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RESEARCH ARTICLE
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Antimicrobial activity of Algerian honey on subclinical mastitis pathogens isolated from goat's milk

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Abstract

Aim: The aim of the present study was to determine the susceptibility of subclinical mastitis pathogens isolated from goat's milk and to evaluate the antimicrobial activity of Algerian honey on mastitis causing bacteria.

Materials and Methods: The antibacterial activity against the isolated bacteria was evaluated by determining the Minimal Inhibitory Concentration (MIC), using the agar incorporation method.

Results: The results showed that both *Micrococcus* spp. and *Klebsiella* spp. were susceptible to Streptomycin and tetracycline, while *Pseudomonas aeruginosa*, *E coli*, *Enterobacter* spp., *Bacillus* spp., and Coagulase Negative Staphyloccoci (CNS) were preferentially susceptible to Streptomycin. However, *Streptococcus* D was the most resistant to the tested antibiotics whereas *Staphylococcus aureus* was the most susceptible to all the studied antibiotics. As regards to the antimicrobial activity of honey, the measured values were comprised between 11 and 14%.

Conclusion: The results reveal that antimicrobial drugs susceptibility tests in goat subclinical mastitis might be necessary before the treatment. Algerian honey exhibited *in vitro* antimicrobial activity against different isolated bacteria in goat mastitis.

Keywords: antibiotics, antimicrobial activity, goat, honey, subclinical mastitis, susceptibility.

Introduction

Mastitis inflammation of the mammary gland is a serious health problem in dairy goats. Therefore, any factor that affects the quantity and quality of goat milk is of great financial interest. The inflammatory response was associated with a marked reduction of milk yield in the infected gland (0.29 kg/milking) compared to that of the uninfected [1]. Many mastitis pathogens were considered as agents of major concern because of their low response to the treatment and their propensity to recur chronically [2]. Targeted antimicrobial therapy plays an important role in mastitis control. It reduces the levels of herd infection and prevents new infection [2]. Now-a-days, the abundant use of antibiotics has resulted in widespread resistance. As far as mastitis is considered, it is important to know the type of antibiotic to recommend for the treatment and the way it should be introduced (parenteral or intramammary). Incorrect or incomplete treatment of infections may cause an antibiotic resistance.

Honey has a valuable role in traditional medicine worldwide for thousands of years. It is recognized as an efficient topical antimicrobial agent in the treatment of many infections [3, 4]. Recently, there is a renewed interest in the study of honey as a natural product for therapeutic purposes including the treatment of infected wounds [5-9]. Note that honey has no unique composition but various types could be collected depending on the botanical source and the geographical origin of the beehive. The change in honey composition is known to affect its antimicrobial and antioxidant properties because of differences in levels of peroxide and non-peroxide factors [10]. This effect has been recently investigated on different types of honey [6, 10-12].

The potent *in vitro* activity of honey against antibiotic-resistant bacteria [13-15] and its successful application in treatment of infections is a promising research topic as confirmed by the published reports of many researchers [7, 9, 11, 15-17]. Furthermore, the large spectrum of the antibacterial activity of honey is multi-factorial in nature. Indeed, hydrogen peroxide and high osmolarity-honey consisting of 80% (w/v) of sugars are the only characterized antibacterial factors in honey [18]. Recently, high concentrations of antibacterial compound consisting of methylglyoxal (MGO) were found [19, 20].

In the present study, we evaluated *in vitro* the antimicrobial drugs susceptibility of isolated bacterial strains from subclinical mastitis goat's milk and then investigated the antimicrobial activity of locally collected Algerian honey against these isolated bacteria.

