Evaluation of the Inhibitory Effect of Sidr Honey from Different Sources Against Methicillin Resistant *S. aureus*

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Abstract

In order to determine the antibacterial effect of 5 different sidr honey samples { from Egypt (E), Libya (L) ,Yemen (Y), Pakistan (P) and Saudi Arabia (S) }against MRSA strains, atotal of 200 quarter milk samples were collected from local breed cows suffering from clinical mastitis and 170 samples were collected from human patients from hospitals in Sharkia Governorate.The collected samples were subjected to bacteriological examination where the percentage of *S.aureus* was 40.86% (n = 152). Out of 152 *S. aureus* strains, 35% were found to be methicillin resistant.The MIC and MBC of the tested strains ranged from 20-60% (w/v). After exposure to sidr honey,about 42% of MRSA strains changed from coagulase positive to coagulase negative, latex agglutination test showed that 3% of MRSA strains changed from positive to negativeand all intermediate methicillin resistant ***S.aureus***strainschanged to sensitive with all sidr honey.The results suggestthe possible use of sidr honey as antibacterial agent against MRSA strains.

Introduction

Mastitis is a multi etiopathogenic condition of mammary gland affecting dairy cows and remains the most economically important disease of dairy industries around the world. It is characterized by physical,chemical and microbiological changes in the milk and pathological changes in the glandular tissues of the udder ***Patnaik et al (2013).*** *S. aureus*is a major pathogen in dairy cattle mastitis ***Piepersetal. (2007).*** The practice of incorporating antibiotics into feeds to control and treat diseases in cows on dairy farms has increased, and antibiotic administration in animals could be one of the main causes of antibiotic

resistance in pathogens ***Jamali et al (2013)***

*S. aureus*is the most common cause of nosocomial infection and is of increasing concern because of their tendency to multiple antibiotic resistances which often complicates treatment. During the past 4 decades MRSA has spread throughout the world and has become highly endemic in many geographic areas ***Mark et al (2002).*** Honey has been reported to have an inhibitory effect to around 60 species of bacteria including aerobes and anaerobes, Gram positives, and Gram negatives ***Hannan et al (2004).*** There are many reports of bactericidal as well as bacteriostatic activity of honey and the antibacterial properties of honey may be particularly useful against bacteria, which have developed resistance to many antibiotics ***(Al-Waili et al, 2012)***.

Sidr honey is made from bees who feed only on the nectar of the Sidr tree, which is native to the South Saudi Arabia and Yemen regions. The Sidr tree is considred sacred and has been used as a natural medicine for centuries. Sidr honey is a "monofloral honey", a type of honey which has a high value in the marketplace because it has a distinctive flavor or other attribute due to its being predominantly from the nectar of one plant species. Sidr honey has wide medicinal applications and uses which include: liver diseases treatment, stomach ulcers, and respiratory infections, diseases resulting from malnutrition, digestive problems, constipation, eye diseases, infected wounds and burns, surgical wounds (caesarian section), speedy recovery after childbirth, general health and vitality. Sidr honey has strong antioxidant and antibacterial properties ***Alandejani et al (2009)*** .

This work was planned to evaluate the antibacterial activity of sidr honey against Methicillin Resistant Staphylocousaureusstrains were isolated from human patients and clinicallymastitic milk.

Material and Methods

Samples for bacterial isolation:

Atotal of 370 samples were collected (200 quarter milk samples were collected from clinically mastitic cows and 170 samples (45wounds, 30 sputum, 25 blood, 30 aspirates, 20 urine, and 20 cerebrospinal fluids) were collected from human patients at hospitals in Sharkia Governorate.

Honey Samples:

Sidrhoney samples were obtained from 5 different sources; from Egypt (E), Libya(L) ,Yemen (Y), Pakistan (P) and Saudi Arabia(S).

Isolation and identification of *S.aureus*:

Samples were inoculated on mannitol salt agar , Baird parker medium and 7% sheep blood agar. All plates were incubated at 37°C for 24-48 hours and examined daily for bacterial growth. Bacterial

colonies were identified morphologically using Gram's stain as well as biochemically using methods described by ***Quinn et al (1994).***

Latex agglutination test:
One drop of reagent was added to one drop of the overnight broth culture of the test organism (McFarland tube 1 or 2), on the test card and mixed. It was observed visually for agglutination. Both positive and negative controls were performed. ***Brown et al (2005).***

Antimicrobial susceptibility testing by disc diffusion method:
The susceptibility testing was performed according to the procedures of ***NCCLS (2007)*** using Ampicilin, Tetracycline, Amoxicillin, Erythromycin. Ciprofloxacin andmethicillin antibiotic discs.

