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Isolation of Lytic Bacteriophage against Some Pathogenic Bacteria from Camel's Urine

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Abstract **Original Research Article**

Background: Antibiotic resistance has been identified as a major hazard to public health in recent years. Using bacteriophages to control bacterial infections seems to be a safer alternative. *Methicillin-resistant Staphylococcus aureus (MRSA) Escherichia coli* (*E.coli*) can cause a variety of infections, ranging in severity from fairly insignificant to potentially fatal. Camels' urine has been utilized for therapeutic purposes and anecdotally hailed as a treatment for a variety of ailments for millennia. Alternative eradication strategies must be taken into consideration because of their increased antibiotic resistance, which frequently causes therapeutic failures. Potential candidates to control MRSA &*E.coli* infections are bacteriophages and their lytic enzymes, lysins. The goal of the current study is to examine the existence of native bacteriophages in camel urine and determine whether they have any antibacterial properties against *methicillin-resistant Staphylococcus aureus (MRSA) and Escherichia coli* (*E.coli).* As well, as the impact of camel's status on the presence of bacteriophages. ***Material and methods****:* In this study, we collected 80 camel urine samples from 6 different areas in the Taif region. The camel's urine was collected from healthy male and female (virgin, mother) camels aged 1–8 years, it has been divided into two groups with different natural feeding patterns G1:which grazes naturally on wild plants (wild feeding)&G2: which grazes naturally on wild plants, fodder, and barley(mixed feeding). Bacteriophages were isolated by spot assay *Results*: The phages had good lytic activity against Methicillin-resistant *Staphylococcus aureus* (MRSA) enterotoxigenic *Escherichia coli* (*E.coli*). When evaluated in vitro the greatest activity occurred 24 hours after phage infection. The highest activity of about 100% was achieved by virgin females out of eighty males and female samples. While the ratio represented in male and mother female, 70% 60% respectively**.** ***Conclusions***: Overall findings showed that strains were excellent lytic activity inhibited by bacteriophages obtained from camel urine. The ability to further describe bacteriophages makes them a possible possibility for phage therapy against *MRSA &E.coli* in the future.

Keywords: Bacteriophage, camel urine, pathogenic, *Ecoli, MRSA.*

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1. INTRODUCTION

The topic of camel urine (*Camelus dromedaries* L.) has become a hot topic among Arabian Peninsula residents, and the subject oscillates between inherited popular experiences and a few scientific investigations on the matter. Muslims dealt with the subject of milk and camel urine treatment with assurance, citing what was their tell in Hadith the of the prophet Mohammad peace be upon him as evidence (Abdalall *et al*., 2010).

Camel urine has been considered a "miraculous" Prophetic Medicine since ancient times and was commonly used in the pre-Islamic era (O'haj *et al*., 1993). Camel urine contains metabolites that exhibit beneficial pharmacological properties similar to those of antibacterial, antifungal (Al-Bashan, 2011; Alzahrani and Alharbi, 2011; Al-Haider *et al.*, 2011& Sumia *et al*., 2016), antiviral, and anticancer agents (Al-Yousef *et al*., 2012). Among the Arabian population, camel urine has been used to treat diabetic neuropathy and enhance the luster of women's hair (Alhaidar *et al*., 2011; Al-Awadi and Al-Judaibi, 2014). According to a study, camel urine's antibacterial properties can shield the liver from harm caused by carbon tetrachloride (Al-Bashan, 2011). Additionally, camel urine has been reported to have gastroprotective and ulcer-healing properties (Hu *et al*., 2017).

Camel urine has a unique and distinct biochemical composition (Gole& Hamido, 2020). Due to the small amounts of urea and absence of ammonia, camel urine is free of toxicity, unlike that of humans and other animals. However, research has found that camel urine contains both creatine and creatinine. Camel urine has roughly ten times more mineral salts than human urine. Also, it is basic, with a pH ≥7.8 whereas human urine is weakly acidic or basic (Read, 1925; Mostafa and Dwedar, 2016).

