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Review

Honey, milk and antibiotics

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The indiscriminate use of antibiotics has made many microorganisms develop resistance to them. This has created immense clinical problems in the treatment of infectious diseases. Therefore, there is a need to develop alternative antimicrobial agents for the treatment of infectious diseases. Non -antibiotic approaches to the treatment and prevention of infection includes the application of honey and milk. Milk naturally contains an array of bioactivities due to lysozyme, lactoferrin, immunoglobulins, growth factors, and hormones, which are secreted in their active form by the mammary gland. The combination of milk and honey may prove to be important source of nutrition and for protection against microbial infection. In this review article we discuss the importance of honey, milk and their combination in providing protection from infection.

Key words: Honey, milk, antibiotics, combination.

INTRODUCTION

Antibiotics are molecules that stop microbes (both bacteria and fungi) from growing or killing them outright. However, antibiotics are sometimes associated with adverse effects on host which include hypersensitivity, depletion of beneficial gut and mucosal microorganisms, immunosuppression and allergic reactions. There is, therefore, a need to develop alternative antimicrobial agents for the treatment of infectious diseases. There are already several non-antibiotic approaches to the treatment and prevention of infection including the use of honey. Bovine and human milk have also been reported to possess antimicrobial activities. Milk is an established and healthy food source for energy, proteins, vitamins, and minerals. In addition to its value as a nutrient source, interest has arisen in the ability of milk to kill bacteria and how this knowledge can be applied to human health. A number of proteins like immunoglobulins found in milk under various conditions exhibit antimicrobial activity and can confer passive immunity from mother to the neonate. The young of many mammalian species are not born with an effective immune system. The immunoglobulins protect the neonate from infection until their own immune system is developed. The use of honey, milk and combination of both may reduce the indiscriminate use of antibiotics.

In this article, we review our knowledge of the antimicrobial effect of honey, milk and combination of both. We also discuss our own experience from *in vitro* tests on the benefit of combining milk and honey against bacterial infection. We will start by giving a brief review of the antimicrobial activity of honey, then of milk and thereafter we will discuss combination of honey with milk and with antibiotics.

ANTIMICROBIAL ACTIVITY OF HONEY

Honey is acceptable in the medical profession as an antibacterial agent for the treatment of some diseases and infections resulting from wounds and burns (Zumla and Lulat, 1989). In many cases, it is used with success on infections not responding to standard antibiotic and antiseptic therapy. Its effectiveness as an antibacterial agent is widely reported (Molan, 1992b). Honey is the substance made when the nectar and sweet deposits from plants are gathered, modified and stored in the honeycomb by honey bees. The definition of honey depends upon who defines it. Most people think of honey as excellent food, but some others consider it an elixir, and still others as medicine (Zaghloul et al., 2001). Essentially, honey is an invert sugar (a mixture of glucose and fructose) dissolved in 14 to 20% water with minor amounts of organic acids, along with traces of minerals and vitamins. Honey is derived from the nectar of

flowering plants which the honey bee collects. Nectar consists primarily of 10 to 50% sucrose, glucose, and fructose, and 50 to 90% water (Audrey et al., 1995). The source of honey determines many of the attributes of honey such as aroma, flavor, color and composition.

Honey has had a valued place in traditional medicine for centuries. The anicient Egyptians, Assyrians, Chinese, Greeks and Romans employed honey for wounds and diseases of the gut. Honey was the most popular Egyptian drug being mentioned 500 times in 900 remedies (Zumla and Lulat, 1989). Whilst Hippocrates (3^{rd} and 4^{th} centuries BC) made little use of drugs in treatment, he prescribed a simple diet, favoring honey given as oxymel (vinegar and honey) for pain, hydromel (water and honey) for thirst and a mixture of honey, water and various medicinal substances for acute fevers (Zumla and Lulat, 1989). During the biblical era honey received a religious endorsement by both Islam and Christianity.

More intensive studies did not commence until the year 1955 where the word 'inhibine' for the antibacterial activity of honey was introduced, a term which has been widely used since the beginning of literature on honey (White and Subers 1963). Since then there have been many reports on the antimicrobial activity of honey. Some have been of simple testing that has shown honey to have antibacterial activities (Molan, 1992a) . Most, however, have involved investigation of the activity spectrum of honey (i.e. determining which species of micro-organisms are sensitive to the action of honey), or comparison of different types of honey for the potency of their action against one or more species of bacteria. Also, there have been many investigations of the antibacterial substances present in honey (Molan, 1992a).

The low pH of honey is inhibitory to many animal pathogens. Under experimental conditions, especially with heavily diluted honeys, the growth medium tends to neutralize the acidity of the honey so that it does not cause inhibition but when honey is used as a dressing on a wound or ulcer, bacteria may be in contact with honey that is much less diluted and the acidity could well be of importance. The fairly strong buffering capacity of body fluids may likely neutralize the acidity of honey in other situations where there is greater dilution of honey.

