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Effect of Asses, Camel and Cow's Milk against Infections Bacteria Isolated from Human

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Abstract

This study was evaluated the effects of Asses, camels and cow's milk on pathogenic bacteria and fungus. Fifty-two isolates representative Gram negative, positive pathogenic bacteria and fungus belong to the genera *(Pseudomonas, Escherichia, Klebsiella, Proteus, Salmonella, Staphylococcus, Streptococcus, Candida albicans)*. Samples were collected during period 1st 2016 to 30th 2017. The inhibition zoon test was used as indicator to determine the antimicrobial activity of milk as cultivated by agar diffusion method. The results shows that all milk types used in this conduct test were capable of inhibited the microbial strain bring up above. Ass milk shows superior activity against Staphylococcus aureus, *Escherichia coli* and *Candia albicans* with LSD values of 6.091*, 6.448 *and 5.609* respectively. Camel milk active against E.*coli, Klebsiela spp,* and *Candida* 1 with LSD values of 6.448*, 6.205* and 7.413* respectively . Cow milk shows lowest effects with LSD value of 5.709 against *Candida albicans* .This study conclude the that Ass milk and camel represents the most effective types of milk against the gram positive and negative pathogenic bacteria and fungus isolates.

Key words: Asses milk; Caw milk; Camels milk; Anti-microbial; pathogenic bacteria.

1. Introduction

The milk proteins mainly responsible for the allergy are α- and β-caseins, followed by β-lactoglobulin and α-lactalbumin to a lesser extent [1]. In children with CMPA, when it is not possible to breast feed or to use cow milk, the clinical use of donkey milk (DM) is considered [1] since several studies have demonstrated the high similarity of DM compared to human milk [2].

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Interest in donkey milk has recently increased, especially in Europe, as it represents an alternative food in cases of bovine milk proteins allergy and in the prevention of metabolic pathologies, [2,3]. Milk is an established and healthy food source of energy, protein, vitamins and minerals, and has a highest quality source of well – balanced nutrients. Milk from different species contains several antimicrobial factors which exert both specific and nonspecific bacteriostatic and bactericidal activity [2]. These factors are transferred from the mother to the neonate and contribute to the protection against infectious diseases [2,4].In practical, Milk is rich in proteins that are classically grouped into two main classes (i) major milk proteins including caseins and the whey proteins and (ii) minor milk proteins including lysozyme lactoferrins, lactoperoxidase and immunoglobulins . Although the constituents represent only a minor fraction of milk protein, they have highest immune stimulation potential when consumed in human diet [5,6]. These proteins are present in the milk of cows , ewes, goat, buffalos , pigs , camel and human [7,8] but their concentration fluctuates depending on species , health status of animal and stage of lactation .Cow's milk has high lactoperoxidase , but low lactoferrin and lysozyme , while human milk has high lactoferrin and 1ysozyme but low lactoperoxidase . Camel's milk contains all essential nutrients as cow's milk and also has some components that are different from those in cow's milk as their values. Insulin, vitamin C, niacin and some fatty acid are higher in camel's milk [9]. She camel's milk has a good biological value due to higher content of antimicrobial factors such as lysozyme , lactoferrin , lactoperoxidase , immunoglobulins G, Peptidolycan recognition protein PGRP , Nacety1 –glucosaminidase (NA Gase) [9].Camel milk's lactoferrin very high levels of bactericidal and bacterio static properties against Gram-positive and Gram –negative bacteria[10] more than cow and human lactoferrin . The action is similar against viruses in this case , for example , it prevents the penetration of hepatitis C virus in leukocytes [11] . Camel and human milk also has a unique property includes the presence of Lactic acid bacteria (LAB). The Lactobacillus spp. and Bifidobacteria that isolated from human milk have antimicrobial activity against *Shigella flexineri, Shigella dysenteriae* , *Vibrio cholera* , Salmonella typhi , pseudomonas spp. Streptococcus pneumoniae , Haemophilus influenzae and *Staphylococcus aureus* [12] .

