels has robust antimicrobial activity against multidrug-resistant *E. coli* strains.

© 2021 The Authors. Published by Elsevier B.V. on behalf of King Saud University. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).
1. Introduction

Mastitis in cattle is one of the most important diseases that lead to great economic loss in animal farms, not only in developing countries but in most countries of the world (Abebe et al., 2016). Costs resulting from mastitis include severe shortages in milk production, exclusion of infected animals from the herd, and expensive veterinary drug costs (Seegers et al., 2003). In addition, mastitis has a thoughtfully zoonotic perspective linked with the detaching of various bacteria and their toxic substances in the milk (González and Wilson, 2003).

Previous studies have shown that mastitis has developed a clear danger to human health, due to the aptitude of disease-causing microorganisms, as well as their toxins, to enter the food chain and then lead to severe foodborne diseases. (Oliver et al., 2005; Hennekinne et al., 2012), particularly via the ingestion of unpasteurized milk (Gillespie et al., 2009). There are many microorganisms that cause mastitis in cows and the bacterium E. coli represents one of the significant reasons for symptomatic and asymptomatic mastitis among dairy farms (Burvenich et al., 2003; Abebe et al., 2014; Bedasa et al., 2018; Ismail and Abutarbush, 2020). The Gram-negative E. coli is rod-shaped bacterium that frequently established in the human’s intestine and animals. However, the majority of E. coli strains are safe, certain strains, for instance, E. coli strains that produce Shiga toxins, has the competence to cause foodborne illnesses (Dhaka et al., 2016; Wang et al., 2016; Ismail and Abutarbush, 2020). Predominantly, this germ is transferred to human beings via ingesting adulterated food, such as unpasteurized milk and dairy products (Bali et al., 2013).

There are many highly virulent genes produced by E. coli. Shiga toxins (Stx1 & Stx2), and intimin (eae) are the most common identified genes from cows suffering from symptomatic mastitis which represents an explicit danger to human healthiness. The development of multi-drug resistant E. coli strains recovered from mastitic milk and clinical samples is considered a public health alarm worldwide (Kahlmeter and Poulsen, 2012; Copur-Cicek et al., 2014). Previous scientific reports have proven that there is a close correlation between the amazing development of multidrug-resistant E. coli strains from different animals and those from human clinical samples (Rasheed et al., 2014; Walther et al., 2017; Ismail and Abutarbush, 2020).

Antibiotic resistance to various pathogens is a thoughtful community health problem that connected with some higher frequency of infections in different areas in the world (Velez and Sloand, 2016; Frieri et al., 2017). Multidrug resistance bacteria are hard to treat and may even be untreatable with conservative antimicrobial drugs (Frieri et al., 2017). The World Health Organization has confirmed that the resistance of various microbes to many antibiotics is one of the most important risks facing public health in the current century. This global problem has forced the researchers to look for novel agents with lesser resistance.

As described previously in Prophetic texts and confirmed by scientific researches, camel’s urine has numerous uses which are beneficial for humans (Osman et al., 2013). The action of camel’s urine on human health was described by Ibn Sayyid Al-Nas who stated that camels feed on warm wood herbs are enormously beneficial in improving human digestive disorders and help detoxification of the liver leading to treatment of hepatitis (Fontenelle et al., 2007). Thus Arabian camel’s urine was an ancient prescription schedule in Arab medicine; and remained until now as a remedy and as a diuretic, snuff tool and delousing hair wash (Kyle and Dahl, 2004).

Camel’s urine has a distinctive biochemical structure. The biochemical ingredients of camel’s urine were reported previously by Read (1925), who stated that dissimilar to all other animals, camels couldn’t excrete ammonia and an only minimal amount of urea, and these particles are accountable for the offensive odor and poisonousness of urine. Nevertheless, an amount of creatine and creatinine was noticed (Mostafa and Dwedar, 2016). Compared with the other mammals including humans, the alkalinity of camel’s urine may be due to high concentrations of salts (e.g. K, Mg) and little amount of uric acid, sodium, and creatinine (Read, 1925; Kamalu et al., 2004).