The rising development and spread of antibiotic resistance among various microbes are currently one of the most significant health issues (bacteria, fungi, viruses, and parasites). As mortality and morbidity rates rise, germs are becoming more resistant to antibiotics in both community and hospital settings. According to estimates, antibiotic-resistant bacteria cause 33,000 deaths in Europe and 700,000 worldwide each year. In the worst-case scenario, bacterial infections will cause 10 million deaths a worldwide year by 2050, surpassing the 1.8 million cancer deaths (Glasner, *et al.*, 2013; Cepas, & Soto, 2020). In the modern setting, antibiotic resistance among bacterial strains is becoming a serious medical and societal issue (WHO., 2014). At the same time, pharmaceutical companies are abandoning new antibiotic research and development due to the unprofitability of the endeavor in addition to the potential for bacterial resistance (Clarke, 2003), sparking interest in alternatives to conventional and existing microbial control technologies In affluent countries, *Staphylococcus aureus* (SA) infections are the most common type of hospital-acquired infections (Deleo & Chambers, 2009). SA is a widespread commensal bacteria that can colonize the nose and skin (Casewell, 1998) and it is found in 50% of the human population transiently and 20% permanently (Casewell & Hill, 1986 and Grice & Segre, 2011). SA can induce life-threatening disorders in a variety of tissue types, including bones, joints, blood, lungs, heart, and brain, (Lowy, 1998 and Jensen *et al*., 2015). MRSA is a group of SA isolates resistant to methicillin, erythromycin, levofloxacin, tetracycline, clindamycin, mupirocin, gentamicin, trimethoprim, and/or doxycycline, but susceptible to vancomycin (McDougal *et al*., 2010).

Theodor Escherich isolated and described *Escherichia coli*, a rod-shaped, gram-negative bacteria belonging to the family, from newborn stool in 1885 (Escobar-Paramo *et al*., 2003). *E. coli* colonizes and inhabits the gastrointestinal system of babies within hours of birth. Humans and commensal, *E. coli* strains survive without any negative consequences in terms of various reciprocal benefits. Commensal *E. coli*, on the other hand, can cause sickness in people with gastrointestinal barriers that have been breached or in immunocompromised hosts (Tenaillon *et al.*, 2010). In humans, *E. coli* mediates a variety of diseases, including intestinal and extraintestinal problems, nine pathogenic factors have been identified to cause diarrheagenic in people (Nash *et al.*, 2010). Enterotoxigenic *Escherichia coli* (ETEC) is a common cause of severe, watery diarrhea in newborn animals (Duan, *et al*., 2012).

Bacteriophages are viruses that can only replicate in bacterial cells, and they can be found practically anywhere there are live bacteria (Zhan, *et al*., 2015). Soil, sewage, and animal secretions, which are populated by bacterial hosts, constitute a unique source of all forms of phages, allowing them to be isolated for therapeutic applications (Naghavi, *et al*., 2013). Felix d'Herelle described phage therapy for the first time in clinical practice, (Fruciano & Bourne 2007).

Bacteriophages therapy or phage therapy is a promising potential of antibiotic replacement in the treatment of bacterial infections, and current research aims to isolate indigenous bacteriophages from camel urine sources in order to assess their lytic activity against Methicillin-resistant Staphylococcus aureus (MRSA) and *Escherichia coli*.

Many scientists believe that bacteriophages can help to fight resistant bacteria like *S. aureus*. The number of Staphylococcus phages has increased dramatically, with great progress in targeting resistant *S. aureus* (O'Flynn, *et al*., 2004 & Nobrega *et al*., 2015). Different MRSA-specific phages belonging to the *Podoviridae* family were recovered from farm animals and observed using electron microscopy in similar work, demonstrating that lytic bacteriophages can be used against MRSA strains (Kraushaar *et al*., 2013). Two novel phages isolated from a farmyard were categorized into the *Siphoviridae* family and found to have lytic activity against MRSA in another investigation, (Vandamme *et al*., 2019). There are several phages on the earth that are capable to remove a wide range of bacterial populations (Golkar *et al.*, 2014).

Phage treatment is less expensive and has fewer side effects on eukaryotic cells than traditional antibiotics. On bacterial cell membranes, phages have complementary receptors (Wang *et al.*, 2000). This study is the first study to isolate bacteriophages from camel (*Camelus dromedaries* L) urine (according to our research) and the effect of the type of nutrition and camel case on the microbiota in the Taif region.