Laref and Guessas , [13], found that Lactoacillus spp. bacteria which isolated from camel milk have the ability to inhibit the germination of Candida and completely inhibited the mycelium growth of Aspergillus spp , Trichoderma spp, pencillium spp ; Fusariumrmroseum Stemphylium spp. *Dheeb* and his colleagues [14] , determined the inhibitory effects of human , camel and cow's milk against some pathogenic fungi in Iraq and confirmed that there is a positive relationship between the concentrations of milk proteins of these species and the inhibitory growth rate of milk against fungi and found that human milk has a stronger inhibitory effect than camel or cow milk. Many studies were done concerning the inhibitory effect of camel and human milk or camel and cow milk on pathogenic bacteria or fungi.

2. Materials and Methods

2.1. Isolation of bacteria and fungus

Fifty two different gram positive and negative pathogenic bacteria isolates belong to the genera *(Pseudomonas, Escherichia, Klebsiella, Proteus, Salmonella, Staphylococcus , Streptococcus ,and Candida albicans)* were isolated from different sources and characterized as shown in (Table1).

Table 1: The clinical bacterial and fungus isolates and their sources of isolations

Strain No.	Bacteria and fungus spp.	Gram stain	sources of isolation
1.	*Pseudomonas aeruginosa* (10)	-	Urinary tract infection
2.	*E coli* (6)	-	Urinary tract infection
3.	*Klebseilla pneumonia* (5)	-	Respiratory tract infection
4.	*Proteus mirabilis* (5)	-	Urinary tract infection
5.	*Salmonella spp.* (10)	-	Blood
6.	*Staphylococcus aureus* (8)	+	Urinary tract infection
7.	*Streptococcus pyogenes* (3)	+	Blood
8.	*Candida albicans* (5)	Fungus	Candida infections

2.2. Collection of Milk samples

Fresh milk samples of Asses, camels and cow's milk, were collected from apparently healthy animals after two months and after labor bred in the living stock station. The milk samples were placed in sterile containers and transported to the laboratory in a cool box. These samples were passed separately through Millipore filter (0.22mm) before determining their anti-bacterial and yeast activity.

2.3. Anti-microbial susceptibility of Milk activity

The effects of Asses, camels and cow's milk on the growth of bacteria and fungus isolates were gritty by the diffusion method on a media following the method described by Silva and his colleagues [1]. Broth stock cultures of bacteria and yeast were spread on its surfaces of brain infusion agar plates (100µl) of each type of milk was pipetted into prepared holes on the same agar plates and incubated for (24-48 hrs.) at 37 ^{o}C, to each milk sample. The inhibition zone rates were calculated by measuring the means of diameters of clearance zone areas in (mm) for the triplicate repeats of each milk sample after incubation with bacterial and fungal isolates.

2.4. Statistical Analysis

The Statistical Analysis System –SAS [15] was used to determine the effect of resource of milk and bacterial and yeast species in Inhibition zone rate (mm) .Least significant difference LSD test was used to compare the significance between means of obtained result .

3. Results

3.1. Distribution of pathogenic bacteria among different genders

Table 2 shows the distribution pathogenic bacteria among gender, the total were 30 (57.69%) male, and 22(32.21) were female, there are no significant difference between gender, P>0.001.

Table 2: The distribution of pathogenic bacteria among gender

Bacterial isolation	Gender		Total	P value
	Male	Female		
Pseudomonas aeruginosa	7	3	10	P>0.001
E coli	2	4	6	
Klebseilla pneumonia	4	1	5	
Proteus mirabilis	2	3	5	
Salmonella spp.	5	5	10	
Staphylococcus aureus	5	3	8	
Streptococcus pyogenes	3	3	0	
Candida albicans	5	0	5	
Total	**30(57.69%)**	**22(32.21%)**	**52**	

3.2. Distribution of pathogenic bacteria according residency

Table 3 shows that the distribution of pathogenic bacteria according residence and it's significant difference in rural area 33(63.46) in comparison with urban area19 (36.54%), P<0.001.