Materials and Methods

Bacterial strains and inoculum standardization: The study was conducted on local crossbreed goat (*Arabia*

Table-1(a): Antibiotic susceptibility of bacterial isolates (*E. coli, Enterobacter* spp. and *Corynebaterium*) from subclinical mastitis goats

Antibiotics	*E. coli* (n=9)				*Enterobacter* spp (n=8)				*Corynebaterium* spp (n=1)			
	Susceptibility		Resistant		Susceptibility		Resistant		Susceptibility		Resistant	
	n	%	n	%	n	%	n	%	n	%	n	%
Streptomycin	8	88.88	1	11.11	7	87.5	1	12.5	0	0	1	100
Penicillin	1	11.11	8	88.88	1	12.5	7	87.5	0	0	1	100
Erytromycin	3	33.33	6	66.66	2	25	8	75	0	0	1	100
Tetracyclin	4	44.44	5	55.55	6	75	2	25	1	100	0	0
Ampicillin	2	22.22	7	77.77	1	87.5	7	12.5	0	0	1	100
Amoxicilin	6	66.66	3	33.33	2	25	6	75	0	0	1	100

Table-1(b): Antibiotic susceptibility of bacterial isolates (*Bacillus* spp, *S. aureus* and CNS) from subclinical mastitis goats

Antibiotics	*Bacillus spp.*(n=3)				*S. aureus* (n=4)				CNS (n=20)			
	Susceptibility		Resistant		Susceptibility		Resistant		Susceptibility		Resistant	
	n	%	n	%	n	%	n	%	n	%	n	%
Streptomycin	3	100	0	0	4	100	0	0	17	85	3	15
Penicillin	0	0	3	100	2	50	2	50	3	15	17	85
Erytromycin	0	0	3	100	4	100	0	0	16	80	4	20
Tetracyclin	1	33.33	2	66.66	3	75	1	25	13	65	7	35
Ampicillin	0	0	3	100	3	57	1	25	7	35	13	65
Amoxicilin	0	0	3	100	4	100	0	0	16	80	4	20

CNS: Coagulase Negative Staphylococci

x *Makatia*) in Tiaret province. The studied bacteria were isolated from subclinical mastitis goats' milk. A total of 298 samples were collected during the year 2010. The isolates were cultivated on various culture media including MacConkey agar, Chapman agar, nutritive agar, and Columbia blood agar, at a temperature 37° C during a lapse time of 24 h. After the purification of bacterial isolates, the selected colonies were subjected to the coloring of Gram finally galleries Api (Api staph 20, Api E 20).

The inoculum suspensions of tested strains were obtained by taking five colonies from 24 hour-old cultures grown on specific media. They were suspended in 5 ml of sterile saline (0.85% NaCl). The obtained inoculum suspensions were shaken for 15 seconds and the density was adjusted to a turbidity of 0.5 MacFarland Standard (equivalent to 1-5 x 10^6 CFU/ml).

Antibiotics susceptibility test: Antibiotic susceptibility test was performed on the mostly isolated pathogens (Coagulase Negative Staphylococci (CNS), *Pseudomonas aeruginosa*, *Streptococcus* D; *Enterobacter* spp., *Escherichia coli*, *Klebsiella* spp., *Bacillus* spp., *Staphylococcus aureus*, *Corynebacterium* spp. and *Micrococcus* spp.). The antibiotic assay was performed using disk containing the following antibiotics (Ctra de Santa Coloma, 7 17176 Sant Esteve de Bas, Girona, SPAIN): Penicillin-G (10IU); Ampicillin (10µg); Amoxicillin (25µg); Streptomycin (10µg); Erythromycin (15µg), and Tetracycline (30µg).

The diffusion method was achieved on Muller Hinton agar according to the National Committee of Clinical Laboratory Standards (NCCLS). The colonies were suspended in 5 ml of sterile saline having a density of approximately 0.5 MacFarland Standard. A dry sterile cotton wool swab was placed in the suspension and excess liquid was pressed against the inside of the tube. The bacterial suspension was inoculated into Muller Hinton agar with the swab such that the whole surface of the agar is covered.

The antibiotic disks were dispensed on the surface of the medium and incubated aerobically at a temperature 37ºC during 24h. The results were recorded as either resistant or susceptible by measuring the diameters (mm) of the inhibition zones according to the interpretive standards of the NCCLS.