Minimum inhibitory concentration of honey against the*S.aureus* strains.

Agar Well Diffusion Assay
All honey samples were screened by agar well diffusion assay as adopted by ***Allen et al (1991)***. The plates were incubated at 37°C for 24 hr under aerobic condition and then examined for inhibition zones***Barry and Thornsberry. (1985).***

Broth dilution method
The test was carried out as described by ***Heunvelink et al (1998)***. The lowest concentration of honey in the series that inhibited the growth of the organism was taken to be the MIC, expressed in mg/ml.

(MBC) of honey against the*S.aureus* strains:
After ascertaining the MIC, the number of bacteria was counted in each of the tubes of broth that showed no visible turbidity after overnight incubation, and was compared with the number of bacteria in the initial microorganism suspension; according to ***NCCLS (1997).***

Results

Table 1: *Showing number ofS. aureus strainsisolated from clinical mastitic milk samples and human patients:*

Samples	No. of examined samples	No. of *S.aureus*strains	% of *S.aureus*strains
Quarter milk samples	200	62	31
From human patients	170	90	52.9
Total	370	152	40.86

Table 2: *Interpretation of antibiotic sensitivity test for S. aureus strains isolated from clinically mastitic milk samples (N=62):*

Antimicrobial agent	Resistant		Intermediate		Sensitive	
	NO.	%	NO.	%	NO.	%
Ampicilin(Am)	30	48.38	13	20.96	19	30.64
Tetracycline(TE)	40	64.51	12	19.35	10	16.12
Amoxicillin(Ax)	9	14.51	10	16.12	43	69.35
Erythromycin(E)	25	40.32	6	9.67	31	50
Methicillin(ME)	8	12.90	6	9.67	48	77.41
Ciprofloxacin(CIP)	0	0	2	3.22	60	96.77

Table 3: *Interpretation of antibiotic sensitivity test S. aureus strains isolated from human patient samples (N=90):*

Antimicrobial agent	Resistant		Intermediate		Sensitive	
	NO.	%	NO.	%	NO.	%
Ampicilin(Am)	25	27.77	30	33.33	35	38.88
Tetracycline(TE)	37	41.11	20	22.22	33	36.66
Amoxicillin(Ax)	27	30	35	38.88	28	31.11
Erythromycin(E)	36	40	30	33.33	24	26.66
Methicillin(ME)	25	27.77	15	16.66	50	55.55
Ciprofloxacin(CIP)	29	32.22	20	22.22	41	45.55

Table 4: *The percentage of MRSA strains isolated from collected samples:*

Types of examined samples	No. of isolated *S.aureus*	No. of MRSA strains	%. Of MRSA strains
MILK	62	14	**23.80**
Human patient	90	40	**44.44**

Latex agglutination test for of MRSA strains:

MRSA strain isolated from collected samples were found to be positive by Latex agglutination test at which a red agglutination in a slight to significant blue background within30seconds after the initial mixing of the specimen and the detection latex reagent.

-MRSA strain was changed from coagulase positive to coagulase negative after exposure to differentsidr honey.

-MRSA strain were changed fromLatex agglutination positive to negative with (S ,Y) sidr honey otherwise, sidr honey (E, L, P,) showed no effect .

Table 5: *MBC and MIC of different sidr honey against MRSA strains*:

Honey Sample	MBC(w/v)	MIC(w/v)
(L) and (Y)	**40-60 mg/ml**	**30-50 mg/ml**
(P) and (S)	**30-40 mg/ml**	**20-30mg/ml**
(E)	**30-60 mg/ml**	**20-50mg/ml**

Table 6: *Diameter of inhibition zone for Methicillin antibiotic disc of MRSA isolated from milk samples:*

Diameter of inhibition zone for methicillin antibiotic disc						
No. of MRSA strains (n=14)	**Before exposure to honey (mm)**	**After exposure to honey (mm)**				
		L	**E**	**P**	**S**	**Y**
6	**12**	**12**	**18**	**16**	**16**	**17**
5	**11**	**11**	**11**	**14**	**12**	**15**
3	**6**	**6**	**6**	**6**	**6**	**6**

Table 7: *Diameter of inhibition zone for Methicillin antibiotic disc of MRSA isolated from human patients samples:*

Diameter of inhibition zone for methicillin antibiotic disc						
No. of MRSA strains (n=40)	**Before exposure to honey (mm)**	**After exposure to honey (mm)**				
		L	**E**	**P**	**S**	**Y**
20	**12**	**12**	**16**	**22**	**14**	**19**
6	**11**	**11**	**11**	**11**	**11**	**21**
14	**6**	**6**	**6**	**6**	**6**	**6**