Table 3: Distribution of pathogenic bacteria according residency

Residency	Pathogenic bacteria		
	Number	%	P value
Urban	19	36.54	**P<0.001**
Rural	33	63.46	
Total	**52**	**100%**	

3.3. The inhibitory effects of milk on the different bacteria

Table 4 shows the inhibition zone rates (mm) for different bacterial and fungus isolates caused by different milk specimens using the diffusion method in brain heart infusion agar medium. The inhibitory effects Ass, camel and cow's milk for each of different bacteria and fungus species were determined as described previously. The results shown in Table 2 indicate that all three types of milk were capable of inhibiting the growth of the fifty two isolates of pathogenic bacteria and fungus with different inhibition zone rates. For the gram negative bacteria, the genus *pseudomonas*, Ass milk gave the highest zone rate of 29 mm with *pseudomonas aeruginosa* that isolated from urinary tract infection patient while camel and cow's milk gave 10mm and 12 mm respectively with LSD value of 7.327 *. For the genus *Escherichia* isolated species and standard strains, the highest zone inhibition rate of 30mm was recorded with *E. coli* 3 that isolated from patient's stool after treated

with human and camel's milk while cow's milk gave 12 mm inhibition zone rate with LSD value of 6.448*. For the genus *klebsiela spp*. that isolated form respiratory tract infections, Ass milk recorded a highest inhibition zone rate of 30mm followed by 20 mm and 10mm for camel and cow's milk respectively, LSD value of 6.205* was recorded for the three types of milk .For the, *proteus* , and *Salmonella* that were isolated from urinary tract infection , blood and respiratory tract infections respectively , the highest zone inhibition rate of 25 mm was estimated with estimated with camel milk on Salmonella spp. followed by 22mm and 14mm for human and cow's milk respectively with LSD value of 4.724* . For the gram positive bacteria , genus *Staphylococcus* , the highest zone inhibition rate of 33mm was recorded with Staphylococcus aureus 2 that isolated from dermal infection after treated with camel milk , Ass and cow's milk capable to give 14mm and 11 mm inhibition zone rate respectively and LSD value was recorded to be 6.091 *. For the genus Streptococcus and the isolate Streptococcus pyogenes that isolated from blood, camel milk gave a gave a highest inhibition zone rate of 22 mm followed by 20mm and 12 mm for each human cow's milk LSD value of 4.791*. For the fungal isolates particularly for the genus candida, Ass milk was determined to give the highest zone inhibition rate of 30mm on Candia albicans that isolated form respiratory tract infection while camel and cow's milk were recorded inhibition zone rates of 25 mm and 9mm respectively, LSD value of 7.413* was determined for the three types of milk. Camel's milk also recorded a highest zone inhibition rate 26mm Candida albicans1 followed by human and cow's milk respectively with LSD value of 5.6.9 *. The total LSD values for Ass, camel and cow's milk for all the fifty two bacteria and fungus isolates were recorded to be 9.273* , and 9.584* respectively .

Table 4: The inhibition zone rates (mm) for different bacteria and fungus isolates caused by different milk specimens using the diffusion method in brain heart infusion agar medium.

Strain No.	Bacterial and fungal isolates	Ass milk	Camel milk	Cow milk	LSD Value
1.	*Pseudomonas aeruginosa (1)*	10	10	7	4.385 NS
2.	*Pseudomonas aeruginosa (2)*	29	10	12	7.327*
3.	*E coli*	20	12	7	6.982*
4.	*Klebsiella pneumonia (1)*	20	12	13	4.873
5.	*Klebsiella spp.(2)*	30	20	10	6.205
6.	*Proteus mirabilis*	6	9	3	4.613*
7.	*Salmonella spp.*	22	25	14	4.724*
8.	*Staphylococcus aureus*	20	15	2	6.217*
9.	*Streptococcus pyogenes*	20	22	12	4.791*
LSD Value		9.273*	8.269*	9.584*	-----