Honey samples and minimum inhibitory concentration measurement: Honey sample was obtained directly from beekeepers in the region of Tiaret, Algeria (350°15' N, 10°26' E). It was stored in dark at temperature 4º C in tan containers before use. The evaluation of the antibacterial activity of honey dilutions were performed according to the method of Hegazi [6]. To test the efficiency of honey against mastitis pathogen isolates varying concentrations of honey (10% - 20%, v/v) were incorporated into the culture media [21-23]. The final volume of honey and culture media in each plate was adjusted to a total of 5 ml. The plates were incubated at a temperature 37°C during 24 h. The minimum inhibitory concentration (MIC) was defined as the lowest concentration of honey inhibiting the visible growth of each organism on the plate. Note that all the MIC values are expressed in percentage (v/v).

Statistical analysis: Calculations were performed using Statistica software (6^{th} version). The data were expressed as mean ± SD (%).

Results

The antibiotic susceptibility rate of the isolated bacteria from clinically normal goats' milk is detailed in Table-1 (a, b, c, d). We noticed that both *Micrococcus* spp and *Klebsiella* spp. showed a susceptibility to Streptomycin and Tetracycline. Whereas *P. aeruginosa*, *E.* coli, *Enterobacter* spp., *Bacillus* spp.

Table-1 (c): Antibiotic susceptibility of bacterial isolates (*Micrococcus* spp. and *Klebsiella* spp.) from subclinical mastitis goats

Antibiotics	*E. coli* (n=9)				*Enterobacter spp.*(n=8)			
	Susceptibility		Resistant		Susceptibility		Resistant	
	n	%	n	%	n	%	n	%
Streptomycin	10	83.33	2	16.66	23	88.46	3	11.53
Penicillin	9	75	3	25	1	3.84	25	26.15
Erytromycin	7	58.33	5	41.66	11	42.3	15	57.69
Tetracyclin	9	75	3	25	15	57.69	11	42.34
Ampicillin	6	50	6	50	3	11.53	23	88.46
Amoxicilin	6	50	6	50	15	57.69	11	42.3

Table-1 (d): Antibiotic susceptibility of bacterial isolates (*Streptococcus D* and *P. aeruginosa*) from subclinical mastitis goats

Antibiotics	*Streptococcus D* (n=3)				*Pseudomenas aeruginosa* (n=24)			
	Susceptibility		Resistant		Susceptibility		Resistant	
	n	%	n	%	n	%	n	%
Streptomycin	0	0	3	100	18	75	6	25
Penicillin	0	0	3	100	0	0	24	100
Erytromycin	1	33.33	2	66.66	11	45.83	13	48.14
Tetracyclin	1	33.33	2	66.66	14	58.33	10	41.66
Ampicillin	0	0	3	100	0	0	24	100
Amoxicilin	2	66.66	1	33.33	16	66.66	8	33.33

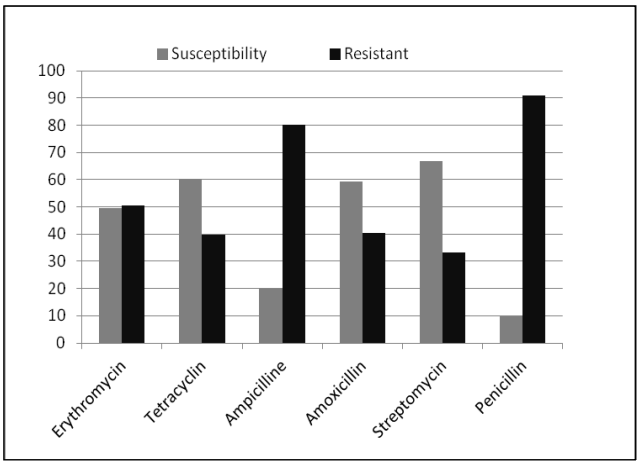

Figure-1: Percentage of resistance of strains isolated from subclinical mastitis goats.

