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Original article

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

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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.

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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 thoughtful 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 hazard 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 serious 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 considered 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 infects 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 healthy cows which exhibited no symptoms of mastitis was identified through California mastitis test (CMT) (Schalm et al., 1971). Under appropriate hygienic conditions, a virtual examination of the udder and teats was performed to find out the heat and any pains or swellings, then the milk secretions were also examined for color and degree of consistency. After performing the virtual examination, about 100 ml of milk samples were taken from the infected cows under precautionary measures, then all samples were preserved at 4 °C and transferred within 2–5 h to Microbiology Laboratory, College of Public Health, to conduct the microbial isolation process. All collected samples that displayed positive reactions to CMT were inoculated onto specific media to identify the *E. coli* isolates. 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 (BioMe′rieux, 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 *Stx1*, *Stx2*, and *eae* genotypes 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 Biomerieux (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), ceftazidime (3rd generation), ceftriaxone (3rd generation), and cefepime 4th generation)]. The Sensititre Nephelometer (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.

Table 1
Oligonucleotide sequences utilized for recognition of *Stx1*, *Stx2*, and *eae* virulence genes of *E. coli* from mastitic milk.

Target gene	Primer sequences (5′–3′)	Base pair
stx1	CAGTTAATGTGGTGGCGAAGG CACCAGACAATGTAACCGCTG	348
Stx2	ATCCTATTCCCGGGAGTTTACG GCGTCATCGTATACACAGGAGC	584
eae	TCAATGCAGTTCCGTTATCAGTT GTAAAGTCCGTTACCCCAACCTG	482

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 tolerated to cefazolin, ceftazidime, 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 cephems 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

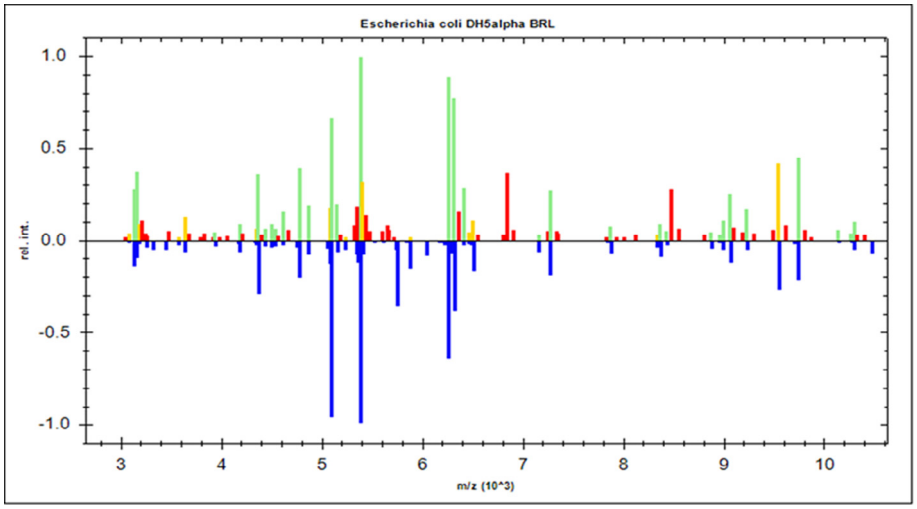

Fig. 1. A comparison between the peak intensities of the field *E. coli* strain from mastitic milk with a reference strain stored in the Compass software of MALDI Biotyper. Matching between peaks are concentrated in the ranging of 3.000–10.400 Da with higher peaks were noticed at 5400 Da and 6300 Da.

Table 2
Presentation of Vitek 2 AST-GN69 card results against 33 *E. coli* isolates from mastitic milk samples.

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
Ceftazidime		22	66.67	0	0.00	11	33.33
Ceftriaxone		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

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 mas-

Table 3
The inhibition zones for various dilutions of virgin and breeding camel's urine against multidrug-resistant E. coli strains from mastitic milk and clinical samples.

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	17				
	50	14				
Breeding camel's urine	100	18				
	75	0				
	50	0				

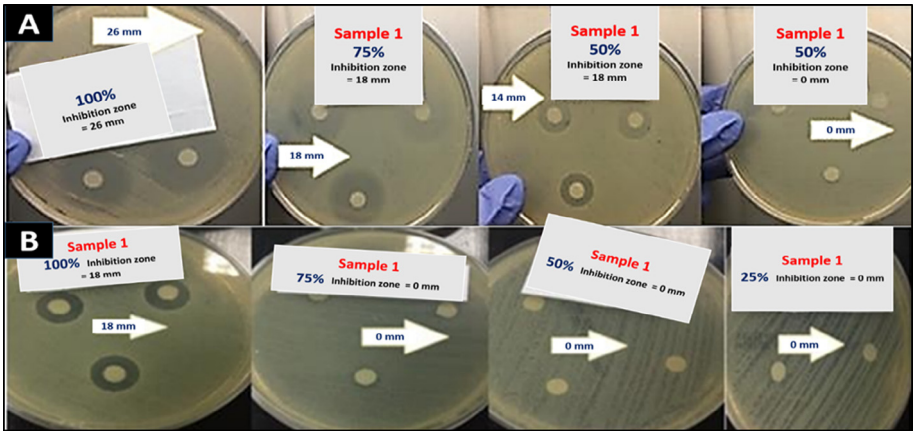

Fig. 2. The efficacy of camel's urine with various concentrations on the *E. coli* strains, (A) Inhibition zones of virgin camel's urine; (B) Inhibition zones of breeding camel's urine.

titis (El-Sayed Lamey et al., 2013). Two hundred and fifty-four milk samples recovered from cows were assessed for the occurrence of *E. coli*. Generally, 33 (12.10%) milk samples were displayed positive results for *E. coli*. Similar findings were recorded by Singh et al. (2018) who observed that 27 (17.19%) *E. coli* strains were 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 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 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.

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