2. MATERIALS AND METHODS

2.1. Samples collection:

Using sterile containers the urine samples were collected from (camels that graze in open places in Taif). The samples were collected with the help of camel patrons after cleaning and sanitizing the skin of

the camels. While camel patrons used sterile gloves and scrubbed the camel skin with alcohol 70%.

The urine samples were collected in 30 mL sterile tubes and the samples were transferred to the lab within 4 hours of collection under cooling.

Sterilize the skin before collecting samples Wear gloves and sterilize hand by 70 % alcohol

Fig 1: Collecting urine samples from camels

Camel farm personnel used gloves and washed hands (right) and camel skin (left) with 70% ethanol before collecting urine samples (Fig 1).

2.2. Period of study:

All samples were studied between the months of November 2019 to June 2020.

2.3. Isolation of Staphylococcus aureus & Escherichia coli (E.coli) strains

Methicillin-resistant Staphylococcus aureus (*MRSA*) and *Escherichia coli* (*E.coli*) strains have been obtained, which was previously defined by a molecular method by the microbiology laboratory in Abdulaziz University Hospital, Jeddah.

2.4. Isolation of bacteriophage

The bacteriophages for use against *Methicillin-resistant Staphylococcus aureus* (*MRSA*) and *Escherichia coli* (*E.coli*) were isolated from camel urine collected in 50 ml falcon tubes from different areas of Taif in the Kingdom of Saudi Arabia. The camel urine samples were then processed for phage isolation as described previously (Sangha *et al*., 2014).

The samples were first centrifuged at 4,000 rpm for 20 minutes, and a supernatant was filtered using a 0.22 µm syringe filter. The filtrate was incubated for 15 minutes at 37°C after the addition of 150 µL of chloroform. Subsequently, 10 mL of fresh bacterial culture and 25mL of broth were mixed with 15mL of the filtrate. Incubated the mixture overnight at 37°C in a shaking incubator at 160 rpm. The centrifugation and filtration steps were repeated, and an active bacterial culture was added to the filtrate once more. Incubated the mixture again. For phage enrichment, the whole process was repeated two to three times. The final enriched filtrate was assessed for lytic activity by means of a spot assay.

2.4.1. Assessment of Filtrate Efficacy by Spot Assay:

The spot assay was performed as previously described (Rasool *et al.*, 2016, O'Flaherty *et al.*, 2005, and Sunagar, *et al.*, 2010). Positive samples were chosen, and the most vulnerable host strains and active camel urine filtrates were further processed.

2.4.2. Plaque Morphology Agar Overlay Assay:

An agar overlay approach was employed to identify plaques, as described previously (Kaur *et al.*, 2012). Each plaque's morphology was recorded in terms of clarity and size.

2.4.3. Phage Purification, Enumeration, and Selection:

Were chosen for the phage count and purification process. Individual plaques were picked using a sterile micro-pipette tip, as follows: first inserted the tip into the center of a well-isolated plaque and was swirled to obtain a single plaque. Then, the plaque was suspended in saline magnesium (SM) buffer, Purified plaques of each type were obtained using an overlay approach on the grass of the specific host bacteria, as described previously (Sangha *et al.*, 2014). Plaque forming unit (PFU) determination for phage, an SM buffer supplemented with gelatin was prepared, and the phages were counted, as described previously.

2.4.4. Effect of CaCl2 on Phage Action

For effective attachment to or intracellular progression into host cells, phages require certain

divalent ions. In this assay, the effect of CaCl2 salts) was assessed on phage action, as described previously, using 5 mM working solutions of divalent salts, namely, CaCl2 (Chhibber *et al*. 2014).

2.4.5. In-Vitro Assessment of the Lytic Activity of Bacteriophages

Following infection by the phage lysate, the bacterial host strain was analyzed for the bacterial count at different time intervals (Rasool *et al*., 2016) Ten test tubes were labeled clearly, and about 3 mL of fresh broth was poured into each test tube. Then, 50 µL of bacterial host strain, with turbidity adjusted to 0.5 McFarland standards, was inoculated into each tube except for the last, which served as a bacterial control. About 100 µL of the phage sample (PFU = 108) was added to all the test tubes. The first tube served as a phage control. All the tubes were then incubated at 37°C. The samples were removed at regular intervals (15 minutes, 30 minutes, and 1, 2, 3, 6, 12, and 24 hours), and 100 µL of the sample was plated and incubated for bacterial count purposes.