Table-2: Antimicrobial activity of honey against bacteria isolated from subclinical mastitis goat

Microorganisms	MIC (Mean ± SD %)
P. aeruginosa	13.00 ± 3.13
CNS	12.66 ± 4.27
Klebsiella spp.	13.37 ± 2.63
Micrococcus spp.	13.55 ± 2.06
Enterobacter spp.	13.09 ± 0.84
E. coli	13.53 ± 3.95
S. aureus	13.25 ± 0.95
Streptococcus D	14.00 ± 1.22
Bacillus spp.	13.00± 4.24
Corynebacterium spp.	11.96 ± 0.00

CNS: Coagulase Negative Staphylococci

and CNS were preferably susceptible to Streptomycin, *Streptococcus* D and *Corynebacterium* spp. were resistant. Indeed, *Streptococcus* D was the most resistant to the tested antibiotics whereas *S aureus* was the most susceptible to all the tested antibiotics (Figure-1).

The results regarding the antimicrobial activity of honey against the isolated bacteria are presented in Table-2. We notice that honey was effective within the concentration range of 11-14% as it completly inhibits the growth of bacteria. The Algerian honey showed higher activity against *Corynebacterium* spp. (13%), *Pseudomonas aeruginosa* (11.96±3.13%), CNS (12.66 ±4.27%), *Klebsiella* spp. (13.37±2.63%), *Micrococcus* spp. (13.55±2.06%), *Enterobacter* spp. (13.09± 0.84%), *E. coli* (13.53±3.95), *S. aureus* (13.25±0.95%), *Streptococcus* D (14 ± 1.22%) and *Bacillus* spp. (13 ± 4.24%).

Discussion

The study of the sensitivity or resistance of mastitis pathogens strains to antibiotics is of high importance from both clinical and economic points of view. Lactam antibiotics are frequently used in the treatment of mastitis in the goats [24]. In the present work, most of the tested bacteria showed a resistance to lactam antibiotics. Some CNS species have been reported to be resistant against Penicillin G in caprine [25] and bovine mastitis [26]. This resistance may be due to the incorrect or incomplete antibiotics treatments [27].

In the present study, the results obtained showed that *S. aureus*, *Bacillus* spp., *Enterobacter, Klebsilla* spp. *and Micrococcus* spp. were susceptible to the most tested antibiotics. Moroni *et al.* [24] and Tras *et al.* [27] have already reported similar susceptibility in the goat mastitis. Moreover, susceptibility to lactams and fluroquinolones was reported in the ewes [28, 29] and bovine mastitis [26]. *Streptococcus* D and *P. aeroginosa* were resistant to the most tested antibiotics in contrast to the results obtained by Aydin and collaborators [30], wherein *Streptococcus* spp. was found to be

susceptible to Lactams, Erythromycin, Oxytetracyclin and Florfenicol.

In the current investigation, the results of Minimum Inhibitory Concentration revealed that honey was effective against *CNS, Staphylococcus aureus, Streptococcus* D, *Corynebacterium* spp., *Klebsiella* spp., *P. aeruginosa, Enterobacter* spp., *E. coli, and Bacillus* spp. All values of MICs registered were between 10% and 14%. Many reports literature revealed that honeys originating from different countries and regions showed wide variability in their antimicrobial activity. The antimicrobial activity of honey may range from concentrations lower than 3-50% or more [6, 31-33]. The presently tested honey had antimicrobial effect against the isolated microorganisms with the concentration range of 10-14%. Some bacteria were inhibited from 25% to 50% [16]. In addition, many species have shown a sensitivity to lower concentrations than 1.8-11% for some honeys [32, 34]. The results discussed in the present paper clearly indicate that the tested honey was of high antimicrobial quality. The inhibition of bacterial growth may be due to many factors including the osmotic effect of honey [18-35], the presence of hydrogen peroxide, non peroxide substances [37], and propolis which contains flavonoid [6].