***Significant (P< 0.05), NS: Non –significant.**

4. Discussion

The results showed that Ass and camel milk represent the most effective type of milk against the gram negative pathogenic bacteria compared to cow's milk which was ranked second .For the gram positive pathogenic bacteria, the most effective type of milk against them was recorded to by camel milk followed by Ass and cow's milk respectively . For fungal isolates, ass milk represents the most effective type of milk followed by camel and cow's milk respectively[16]. Ljubiša Ć. Šari (2016), [17], who found that Ass breast milk have a strong inhibitory activity against *Escherichia coli, Vibrio cholera , Haemophilus infuenzae , Streptococcus pneumoniae ,Clostridium difficile , Salmonella, Klebsiella pneumonia , Staphylococcus aureus Staphylococcus epidermidis and candida albicans* . Trinchese and his colleagues [3], found that camel milk and cow's milk have a bacteriostatic effect against *Listria monocytogenes* and *Escherichia coli* Dheeb and his colleagues [14], confirmed that there is a positive relationship between the concentrations of human , camel in Iraq and founded that Ass milk has a stronger inhibitory effect than camel and cow milk which were ranked second and third respectively . The qualitative results in the present study may by indicative of the effects of some of the constituents of the different types of milk as inhibitors of bacterial and yeasts species growth. Richard Alleyne [18], determined the presence of a multitude of proteins especially in Ass milk such as SigA , lactoferrin , 1ysozyme , 1actoperoxidase , hepatocorrin and α –lactoalbumin have inhibitory activity against pathogenic bacteria , viruses and fungi. Some of these proteins are likely to act independently, whereas others may act synergistically M. Million [19]. The positive health effects of milk proteins can be presented as antioxidative, antimicrobial, antihypertensive immunomodulatory and anti –thrombotic [20]. Filippo Fratini and his colleagues [21], found that the antimicrobial components in Ass milk inhibit the growth of pathogenic bacteria; it is also likely that some substances stimulate the growth of beneficial bacteria, so they have prebiotic bacterial activity [19]. This was originally called the bifid us factor, which can limit the growth of several pathogens by decreasing intestinal pH. [22] , indicated that milk is capable to protect against microbial contaminations by natural inhibitory system including the lactoperoxidase / thioxcyanate / hydrogen peroxide (LP) system lactoferrins, 1ysozyme , immunoglobulins and free fatty acid .Cow's milk inhibited the metabolic activity of *E. coli* through the presence of both xanthine oxidase (xo) activity and the presence of nitrite , implying that xo-generated nitric oxide functions as an antibacterial agent Abdel Galil M [22]. The concentrations and activity of each of these microbial system substances depend on animal species, stage of lactation and health status [22]. Shamsia , (33) determined the antimicrobial factors of both camel and Ass milk. Its concluded that camel milk is richer in immunoglobulin (1.54mg/ml) than human milk (1.14mg/ml). However contents of lactoferrin and 1ysozyme were very low (0.24mg/ml) and (0.06mg/ml) respectively as compared with human milk which contains (1.95mg/ml) lactoferrin and (0.65mg/ml) lysozyme. Camel milk contained more fat, protein, specially casein and ash contents but lower whey protein and lactose contuse and lactose contents than human milk. Casein and whey protein contents in human milk make it very nutritious for the new born baby. El-Agamy and Nawar , [22], determined the level of immunoglobulin –G in camel milk is (1.64mg /ml) compared to 0.67 and 0.86mg/ml for cow and human milk respectively . While the content of lacoferrin in camel milk 0.22 mg /ml is significantly higher than that in cow's milk and very low compared with that of human milk.Siseciaglu and his colleagues [6,23], explained that the Lactoperoxidase (LPO) system of bovine milk exhibits inhibition property against *Escherichia coli , Streptococcus pneumoniae,* Staphylococcus epidermidis , *Staphylococcus intermedius,*

Candida albicans and *Candida krusei*. Jeffrey K. Actor, [23], showed that lactoferrin is an essential element of non –specific innate immunity in human and other mammels (the concentration of lactoferrin in cow's milk is lower than it is human's milk) . Jrad Zeineb1 [14], revealed that camel milk's lactoferrin has very high levels of bactericidal and bacteriostatic properties against (Gram –positive and Gram –negative bacteria) more than cow and human lactoferrin .Cardoso and his colleagues [25], explained the ability of camel's milk to protect the mice that inoculated with *Salmonella* in addition to lactoferrin and Immunoglobulin –G, other substances present in camel milk could be responsible for the protection of the mice, such as lysozyme , lactoperoxidase , Vitamin C (present in large amounts) and carbohydrates through their proven immunomodulatory action, Heike Stier and his colleagues [26] .

5. Conclusion

The present study confirms that there is a strong relationship between the concentrations of the milk proteins that present in Ass's, camel and cow's milk and the inhibition rates camel milk and Ass's milk represents the most effective types of milk against the gram positive and negative bacteria and yeast isolates followed by cow's milk which ranked second in its inhibitory activity.

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