Although some studies had proved that camel’s urine has a lethal effect on various types of bacteria and fungi, there is a little information about its antimicrobial effects (Osman et al., 2013). However, some previous reports showed that camel’s urine has significant antimicrobial activities against various pathogenic microorganisms that infect human such as Staphylococcus aureus, Pseudomonas aeruginosa, and Escherichia coli isolates (AL-Talhi and AL-Bashan, 2006). Another study conducted by Al-Bashan (2011) who confirmed the broad spectrum of camel’s urine as an antimicrobial agent against different types of highly virulent bacteria comprising Escherichia coli and Pseudomonas aeruginosa as well as certain types of fungi such as Aspergillus niger, Aspergillus flavus and Candida albicans. They proved that the camel’s urine has a strong antimicrobial activity against the tested microorganisms. Another investigation achieved by Khalifa et al. (2005) who used the camel’s urine (up to 100%) as antibacterial to treat E. coli in liver tissue of experimental rabbits and they found the camel’s urine was able to kill E. coli without any pathological changes.

The antibacterial effect of camel’s urine is correlated to numerous aspects for example its concentrations of salts, pH (8.15–9.01), in addition the camels are able to feed on plants with active natural compounds, together with the inhabitant microorganisms, and excreted antibacterial ingredients (Kamalu et al., 2004; Mostafa and Dwedar, 2016). Hence, the goal of this study was to identify the Gram-negative E. coli recovered from raw milk of cows showed signs of mastitis. As well, studying the potential impact of camel’s urine on the E. coli strains that exhibited several resistances to various antibiotics.

2. Materials and methods

2.1. Samples collection and bacterial isolation

Two hundred and fifty-four samples of seemingly healthy cows’ milk and that showed signs of mastitis were collected from November 2018 to January 2019 from different cattle farms with a history of mastitis in the Al-Qassim region, KSA. The apparently infected cows under precautionary measures, then all samples were collected from raw milk of cows showed signs of mastitis, which is more specific media for isolation of Staphylococcus aureus. Thus Arabian cattle from Al-Qassim region were inoculated on Coliform ChromoSelect Agar (Sigma-Aldrich, USA) and then incubated for 24 h at 37° C. All positive isolates were streaked on Brain Heart Infusion (BHI) media (Sigma-Aldrich, USA) and then incubated for 24 h at 37° C. All positive isolates on BHI media were also inoculated on Coliform ChromoSelect Agar (Sigma-Aldrich, USA) which is more specific media for isolation of E. coli to obtain the growth culture characteristics of pure colonies. Finally, Gram staining was accomplished to confirm our findings.
2.2. Biochemical and proteomic identifications of E. coli isolates

The potential detection of E. coli isolates was applied through the colony morphology. The confirmatory identification was carried out biochemical and proteomic analyses using Vitek 2 Compact System (BioMérieux, Paris, France) and Peptide Mass Fingerprinting Technique (PMFT) (Bruker, Germany), respectively. E. coli ATCC 35,218 and E. coli DH5 alpha were used as reference strains for Vitek 2 Compact System and PMFT, correspondingly. All processed samples for MALDI-TOF MS were prepared by culturing on BHI media, and then were incubated for 18–24 h at 37 °C. Ethanol-formic acid-acetonitrile extraction protocol (Barreiro et al., 2010) was applied for proteomic identification of different isolates of E. coli recovered from the milk of mastitic cows. Furthermore, the PCR was performed for molecular analysis of E. coli strains based on the protocol designated formerly by Vidal et al. (2005). The amplifications were implemented with three oligonucleotide primers (forward and reverse) as can be seen in Table 1.