Many authors studied the antibacterial activity of honey and found that different bacteria (*S. aureus, Pseudomonas, E. coli, Proteus, Streptococcus*) were inhibited with Manuka honey [38, 39]. Likewise, Jeddar and co-authors [40] found that most pathogenic bacteria (Gram+ve and Gram-ve) failed to grow in honey at concentration of 40% or above. Mullai and Menon [41] assessed the antibacterial activity of different types of honey (Manuka honey, heather honey and locally marketed Indian honey). They found that local (Khadikraft) honey produced the best activity against *P. aeruginosa, E. coli and S. aureus*. Khalil and collaborators [42] found that the tested unifloral honeys available at the Northern Region of Bangladesh showed a significant antibacterial activity against the wound infecting enteric pathogens. Also, Selcuk and Nevin [43] found that honey of Turkey origin (Rize-Anzer region) was the most effective on clinically isolated bacteria. Importantly, many studies suggested that there is a variation in antibacterial activities of honeys from different geographical locations. This could be attributed to the raw materials utilized by bees in preparing their valuable honey [6, 12]. The antibacterial activity of Algeria honey is more significant than the results found by Hassanein *et al.* [14], Basson *et al*. [16] and Chute *et al.* [11] (25-30% v/v, 25% v/v and 20-80% v/v, respectively). On the other hand, it remains less efficient than the honey grade tested by Omaya and Akharaiyi [8] (4-5% v/v).

Conclusion

In conclusion, the results show clearly that antimicrobial drug susceptibility tests in goat subclinical mastitis might be necessary before the treatment. The study also shows that Algerian honey exhibited *in vitro* antimicrobial activity against different bacteria isolated from goat mastitis. The honey could be a promising alternative therapy in the treatment of infected udder. However, future investigations are needed to confirm the *in vivo* antimicrobial activity of honey in the treatment of subclinical mastitis in goat.

Authors' contributions

AB designed the study and performed the research. AA drafted and reviewed the manuscript. SMH and LB analyzed the data and reviewed the manuscript. HB conceived and supervised the work and reviewed the manuscript. All authors read and approved the final manuscript.

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Competing interests

The authors declare that they have no competing interests.

References

1. Leitner, G., Merin, U. and Silanikove, N. (2004) Changes in milk composition as affected by subclinical mastitis in goats. *J. Dairy Sci.* 87: 1719-1726.
2. Shi, D., Hao, Y., Zhang, A., Wulan, B. and Fan, X. (2010) Antimicrobial Resistance of *Staphylococcus* Aureus Isolated From Bovine Mastitis in China. *Transbound. Emerg. Dis.*, 57(4): 1865-1682.
3. Brudzynski, K. (2006) Effect of hydrogen peroxide on antibacterial activities of Canadian honeys. *Can. J. Microbial.* 52: 1228-1237.
4. Jalali, F.S.S., Tajik, S. and Fartash, B. (2007a) Topical application of natural Urmia honey on experimental burn wounds in the dog. *Asian J. Anim. Vet. Adv. Med.* 2: 133-139.
5. Jalali, F.S.S., Farshid, A.A., Saifzadeh, S. and Javanmardi, S. (2007b) Efficacy of intra-peritoneal administration of Iranian honey in prevention of post-operative peritoneal adhesions. *Asian. J. Anim. Vet. Adv.* 2: 212-217.
6. Hegazi, A.G. (2011) Antimicrobial activity of different Egyptian honeys as comparison of Saudi Arabia honey. *Res. J. Microbiol.* 6(5): 488-495.
7. Ibarguren, C., Raya, R.R., Maria, C. and Pella, A. (2010) Carina Audisio M: *Enterococcus faecium* isolated from honey synthesized bacteriocin-like substances active against different *Listeria monocytogenes* strains. *J. Microbiol.* 48 (1): 44-52.
8. Omoya, F.O. and Akharaiyi, F.C.A. (2010) Pasture honey for antibacterial potency on some selected pathogenic bacteria. *J. Nat. Prod.* 3: 05-11.
9. Al-Waili, N., Al-Ghamdi, A., Ansari, M.J., Al-Attalm, Y. and Salom, K. (2012) Synergitic effects of honey and propolis toward drug multi-resistant and *Candida albicans* isolates in single and polymicrobial cultures. *Int. J. Med. Sci.* 9(9): 793-800.
10. Alzahrani, H., Boukraa, L., Bellik, Y., Abdellah, F.,