3. RESULTS

3.1. Bacteriophage isolation from camel urine is a possibility

A total of 80 camel urine samples (30 virgins, 30 mothers &20 males) collected from different areas located in and around Taif, were processed for phage isolation. Each sample's final filtrate was tested against (*MRSA*) *and (E.coli)* strains individually following processing and enrichment. Except for 17 all of the samples were found to be positive in terms of the presence of bacteriophages (Table 1).

3.1.1. Spot Assay:

The purpose of this essay was to evaluate the efficacy of enriched filtrates obtained from 80 camel urine samples from females and males. The majority of the samples were positive for the presence of bacteriophages against the selected host. The highest activity of about 30% was achieved by virgin females out of eighty males and female samples and the bacteriophages were isolated from all virgin samples under study (100%). While the ratio represented in male and mother female, 26% 15% straight. It also represented 21 positive mother samples (70%) and 12 positive male samples (60%) (Table 2 & graph 1). According to the results table (3), the percentage of camels carrying bacteriophages was greater in those grazing wild (36%) than those grazing in a mixed way (30%).

3.1.2. Plaque Morphology and Cell Imaging:

The most effective filtrates and the most susceptible (MRSA) *and (E.coli) i*solates were processed for the isolation of bacteriophages isolated from virgin females. Small- to medium-sized plaques were obtained using the agar overlay method. The plaques were clear, with sharp and undefined boundaries. The presence of plaques and their morphology were confirmed using the ZOETM cell imager (Bio-Rad, USA).

3.1.3. Phage Purification, Enumeration, and Selection:

Each form of plaque was selected and purified independently, with the exception of pinheads. The purified phage plates were then subjected to a lysate preparation followed by a tenfold dilution technique for phage enumeration. Finally, the PFU was determined.

For the following tests, the lysate produced from a plate of medium-sized clear plaques was chosen at random and was termed pq/48. Its activity was assessed against *MRSA & E.coli* as host strains and was found to be effective against 79% of that host (Fig 5).

3.1.4. In-Vitro Evaluation of Phage Lytic Activity

After 24 hours of incubation, the bacterial host strains (108 CFU/mL) were infected with phage lysate at 108 PFU/mL, and 100 µL of this suspension was plated. After 48 hours of incubation, the bacterial count began to decline (Fig 2, 3 & 4).

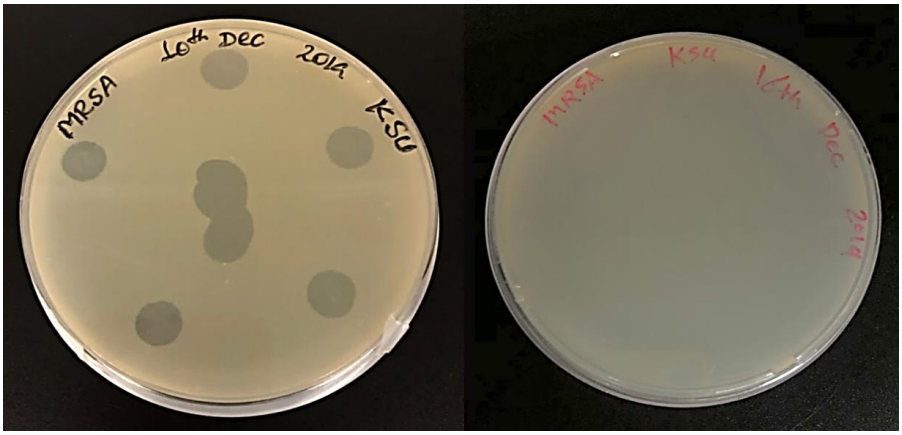


Fig 2: Plaque Assay of lytic Phage of MRSA .left: Plaques produced by MRSA bacteriophage. right: The growth control of MRSA without phages