2.3. Antimicrobial resistance of E. coli isolates using VITEK® 2 AST cards

According to the protocol designated by the company of BioMérieux (France), we utilized the Vitek 2 AST-GN69 (CLSI, 2014) to detect the degree of susceptibility and the resistance of 33 E. coli isolates. Three classes of antibiotics were examined with the Vitek 2 AST-GN69 card as follows: Beta-lactam (aztreonam and doripenem), carbapenems (ertapenem, imipenem, and meropenem, and cephalosporins [cefazolin (1st generation), cefotaxime (3rd generation), ceftriaxone (3rd generation), and cefepime 4th generation]). The Sensititre Nephometer (TREK Diagnostic Systems, Ashford, Kent, England) was performed to adjust the bacterial turbidity using NaCl (0.9%) to obtain turbidity equivalent ca 1 × 10^8 CFU/ml after comparing with 0.5 McFarland standards. The E. coli ATCC 25,922 was used in the current investigation as a quality control strain.

2.4. Camel’s urine used in the study

2.4.1. Samples collection

Camel’s urine was obtained from healthy, domesticated camels in the Al-Qassim region. All animals were females and aged between 2 and 10 years. All animals were apparently healthy and raised in a private farm. The samples were obtained during feeding with the help of experienced camel attendants. A total of 300 ml of urine collected from each camel, were kept in insulated boxes using freezing packs and transferred to the laboratory. Twenty E. coli isolates from mastitic milk and 10 clinical isolates from King Fahad Specialist Hospital–Buraydah, Saudi Arabia were used in our investigation. All E. coli strains were considered as multiple drug resistance organisms by being tolerant of ≥three antimicrobial drugs.

2.4.2. Preparation of paper disk diffusion test (disks with the camel urine)

A bacterial suspension of each isolate was prepared. We used 0.5 McFarland standard solutions to adjust the turbidity of the bacterial suspension. All E. coli isolates were inoculated on Müller-Hinton agar using a sterile cotton swab, then the prepared concentrations of camel’s urine discs were placed on the selected bacterial cultures. The plates incubated at 37 °C for 24 h. Then the examination was carried out for the presence of clear zones of inhibition and measured in millimeters (mm). The presence of zones of inhibition indicates antimicrobial activity. The inhibition zones of camel’s urine were compared with five standards of antimicrobial agents (Amikacin, Chloramphenicol, Amoxicillin, Gentamicin and Metronidazole).

2.4.3. Determination of the antimicrobial activity of camel’s urine

To determine the antimicrobial activity of camel’s urine, samples were initially sterilized using autoclave then, the paper disc diffusion method was carried out. The camel’s urine with 4 different concentrations (100%, 75%, 50% and 25%) were performed through addition of 100, 75, 50, 25 ml urine to 0, 25, 50, 75 ml distilled water in a sterile test tube, respectively. A punch machine was used to prepare the discs of filter paper (Whatman No. 1, Sigma-Aldrich, USA) with a diameter of 6 mm. A dry heat sterilizer was then used to sterilize all discs. The ready to use disc was soaked in diluted urine and then placed onto the plates and incubated for 24 h at 38 °C.

2.5. Statistical analysis

The statistics from the antibacterial effect of camel’s urine will be transported into the SPSS, and all assessments will be completed via SPSS version 20.0.

3. Results

3.1. Identification of E. coli isolates

Out of 254 milk samples exhibited positive reactions to CMT, 33 (12.1%) E. coli isolates were isolated using culture technique, and 31 (93.94%) of them were appropriately identified by the Vitek™ 2 system. The results of MALDI-TOF MS revealed that all E. coli strains (100%) were identified at log values ≥ 2.00. According to the graphic inspection of mass regions, a number of variable peak intensities were noticed between 3.000 Da and 10.400 Da. The highest signal of intensity was identified at 5.400 Da and 6.300 Da (Fig. 1). The genotypic identification of Stx1, Stx2, and eae virulence genes was performed using PCR and our findings revealed that out of 33 E. coli strains, 30 (90.1%), 11 (30.55%), and 21 (63.64%) harbored the Stx1, Stx2, and eae virulence genes, respectively.