Bakhotmah, B.A., Kolayli, S. and Sahin, H. (2012) Evaluation of the antioxidant activity of three varieties of honey from different botanical and geographical origins. *Glob. J. Health Sci.* 4(6): 191-6.
11. Chute, R.K., Deogade, N.G. and Kawale, M. (2010) Antimicrobial activity of Indian honey against clinical isolates. *Asiatic J. Biotech. Res.* 1: 35-38.
12. Bradshaw, C.E. (2011) An in vitro of the antimicrobial activity of honey, iodine and silver wound dressings. *Biohorizons,* 4(1): 61-70.
13. Cooper, R.A., Halas, E. and Molan, P.C. (2002) The efficacy of honey in inhibiting strains of *Pseudomonas aeruginosa* from infected burns. *J. Burn. Care Rehabil.* 23: 366-370.
14. Hassanein, S.M., Gebreel, H.M. and Hassan, A.A. (2010) Honey compared with some antibiotics against bacteria isolated from burn-wound infections of patients in Ain Shams university hospital. *J. American Sci.* 6(10): 301-320.
15. Lehtopolku, M., Nakari, U.M., Kotilainen, P., Huovinen, P., Siitonen, A. and Hakanen, A.J. (2010) Antimicrobial susceptibilities of multidrug-resistant *Campylobacter jejuni* and *C. coli* strains: *in vitro* activities of 20 antimicrobial agents. *Antimicrob. Agents Chemother.,* 54(3):1232-1236.
16. Basson, N.J. and Grobler, S.R. (2008) Antimicrobial activity of two South African honeys produced from indigenous *Leucospermum cordifolium* and *Erica* species on selected micro-organisms. *BMC Complem. Alternative Med.,* 8: 41-45.
17. Elmenoufy, G.A.M. (2012) Bee honey dose-dependently ameliorates lead acetate-mediated hepatorenal toxicity in rats. *Life Sci. J.* 9(4): 780-788.
18. Molan, P.C. (1992) The antibacterial activity of honey.1. The nature of antibacterial activity. *Bee World* 73: 5-28.
19. Adam, C.J., Boult, C.H., Deadman, B.J., Farr, J.M., Grainger, M.N., Manley-Harris, M. and Snow, M.J. (2008) Isolation by HPLC and characterisation of the bioactive fraction of New Zealand manuka (*Leptospermum scoparium*) honey. *Carbohydr. Res.* 343: 651-659.
20. Mavric, E., Wittmann, S., Barth, G. and Henle, T. (2008) Identification and quantification of methylglyoxal as the dominant antibacterial constituent of Manuka honey from New Zealand. *Mol. Nutr. Food Res.* 52(4): 483-489.
21. Tan, H.T., Rahman, R.A., Gan, S.H., Halim, A.S., Sulaiman, S.A. and Kirnpal-Kaur, B. (2009) The antibacterial properties of Malaysian tualang honey against wound and enteric microorganisms in comparison to manuka honey. *BMC Complement. Alternat. Med.* 9: 34.
22. Sherlock, O., Dolan, A., Athman, R., Power, A., Gethin, G., Cowman, S. and Humphreys, H. (2010) Comparison of the antimicrobial activity of ulmo honey from Chile and manuka honey against methicillin-resistant *Staphylococcus aureus, Escherichia coli* and *Pseudomonas aeruginosa*. *BMC Complement. Alternat. Med.* 10:47.
23. Mullai, V. and Menon, T. (2007) Bactericidal activity of different types of honey against clinical and environmental isolates of Pseudomonas aeruginosa. *J. Alternat. Complement. Med.* 13: 439-441.
24. Moroni, P., Vellere, F., Antonini, M., Pisoni, G., Ruffo, G. and Carli, S. (2004) Characterization of *Staphylococcus aureus* isolated from chronically infected dairy goats. *Int. J. Antimicrob. Agents* 23: 637-640.
25. Da Silva, E.R., Siqueira, A.P., Dias Martins, J.C., Barbosa Ferreira, W.P. and Da Silva, N. (2004) Identification and in vitro antimicrobial susceptibility of *Staphylococcus* species isolated from goat mastitis in the northeast of Brazil. *Small Ruminant Res.* 55: 45-49.