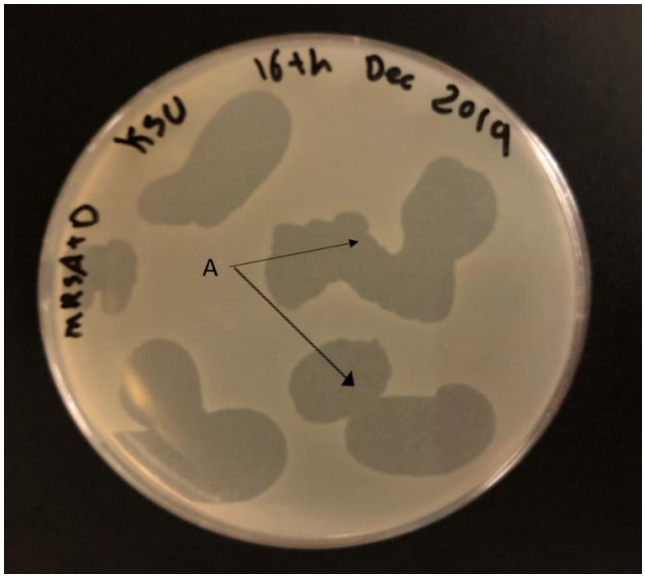


Fig 3: Plaque Assay by lytic Phage on MRSA strain

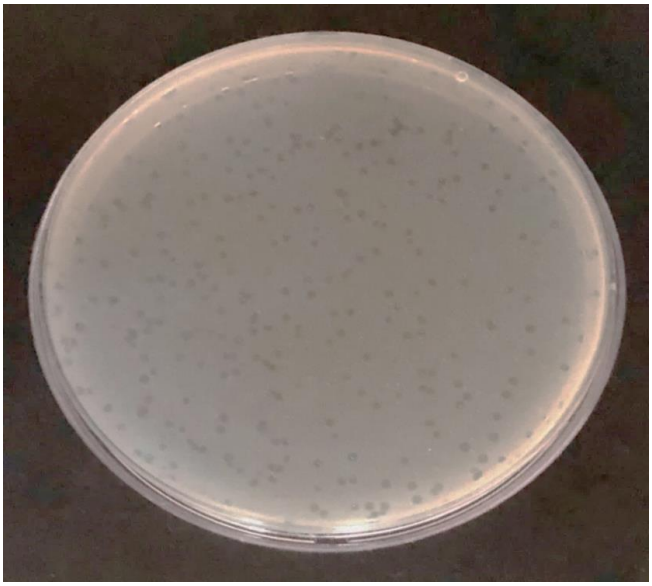


Fig 4: Plaque Assay Of Lytic Phage On MRSA Strain

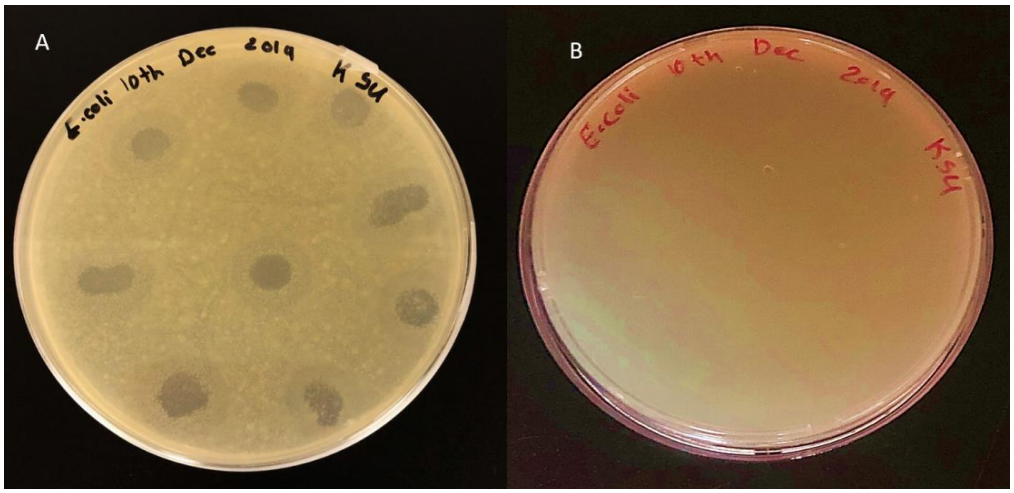


Fig 5: Plaque Assay of lytic Phage of E.coli strain: left(A): Plaques produced by E.coli bacteriophage. Right(B): The growth control of E.coli without phages