3.2. Antimicrobial resistance of E. coli strains

According to the 2014 CLSI M100-S24 breakpoints, 33 E. coli isolates from mastitic milk were tested against various antibiotics. As demonstrated in Table 2, 78.79% (26/33), 66.67% (22/33), 60.61% (20/33), 54.55% (18/33), and 39.40% (13/33) of E. coli isolates were tolerant to cefazolin, cefazidime, cefotaxime, ceftriaxone, and cefepime, respectively. It is evident from the previous results that most strains of E. coli recovered from mastitic milk are resistant to cephalosporins and aztreonam group of antibiotics. In contrast, the results of carbapenems (class of beta-lactam antibiotic) showed that the majority of E. coli strains resisted this group of antibiotics to a small degree, ranging from 12 to 21%. Therefore, the current

Table 1

| Target gene | Primer sequences (5‘-3’) | Base pair |
|-------------|--------------------------|-----------|
| Stx1        | CAGTTAATGGTGTCGCCAACGG  | 348       |
|             | CAGCAGACATGAAACCGTGGT    |           |
|             | ATTCTTAATCCGGGAGTTTACAG  | 584       |
|             | GCGTCATCGTATACACAGGAGC   |           |
| Stx2        | TCAATGCGATCTCCGTATTACAGT | 482       |
|             | GTAAAGTCCGTTACCCCAACTG   |           |
| eae         |                          |           |
study confirmed that the *E. coli* strains recovered from the milk of cows suffering from mastitis were multi-drug resistant.

3.3. Evaluation of camel’s urine bioactivity

In this investigation, we used various concentrations of camel’s urine to determine its antibacterial effect against a total of 30 strains of multidrug-resistant *E. coli* (20 from mastitic milk and 10 clinical isolates from the Strain Bank. As shown in Table 3 and Fig. 2, the antibacterial activity of virgin and breeding camel’s urine was compared with 4 standard antibiotics (amoxicillin, AML, amikacin, AK, chloramphenicol, C and gentamycin, GEN) against the above-mentioned bacteria. The results revealed that the inhibitory zones of virgin camel’s urine against multi-drug resistant *E. coli* strains were 28 mm, 17 mm, and 14 mm, for the concentrations of 100%, 75%, and 50%, respectively. Whereas; the inhibitory zones for the breeding camel’s urine were 18 mm, 0 mm, and 0 mm, for the concentrations of 100%, 75%, and 50%, respectively. Whereas, the inhibition zones for AML, AK, C, and GEN were 11, 24, 22, 23 mm, respectively. This finding indicated that camel’s urine is more potent than the commercial antibiotic against *E. coli* strains. Interestingly, the virgin camel urine has more antibacterial activity than the breeding camel’s urine.

4. Discussion

*E. coli* represents one of the most significant environmental microorganisms that cause bovine mastitis and represents one of the significant coliforms that have received great attention, due to their higher incidence rate than other microbes that cause mastitis.

Table 2

| Antimicrobial agent | Degree of resistance and susceptibility |
|---------------------|----------------------------------------|
|                     | R | I | S |
| No. of isolates     | % | No. of isolates | % | No. of isolates | % |
| Aztreonam Cephalosporins and aztreonam | 11 | 33.33 | 3 | 9.10 | 19 | 57.58 |
| Cefazolin           | 26 | 78.79 | 0 | 0.00 | 7 | 21.21 |
| Cefepime            | 13 | 39.40 | 2 | 6.10 | 18 | 54.55 |
| Cefotaxime          | 20 | 60.61 | 1 | 3.03 | 12 | 36.36 |
| Cefazidime          | 22 | 66.67 | 0 | 0.00 | 11 | 33.33 |
| Ceftriazone         | 18 | 54.55 | 1 | 0.03 | 14 | 42.42 |
| Doripenem Carbapenems | 5 | 15.15 | 2 | 6.10 | 26 | 78.79 |
| Ertapenem           | 7 | 21.21 | 6 | 18.18 | 20 | 60.61 |
| Imipenem            | 4 | 12.12 | 2 | 6.10 | 27 | 81.82 |
| Meropenem           | 5 | 15.15 | 3 | 9.10 | 25 | 75.76 |