Discussion

In the present study,as shown in Table (1) the percentage of *S.aureus*clincal mastitis was (31%) which agreeswith that was reported by ***Enany et al (2013).*** *S.aureus* is responsible for approximately 30% to 40% of all mastitis cases. *S. aureus* can gain access to milk either by direct excretion from udders with clinical or subclinical staphylococcal mastitis or by contamination from the environment during handling and processing of raw milk ***Scherrer et al (2004)***

Results illustrated in Table (4) revealed that the percentage of MRSA strains isolated from milk samples was (22.8 %) and from human patients was (44.4%), our results partially in agreement with those obtained by ***Poonam and Pratibha., (2012).*** Methicillin resistant *S.aureus*is one of the most significant human pathogens that cause both nosocomial and community-acquired infections worldwide which are associated with high morbidity and mortality rates with rapid development of resistance.

our study was undertaken to investigate *in vitro* antibacterial activity of 5 different types of sidr honey samples against MRSA strains, as shown in Table (5),the minimum inhibitory concentrations (MICs) and minimum bactericidal concentrations (MBCs) for sidr honey ranged from 20-60 mg/ml . our results is are in agreement with that reported by ***Alqurashi et al (2013)*** and ***Poonam and Pratibha (2012)***. The antimicrobial activity of honey is derived from multiple factors, with contributions from high sugar content, low water content, low acidity, hydrogen peroxide and phytochemicals ***Molan (1992)***The effective antimicrobial properties of honey against antibiotic-resistant organisms such as MRSA also detected by ***Church et al (2006)*** **and** ***Erol et al (2004)***.

Results shown in Tables (6-7), revealed thatall intermediate methicillin resistant *S.aureus* strainschanged to sensitive after exposureto all different types of sidr honey, this may be attributed to the inhibitory effect of sidr honey on mec gene of MRSA strains ***De, N et al (2010).***

In conclusion, Sidr honey has an effective antimicrobial properties against Methicillin resistant *S. aureus* strains isolated from both clinically mastitic milk samples and human patients. It has both a bacteriostatic and bactericidal activity when tested in vitro.

References

Alandejani ,T.; Joseph, M.; Wendy, F.; Robert, S.and Frank, C. (2009): Effectiveness of honey on ***S.aureus***and ***Pseudomonas aeruginosa*** biofilms**.** Otolaryngology–Head and Neck Surgery . 141, 114-118

Allen, K.L.; Molan, P.C.; and Reid,G.M.(1991): A survey of the antibacterial activity of some New Zealand honeys. J Pharm Pharmacol. 43(12), - pp. 817–822

Alqurashi, A. M.; Masoud, E. A.; and Alamin, M. A. (2013): Antibacterial activity of Saudi honey against Gram negative bacteria Journal of Microbiology and Antimicrobials. Academic Journals. Vol. 5(1), pp. 1-5.

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.

Barry, A.andThornsberry, C. (1985): Susceptibility test: diffusion test procedures. In:lennetteEA,BalowsA,HausllerWJ ,Shadomy HJ (eds). Manual of Clinical Microbiology, Washington, DC pp. 978-987

Brown, D.F.;Edwards, D.andHawkey, P.M.(2005): Guidelines for the laboratory diagnosis and susceptibility testing of methicillin-resistant ***S. aureus***(MRSA). Journal ofAntimicrobial Chemotherapy56:1000-1018.

Church, D.; Elsayed, S.; Reid, O.;Winston, B.and Lindsay, R.(2006): Burn wound infections. ClinMicrobiol Rev; 19: 403-434.

De, N., Yenda, E. N., Lynn, M and , Aliyu, T. B (2010). Studies On Susceptibility Of Methicillin–Resistant***S. aureus*** To Some Nigerian Honey. Nature and Science:8(6)

Enany,M.E.; Younes,S.;ALgammal,A.M.;Sale m,M.and EL Dieb,H.A(2013): phenotypic and genotypic characterization of ***S.aureus*** isolated from clinical and subclinical bovine mastitis. Suez CanalVeterinary Medicine Journal(SCVMJ,XVIIII(1).

Erol, S.; Altoparlak, U.; Akcay, M.N.; Celebi, F. and Parlak, M. (2004): Changes of microbial flora and wound colonization in burned patients. Burns, 30: 357-361

Hannan, A.; Barkaat, M.; Saleem, S.; Usman, M.andGilani, W.A. (2004): Manuka honey and its antimicrobial potential against multi drug resistant strains of Typhoidal***salmonellae***, Ph.D. thesis, Department of Microbiology, University of Health Science, Lahore, Pakistan.