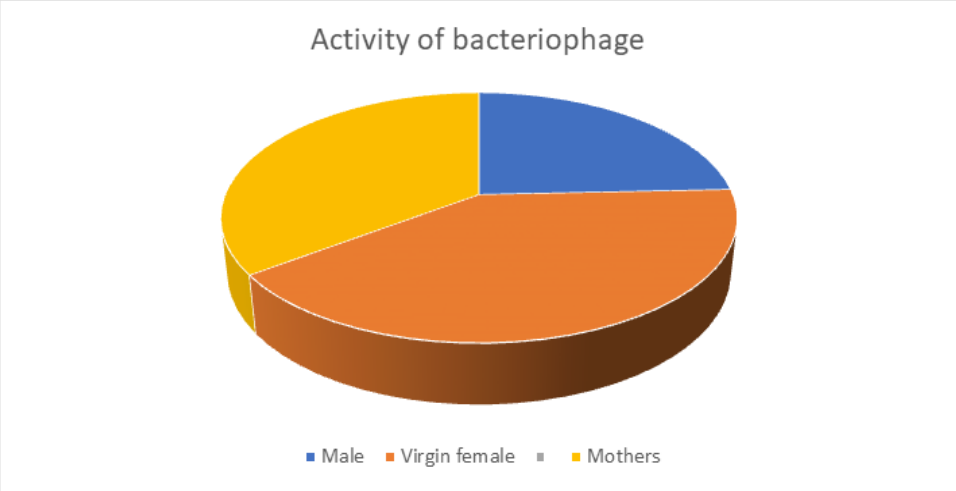
Table 1: Spot Assay of the Activity of Bacteriophages Isolated From Camel urine Samples Against S. aureus

Status	Age	Sex	Phage activity	No.
m	7year	F	+	1
	6year	M		2
	7year	M	+	3
	8year	M		4
V	1 YEAR	F	+	5
	4 year	M	+	6
V	4year	F	+	7
m	7year	F	+	8
V	1 YEAR	F	+	9
V	5year	F	+	10
m	6year	F	+	11
m	8year	F	+	12
V	1YEAR	F	+	13
m	8year	F	+	14
m	8year	F	+	15
	7year	M		16
	6year	M	+	17
	6year	M		18
	6year	M	+	19
V	8year	F	+	20
m	8year	F		21
V	8year	F	+	22
m	7year	F		23
m	7year	F	+	24
m	8year	F		25
V	6year	F	+	26
V	7year	F	+	27
m	8year	F	+	28
m	7year	F	+	29
V	1year	F	+	30
V	2year	F	+	31
V	3year	F	+	32
V	2year	F	+	33
m	5year	F	+	34
m	7year	F	+	35
V	7year	F	+	36
m	7year	F	+	37
m	5year	F	+	38
	6year	M	+	39
m	5year	F	+	40
V	1YEAR	F	+	41
	6year	M	+	42
m	6year	F	+	43
m	8year	F	+	44
V	3year	F	+	45
V	3year	F	+	46
V	1YEAR	F	+	47
	7year	M		48
V	4year	F	+	49
m	6year	F		50
V	3year	F	+	51
m	7year	F		52
m	6year	F		53
V	4year	F	+	54
V	3year	F	+	55
V	4year	F	+	56
	7year	M	+	57
m	6year	F	+	58
V	1year	F	+	59
V	1year	F	+	60
V	2year	F	+	61
V	3year	F	+	62
	4year	M		63
m	6year	F	+	64
m	5year	F	+	65
m	6year	F		66
V	3year	F	+	67
m	6year	F		68
	5year	M	+	69
m	6year	F	+	70
	5year	M		71
	5 YEAR	M	+	72
V	1year	F	+	73

V	2year	F	+	74
	7year	M	+	75
	3year	M	+	76
m	5year	F	+	77
	2year	M	+	78
m	6year	F		79
	3year	M		80
Total:80 F:60=Female M:20=Male		m=Mother=30 V=Virgin=30 (+) positive		

Table 2: Percentage of urine samples that contains bacteriophage against MRSA & E.coli

	Mothers	Virgin female	Male
Percentage	38%	38%	25%
Phage activity Percentage	26%	30%	15%



Graph 1: Percentage of urine samples that contains bacteriophage against MRSA &E.coli with different status of camel

Table 3: Percentage of urine samples that contains bacteriophage against MRSA & E.coli strains with different types of grazing

	Mix feeding	Wild feeding
Percentage	50%	50%
Phage activity Percentage	30%	36%
Total=80 camel	Mix feeding=40	Wild feeding=40

4. DISCUSSION

The specialized affinity of bacteriophages for specific bacterial strains can help to avoid the frequent adverse effects of antibiotic therapy. Furthermore, phage gene modification can improve bacteriophage specificity and sensitivity to bacterial strains (Nobrega *et al.*, 2015).