Table 3

| Antimicrobial agent | Susceptibility % | Inhibition zone of *E. coli* (mm) | Inhibition zones (mm) of the control group of antibiotics |
|---------------------|------------------|----------------------------------|----------------------------------------------------------|
|                     |                  |                                  | Amoxicillin | Amikacin | Chloramphenicol | Gentamicin |
| Virgin camel’s urine| 100              | 28                               | 0           | 13       | 25              | 19         |
|                     | 75               |                                  |             |          |                 |            |
|                     | 50               |                                  |             |          |                 |            |
| Breeding camel’s urine | 100             | 18                               |             |          |                 |            |
|                     | 75               |                                  |             |          |                 |            |
|                     | 50               |                                  |             |          |                 |            |
are present in most of our current study, it became clear that the among humans (Tavakoli and Pourtaghi, 2017). A closer look at conducted by Dong et al. (2017) indicated that E. coli samples recovered from 157 milk samples of buffalo mastitis. Several previous studies were largely similar to the current results, and the percentage of E. coli from the milk of buffaloes infected with mastitis ranged between 15 and 18% (Ali et al., 2011; Bhanot et al., 2012; El-Sayed Lamey et al., 2013).

In the current investigation, all identified strains of E. coli from dairy cows with clinical and sub-clinical mastitis exhibited a higher degree of resistance for at least 4 antimicrobial drugs out of ten belonging to two various common classes of antibiotics. These results are of great concern as they indicate a direct relationship between the genes responsible for antibiotic resistance and this may lead to the ability of different bacteria to resist antibiotics on the largest scale, which negatively affects public health. There are many studies in the field of animal products that have suggested that the repeated use of antibiotics has increased the prevalence of different bacterial strains that carry many highly pathogenic genes against the many antibiotics used to treat these bacteria (Srinivasan et al., 2007). Consequently, truthful identification, careful usage of antibiotics, and the application of an effective antimicrobial drug to treat the various contagious illnesses should be applied to restrict the development and distribution of multidrug-resistant microorganisms among animals and humans (Ismail and Abutarbush, 2020).

Concerning the genotypic analysis of certain genotypes in E. coli strains in the present investigation, it was observed that the majority of E. coli strains were established to harbor the Stx1, Stx2, and eae genes. Parallel outcomes were stated formerly by Ashraf et al. (2018) who revealed that, the majority of the E. coli isolates recovered from raw milk were able to harbor several virulence genes (e.g. Stx1, Stx2, and eae). In contrast, another study conducted by Dong et al. (2017) indicated that E. coli isolates were found to carry neither stx1 nor stx2 genes. Therefore, it is worth noting that there was a strong relationship between the existence of eae gene and the capability of E. coli to cause severe diseases among humans (Tavakoli and Pourtaghi, 2017). A closer look at our current study, it became clear that the Stx1 and Stx2 genes are present in most E. coli isolates, and it is already recognized that these genes are found in Shiga toxin-producing E. coli (STEC), which represents a direct threat to human health (Montso et al., 2019).