26. Turutoglu, H., Ercilik, S. and Ozturk, D. (2006) Antibiotic resistance of Staphylococcus aureus and Coagulase-Negative Staphylococci isolated from bovine mastitis. *Bull. Vet. Inst. Pulway* 50, 41- 45.
27. Tras, B., Yazar, E. and Elmas, M. (2007) Practical and rational drug use in veterinary profession. Olgun Press, Konya,Turkey. Page no. 29-89.
28. Fthenakis, G.C. (1994) Prevalence and aetiology of subclinical mastitis in ewes of Southern Greece. *Small Rum. Res.* 13: 293-300.
29. Pengov, A. and Ceru, S. (2003) Antimicrobial drug susceptibility of *Staphylococcus aureus* strains isolated from bovine and ovine mammary glands. *J. Dairy Sci.* 10: 3157-63.
30. Aydin, I., Kursat, K. and Celik, H.A. (2009) Identification and Antimicrobial Susceptibility of Subclinical Mastitis Pathogens Isolated from Hair Goats' Milk. *J. Anim. Vet. Adv.* 8(6): 1086-1090.
31. Lusby, P.E. and Combes, A.L. and Wilkinson, JM. (2005) Bactericidal activity of different honeys against pathogenic bacteria. *Arch. Med. Res.* 36: 464-467.
32. Wilikinson, J.M. and Cavanaghn H.M. (2005) Antibacterial activity of 13 honeys against Escherichia coli and Pseudomonas aeruginosa. *J. Med. Food* 8: 100-103.
33. French, V.M., Cooper, R.A. and Molan, P.C. (2005) The antibacterial activity of homey against coagulase-negative staphylococci. *J. Antimicrob. Chemother.* 56: 228-231.
34. Willis, D.J., Molan, P.C. and Harfoot, C.G. (1992) A comparison of the sensitivity of wound-infecting species of bacteria to the antibacterial activity of Manuka honey and other honey. *J. Appl. Bacteriol.* 73: 388-394.
35. Listner, W. (1975) The significance of water activity for microorganisms in meat. In: Water relations of food. Academic Press, London, UK. Page no. 309-323.
36. Cherife, J., Scanmato, G. and Herzage, L. (1982) Scientific basic for use of granulated sugar in treatment of infected wounds. *Lancet* 319: 560-561.
37. Radwan, S.S., El- Essawy, M. and Sharhan, M. (1984) Experimental evidence for the occurrence in honey of specific substances active against micro-organisms. *Zentralblat Microbial.* 139: 249-255.
38. Hassanein, S.M., Gebreel, H.M. and Hassan, A.A. (2010) Honey compared with some antibiotics against bacteria isolated from burn- wound infections of patients in Ain Shams University hospital. *J. American Sci.* 6: 301-320.
39. Kwakman, P.H.S., Johannes, P.C., Van den Akker, A.G., Aslami, H., Binnekade, J.M., Leonie de Boer, Boszhard, L., Paulus, F., Middelhoek, P., Te Velde, A.A., Vandenbroucke-Grauls, C.M.J.E., Schultz, M.J. and Zaat, S.A.J. (2008) Medical–grade honey kills antibiotic-resistant bacteria in vitro and eradicates skin colonization. *Clin. Infect. Dis.* 46(11): 1677-1682.
40. Jeddar, A., Kharsany, A., Ramsaroop, U.G., Bhamjee, A., Haffejee, I.E. and Moosa, A. (1985) The antibacterial action of honey: an *in vitro* study. *S. Afr. Med. J.* 67: 257-258.
41. Mullai, V. and Menon, T. (2007) Bactericidal activity of different types of honey against clinical and environment isolates of *Pseudomonas aeruginosa*. *J. Altern. Complement. Med.* 13: 439-441.
42. Khalil, M.I., Adbul-Motallib, M., Anisuzzaman, A.S.M., Sathi, Z.S., Hye, M.A. and Shahjahan, M. (2001) Antibacterial activities of different brands of unifloral honey available at the northern region of Bangladesh. *J. Med. Sci.* 1: 389-392.
43. Selcuk, H. and Nevin, K. (2002) Investigation of antimicrobial effect of honey collected from various regions of Turkey. *Pak. J. Biol. Sci.* 5: 325-328.

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