Bacteriophages are a potential antibiotic replacement in the treatment of bacterial infections, and current research aims to isolate and characterize indigenous bacteriophages from camel urine sources in order to assess their lytic activity against *MRSA.* Camel urine samples were collected from healthy camels from different locations in and around Taif city. In our study, 79% of the samples were found to be positive for the presence of bacteriophages, which is in accordance with previous research reporting the presence of bacteriophages in sewage water (Gupta &Prasad, 2011, Han *et al.*, 2013, Sangha,*et al.*, 2014 and Rasool *et al.*, 2016).

Individual enriched filtrates were assessed for the selection of the best filtrate for phage isolation by spotting the enriched filtrate over the plates of host strains.

Again, more than one type of phage can infect a single host strain, making some of the host strains most susceptible and others less susceptible.

In addition, phages were characterized by the presence of clear zones. The plaques thus obtained were confirmed using a cell imager (Bio-Rad, USA). Plaques were of two types, namely, clear and turbid. A similar morphology of plaques has been reported previously (Han *et al.*, 2013).

The bacterial count began to reduce after an incubation period of 30 minutes, but a significant reduction was seen 24 hours after the phage infection.

The ability of the lytic phage binding protein to bind to the phage receptor of the susceptible bacteria and the lysis of the host cell are both important components in the lytic phage life cycle.

In this study, we isolated bacteriophages with lytic activity against *Methicillin-resistant Staphylococcus aureus* (MRSA) and *Escherichia coli* (*E.coli*) by spot testing and spectrophotometric assays of phage-treated bacterial cultures. We found that our phages were able to significantly reduce *MRSA* and *E.coli* growth in culture.

Through the results, it was noted that there is a difference in the percentage of samples from which the bacteriophages were isolated according to the condition of the camels.

While the highest percentage was from virgin female camel urine (100%) and then, mother female (70%) and male (60%). According to a similar study that virgin camels have robust antimicrobial activity against multidrug-resistant *E. coli* strains. Inhibitory zones in virgin camel urine were on average, 28 mm, 17 mm, and 14 mm, respectively, for concentrations of 100%, 75%, and 50%. Whereas, for the concentrations of 100%, 75%, and 50%, respectively, the inhibitory zones for the urine of mother camels were 18 mm, 0 mm, and 0 mm (Elbehiry *et al*., 2021). In another study conducted by (AL-Talhi and AL-Bashan, 2006) the camel's urine has significant antimicrobial activities against various pathogenic microorganisms that infect humans such as *Staphylococcus aureus, Pseudomonas aeruginosa, and Escherichia coli* isolates. According to the results, we noticed that the camels that were fed wild contain the highest percentage of bacteriophages compared to those fed a mixed diet (Table 3) This indicates the effect of different grazing methods on the microbial diversity of camel urine microbiota. Khalifa *et al*., (2005) *conducted a second study in which they utilized camel urine (up to 100%) as an antibiotic to treat E. coli in the liver tissue of experimental rabbits. They discovered that the urine was able to eradicate E. coli without causing any pathological alterations.* Isolation and characterization of effective bacteriophages with lytic activity against *MRSA* and *E.coli* were investigated in this study. Phage therapy, based on our findings, could be a strong candidate for combining with established therapies.

In addition to the difference in the proportion of bacteriophages isolated from the two groups. This indicates the effect of different grazing methods on the microbial diversity of camel urine microbiota (Table 3).

5. CONCLUSION

Finally, the phages described in this paper have good bacterial lysis activity. However, these isolated phages must be further described before being employed in commercial lysate preparations in the near future, when their therapeutic potential against a larger spectrum of bacterial strains can be studied.

This topic will be highlighted by research employing animal models of phage-bacterial interactions, which may eventually lead to human trials, and could give a powerful alternative treatment against the threats of multi-resistant diseases.

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