Various antibiotics are frequently utilized in the control of different types of bacteria causing mastitis. It is unfortunate that the misapplication of antibiotics may lead to the development of multi-drug resistant bacteria. Therefore, our current study also examined the extent of resistance of E. coli isolates to various antibiotics. It was observed that most of the isolates resisted many of the tested antibiotics, especially cefazolin, ceftazidime, cefotaxime, ceftriaxone, and cefepime by 78.79%, 66.67%, 60.61%, 54.55%, and 39.40%, respectively. Parallel results were shown by Hinthong et al. (2017), who studied the antimicrobial resistance of E. coli strains from milk and water samples. They stated that ampicillin, carbenicillin, ceftriaxone, and cefotaxime were the most frequently resistant antibiotics to E. coli isolates recovered from water samples, whereas; ampicillin, carbenicillin, ciprofloxacin and norfloxacin were commonly resistant to E. coli strains from milk samples. This may perhaps explain that E. coli strains recovered from milk could possibly originate from various environmental sources such as water.

Another study was carried out Todorovic et al. (2018) stated that 45.8% E. coli strains from mastitic milk were resistant to 13 various antimicrobial agents. Hence, the persistent utilization of antimicrobial drugs may lead multi-drug resistant bacteria in the dairy farms (Suojala et al., 2011; Lan et al., 2020). Therefore, results of the current study confirms that the necessary precautions must be applied to prevent the repeated use of antibiotics in different dairy farms in the Al-Qassim region, because the antibiotics to which the E. coli isolates were susceptible are of cephalosporins (3rd and 4th generations) and Carbapenems, which are currently used against antibiotic-resistant bacteria.

In view of the significant antimicrobial resistance shown by E. coli in our current and previous studies, it was publicly necessary to search for alternative treatment methods to antibiotics. Therefore, the current study was interested in using the virgin and breeding camel’s urine as an antibacterial agent. The results of the current study showed that the virgin camel’s urine particularly in concentrations of 100% and 75% has revealed a robust antibacterial effect of camel’s urine against multidrug-resistant E. coli strains from clinical and mastitic milk samples more than the breeding camel’s urine. Similar findings were obtained by Al-Awadi and Al-Judaibi (2014) and Mostafa and Dwedar (2016). They indicated that the camel’s urine has a broad spectrum of antibacterial activity against various types of bacteria and this activity was increased after the storage and heating of camel’s urine up to 100 °C. It is believed that the heating process increased the active components of urine (Al-Awadi and Al-Judaibi, 2014).
According to the information available to us, it becomes clear that there are few scientific studies on the use of virgin camel's urine as an antibacterial agent in the Kingdom of Saudi Arabia, which is considered one of the most important camel producing countries worldwide. The strong effect of camel urine as an antibacterial agent can be explained by its high alkalinity as a result of its higher contents of potassium, magnesium, calcium, proteins and a low percentage of carbohydrates and cellulose (Kamalu et al., 2004). It is worth noting that the feeding behavior of camels is completely different from the behavior of other ruminants such as cows, buffaloes, sheep, and goats. The camels are able to feed on different types of plants such as thorny shrubs and plants that contain a high percentage of salts, and this behavior is not available to other animals (Iqbal and Khan, 2001; Mostafa and Dwedar, 2016).

5. Conclusions
The current study showed that E. coli strains isolated from cows with clinical and subclinical mastitis in different dairy farms in the Al-Qassim region were able to resist many antibiotics, especially the third and fourth generation cephalosporins group, which may cause a public health concern as a result of the repeated and improper use of antibiotics in this field. This study was also provided a robust evidence that the camel’s urine has an antibacterial activity against multidrug resistant E. coli strains. There is an urgent need for many future studies to thoroughly investigate the components of camel’s urine and its role as an antibacterial as a step on the road to introduce the camel urine as well as its active ingredients in the local and systemic anti-microbial pharmaceutical drugs.

Declaration of Competing Interest
The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements
This work was supported by the Deanship of Scientific Research at King Saud University RG- 1442-162.