Heunvelink, A.E.; van, B. F.; Zwartkruis,N. J.; Herbes, R.G.; Huyben, R.; Nagelkerke, N.; Melchers, W.J.; Monnens, L.and de Boer, E. (1998): Occurrence of verocytotoxin-producing E. coli O157 on Dutch dairy farms. J. Clin. Microbiol. 36(12): 3480-3487 . Identification of ***Staphylococcus*** species of bovine origin with the DMSTrac system. Journal of Clinical Microbiology 20,227-230.

Jamali, H.;Behrad, R.andKwai, L. T. (2013): Prevalence, characterization, and antimicrobial resistance of ***Listeria*** species and

Listeria monocytogenes isolates from raw milk in farm bulk tanks.Food Control 34 :121e125

Mark, C. E .; D. Ashley, R.;Gaynor, R.;Edward, J.;Feil.; HajoGrundmann, and Brian, G. S. (2002): The evolutionary history of methicillin-resistant***S.aureus*** (MRSA). PNAS 2002 99 (11) 7687-7692

Molan P.C. (1992): The antibacterial activity of honey. Bee World; 73: 5-28.

NCCLS (1997). National Committee for Clinical Laboratory Standards. Methods for dilution antimicobial susceptibility tests for bacteria that grows aerobically. Approved Standards, M7-A4, Wayne, Pa. Nzeako BC, Hamdi J. Antimicrobial potential of Honey. Med. Sci. 2:75-79.

NCCLS (National Committee for Clinical Laboratory standard) (2007): performance standards for Antimicrobial Susceptibility Testing;fifteenth Informational Supplement According to CLSI. CLSI document M100-s15. Clinical Laboratory standard Institute,Wayne,

Patnaik, S.; Arun, P. and Subha, G. (2013): Antigenic and Biological Interaction of ***Staphylococcus*** spp. in Host Tissues for Causing Mastitis. J. Chem. Bio. Phy. Sci. Vol.3, No.4; 2649-2651.

Piepers, S.; De Meulemeester, L.; de Kruif, A.; Opsomer, G.; Barkema, H.W. and De Vliegher, S. (2007): Prevalence and distribution of mastitis pathogens in subclinically infected dairy cows in Flanders, Belgium. Journal of Dairy Research, 74:478- 483

Poonam, B. C. and Pratibha, B. D.i (2012): The antibacterial activity of honey against methicillin-resistant ***S. aureus*** isolated from pus samples. ActaBiologicaIndica, 1(1):55-59

Quinn,P.J.;Carter,M.E;Makrkey, B.K. andCarter,G.R.(1994): Clinical veterinary microbiology .mosby year book EuropLimited,Lynton House,London,.109-126

Robert S.; Conrad.; Marsha, J. H.; Roger, C. G.;Stacey, W. and David, A. H. (1998): The Effects of Daptomycin on Chemical Composition and Morphology of ***S. aureus***. Proceedingsof the Oklahoma cademy of Scince .V (78).

Scherrer, D.; Corti, S.; Muehlherr, J. E.; Zweife, C. and Stephan, R. (2004): Phenotypic and genotypic characteristics of ***S. aureus***isolates from raw bulk-tank milk samples of goats and sheep.Veterinary Microbiology ;101 :101–107**.**

الملخص العربى

" تقييم تأثير عسل السدر الجبلى من مصادر مختلفة على الميكروب المكور العنقودى الذهبى المقاوم للمثيسيلين"

من أجل إكتشاف تأثير عسل السدر المضاد للبكتيريا ضد الميكروب المكور العنقودى الذهبى المقاوم للميثيسلين، تم تجميع ٢٠٠ عينة لبن من أبقار محلية تعانى من إلتهاب الضرع و١٧٠ عينة من مرضى من مستشفيات مختلفة فى محافظة الشرقية. ثم تم عمل الفحص البكتريولوجى للعينات المجمعة وكانت نسبة المكور العنقودى الذهبى ٤٠,٨٦% (عدد ١٥٢) وكان منهم ٣٥% مقاوم للميثيسيلين. وكانت نسبة MBC و MIC للعسل ضد الميكروب تتراوح من ٢٠- ٦٠%. وبعد تعرض الميكروب للعسل تحول ٤٢% منه من موجب لإختبار التلزن إلى سالب، وتحولت الميكروبات متوسطة المقاومة للميثيسلين الى حساسة له. وبذلك ومن النتائج المسبقة نقترح إستخدام عسل السدر كمضاد بكتيرى ضد المكور العنقودى الذهبى المقاوم للميثيسلين.