References
Abebe, M., Hailelule, A., Abraha, B., Nigus, A., Birhanu, M., Adane, H., Genene, T., Getachew, G., Merga, G., Haftay, A., 2014. Antibiotic of Escherichia coli strains isolated from food of bovine origin in selected Woredas of Tigray, Ethiopia. African J. Bacteriol. Res. 6, 17–22.
Abebe, R., Hatiya, H., Abera, M., Megersa, B., Asmare, K., 2016. Bovine mastitis: prevalence, risk factors and isolation of Staphylococcus aureus in dairy herds at Hawassa milk shed, South Ethiopia. BMC Vet. Res. 12 (1), 270.
Al-Awadi, A., Al-Judaibi, A., 2014. Effects of heating and storage on the antifungal activity of camel urine. Clin. Microbiol. 3 (6).
Al-Bashan, M.M., 2011. In vitro assessment of the antimicrobial activity and biochemical properties of camel’s urine against some human pathogenic microbes. Middle-East J. Sci. Res. 7, 947–958.
Ali, M.A., Ahmad, M.D., Muhammad, K., Anjum, A.A., 2011. Prevalence of Subclinical mastitis in dairy buffaloes of Punjab. Pak. J. Anim. Plant Sci. 21 (3), 477–480.
Montsio, P.K., Mlambo, V., Abate, C., 2019. The first isolation and molecular characterisation of bovine mastitis causing Mycoplasma bovis in smallholder livestock in KwaZulu-Natal, South Africa. J. Anim. Vet. Adv. 8 (12), 4853–4858.
Mostafa, M.-S., Dwedar, R.A., 2016. Antimicrobial activity of camel’s urine and its effect on multidrug resistant clinical bacterial and fungal isolates. Br. J. Pharm. Res. 13 (1), 1–6.
Rasheed, M.U., Thajuddin, N., Ahamed, P., Teklemariam, Z., Jamil, K., 2014. Characterization and outbreak investigation. FEMS Microbiol. Rev. 36 (4), 815–836.
Sibrim, J.J.C., 2003. Chemical composition, toxicological aspects and antifungal activity of essential oil from Lippia sidoides Cham. J. Antimicrob. Chemother. 59 (5), 934–940.
Srivastava, R.B., 2017. Antibiotic resistance. J. Infect. Public Health 10 (4), 369–378.
Gillespie, B.E., Headrick, S.L., Boonyayatra, S., Oliver, S.P., 2009. Prevalence and persistence of coagulase-negative Staphylococcus species in three dairy research herds. Vet. Microbiol. 134, 65–72.
Gonzalez, R.N., Wilson, D.J., 2007. Mycoplasmal mastitis in dairy herds. Vet. Clin. North Am. Food Anim. Pract. 19 (1), 199–221.
Kahlmeter, G., Poulsen, H.O., 2012. Antimicrobial Susceptibility Testing; 24th Informational Supplement. CLSI document M100-S24. Clinical and Laboratory Standards Institute, Wayne, PA.
Kemper, J., Caldow, R.W.G., 2007. Chemical composition, toxicological aspects and antifungal activity of essential oil from Lippia sidoides Cham. J. Antimicrob. Chemother. 59 (5), 934–940.
Khalifa, S., Al-Elyani, R., Al-Awadi, A., 2005. Histological, cytological and histochemical studies on the effect of camels’ urine on liver of rabbits infected by Escherichia coli. Saudi J. Biol. Sci. 12, 66–80.
Kyle, A.A., Dahl, M.V., 2004. Topical therapy for fungal infections. Am. J. Clinical Dermatol. 5 (6), 443–451.
Lan, T., Liu, H., Meng, L., Xing, M., Dong, L., Gu, M., Wang, J., Zheng, N., 2020. Antimicrobial resistance of E. coli isolates from subclinical mastitis cows and water supply in dairy farms in Saraburi Province, Thailand. Pear J. 13 (5), e4341.
Iqbal, A., Khan, B.B., 2001. Feeding behavior of camel. Rev. Pak. J. Agric. Sci. 38, 58–63.
Issmail, Z.R., Abutarbush, S.M., 2020. Molecular characterization of antimicrobial resistance and virulence genes of Escherichia coli isolates from bovine mastitis. Vet. World 13 (8), 1588–1593.
Ashraf, A., Imran, M., Chang, Y., 2018. Antimicrobial resistance of Escherichia coli isolates from mastitic milk and its possible relationship with resistance and virulence genes. Pak. J. Zool. 50 (4), 1435–1441.
Gal, D.S., Lajnef, R., Felfoul, I., Arru, A., Ayadi, M.A., 2016. Antimicrobial activity of camel urine at Taif City. In: The Proceeding of the International Scientific Conference on Camels, Qassim University, College of Agriculture and Veterinary Medicine, Kingdom of Saudi Arabia, pp. 533–552.
Ashraf, A., Imran, M., Chang, Y., 2018. Antimicrobial resistance of Escherichia coli isolates from mastitic milk and its possible relationship with resistance and virulence genes. Pak. J. Zool. 50 (4), 1435–1441.
Gal, D.S., Lajnef, R., Felfoul, I., Arru, A., Ayadi, M.A., 2016. Antimicrobial activity of camel urine at Taif City. In: The Proceeding of the International Scientific Conference on Camels, Qassim University, College of Agriculture and Veterinary Medicine, Kingdom of Saudi Arabia, pp. 533–552.
Singh, A., Chhabra, D., Sikrodia, R., Shukla, S., Sharda, R., Audarya, S., 2018. Isolation of E. coli from bovine mastitis and their antibiotic sensitivity pattern. Int. J. Curr. Microbiol. App. Sci. 7 (10), 11–18.

Srinivasan, V., Gillespie, B.E., Lewis, M.L., Nguyen, L.T., Headrick, S.L., Schukken, Y.H., Oliver, S.P., 2007. Phenotypic and genotypic antimicrobial resistance patterns of Escherichia coli isolated from dairy cows with mastitis. Vet. Microbiol. 124 (3), 319–328.

Suojala, L., Pohjanvirta, T., Simojoki, H., Mäkyniemi, A.L., Pitkala, A., Pelkonen, S., Pyorala, S., 2011. Phylogeny, virulence factors and antimicrobial susceptibility of Escherichia coli isolated in clinical bovine mastitis. Vet. Microbiol. 147, 383–388.

Tavakoli, M., Pourtaghi, H., 2017. Molecular detection of virulence genes and multidrug resistance patterns in Escherichia coli (STEC) in clinical bovine mastitis: Alborz Province, Iran. Iran J. Vet. Res. 18 (3), 208–211.

Todorovic, D., Velhner, M., Grego, E., Vidanovic, D., Milanov, D., Krnjaic, D., Kehrenberg, C., 2018. Molecular characterization of multidrug-resistant Escherichia coli isolates from bovine clinical mastitis and pigs in the Vojvodina Province, Serbia. Microb. Drug Resist. 24, 95–103.

Velez, R., Sloand, E., 2016. Combating antibiotic resistance, mitigating future threats and ongoing initiatives. J. Clin. Nurs. 25 (13–14), 1886–1889.

Vidal, M., Kruger, E., Duran, C., Lagos, R., Levine, M., Prado, V., Toro, C., Vidal, R., 2005. Single multiplex PCR Assay to identify simultaneously the six categories of diarrheagenic E. coli associated with enteric infections. J. Clin. Microbiol. 43, 5362–5365.

Walther, B., Tedin, K., Lübbe-Becker, A., 2017. Multidrug-resistant opportunistic pathogens challenging veterinary infection control. Vet. Microbiol. 200, 71–78.

Wang, J., Stanford, K., McAllister, T.A., Johnson, R.P., Chen, J., Hou, H., Zhang, G., Niu, Y.D., 2016. Biofilm formation, virulence gene profiles, and antimicrobial resistance of nine serogroups of non-O157 Shiga toxin-producing Escherichia coli. Foodborne Pathog. Dis. 13 (6), 316–324.