Hydrogen peroxide, a component of honey is well known as an antibacterial agent, although it is mostly used as an antiseptic rather than antibacterial agent. It has been out of favor with the medical profession since it first came into use in the late 19^{th} century (Molan, 2001). It has been suggested that it readily decomposes in solutions containing traces of catalytic metals such as iron or copper. This may be the reason why hydrogen peroxide went out of favor as an antiseptic after initially being hailed as an antibacterial and cleansing agent when first introduced (Turner, 1983) . There was an upsurge of interest in its use later when stabilized preparations became available, with good germicidal activity being reported (Turner, 1983). But in more recent times, it has again gone out of favor as awareness has developed of its inflammatory properties and damage caused to tissue by its oxygen free radicals (Salahudeen et al., 1991; Halliwell and Cross, 1994; Saissy et al., 1995). However, the hydrogen peroxide concentration produced in honey activated by dilution is typically around 1 mmol/l, about one thousand times the level in the 3% solution that is commonly used as an antiseptic (Molan and Russel 1988; Molan, 1992b) . There is also a potential for honey to sequester and inactivate the metal ions which catalyse the formation of oxygen radicals from hydrogen peroxide, and the antioxidant components of honey to mop up any free radicals that may be formed.

Aristotle, in 350 BC, and Discorides, in AD 50, recommended that honey collected in specific regions and seasons could be used for the treatment of different ailments (Molan, 1992b). This consideration is continued into present-day folk medicine. The strawberry-tree (*Arbutus unedo*) is valued for its therapeutic properties, while in India lotus (*Nulumbium sceiosum*) is said to be a panacea for eye diseases. In modern clinical practice, however, these views have gone unnoticed, though laboratory findings have found large differences in the antibacterial potency of honey from different floral sources.

In almost all studies in which more than one type of honey has been used, differences in the antibacterial activity of honey have been observed (Al-Jabri et al., 2003; Molan, 1992b). The degree of difference observed has in some cases been very large and in many others very small. The differences are attributable to limited range of testing rather than variation in the activity of the honeys (Molan, 1992b).

THERAPEUTIC PROPERTIES OF HONEY

The antimicrobial activity of honey is very important therapeutically, especially in situations where the body's immune response is insufficient to clear infection. Bacteria often produce protein-digesting enzymes, which can be very destructive to tissues (Church, 1954) and can destroy the protein growth factors that are produced by the body to stimulate the regeneration of damaged tissues in the healing process (Postmes and Vandeputte, 1999). Furthermore, some bacteria produce toxins that kill tissue cells (Davis and Arnold, 1974). Additional damage is often caused by bacteria carrying antigens that stimulate a prolonged inflammatory immune response which give excessive production of free radicals that are very damaging to tissues. Bacteria in wounds can also consume oxygen, and thus make the level of oxygen available in wound tissues drop to a point where tissue growth is impaired (Christopher, 2001). The consequences of bacterial infection are; non-healing of wounds; increase in size of wounds and development of ulcers and abscesses; failure of skin grafts; and

inflammation causing swelling and pain. All these can be avoided by administering honey to clear infection. In addition to having a direct antibacterial action, honey may clear infection through a number of properties including boosting the immune system, its anti- inflammatory action, its antioxidant activity and stimulation of cell growth.

It has been reported that honey stimulates T-lymphocytes in cell culture to multiply, and activates neutrophils (Abuharfeil et al., 1999). It has also been reported that honey stimulates monocytes in cell cultures to release the cytokines TNF-alpha, IL-1 and IL-6, the cell 'messengers' that activate the many facets of the immune response to infection (Jones et al., 2000). In addition to stimulation of these leucocytes, honey provides a supply of glucose, which is essential for the 'respiratory burst' in macrophages that produce hydrogen peroxide, the dominant component of their bacteria-destroying activity (Molan, 2001). Furthermore, it provides substrates for glycolysis, which is the major mechanism for energy production in the macrophages, and thus allows them to function in damaged tissue and exudates where the oxygen supply is often poor. The acidity of honey may also assist in the bacteria-destroying action of macrophages, as an acid pH inside the phagocytotic vacuole is involved in killing ingested bacteria (Molan, 2001).

The anti-inflammatory properties of honey have been well established. It has been observed clinically that when honey is applied to wound, it visibly reduces inflammation (Subrahmanyam, 1998). It has also been observed to reduce oedema around wounds (Subrahmanyam, 1996; Effem, 1993 and 1988; Dumronglert, 1983) and exudates from wounds (Hejase et al., 1996), both of which result from inflammation. Pain is another feature of inflammation, and honey has been observed to be soothing when applied to wounds (Subrahmanyam, 1993). A histological study of biopsy samples from wounds has also shown that there are fewer of the leucocytes associated with inflammation present in the wound tissues (Subrahmanyam, 1998). What is responsible for these observation is a direct anti-inflammatory effect, not a secondary effect resulting from the antibacterial action removing inflammation-causing bacteria. The anti-inflammatory effects of honey have been demonstrated in histological studies of wounds in animals where there was no infection involved (Gupta et al., 1992; Postmes et al., 1997; Oryan and Zaker, 1998).

The anti-inflammatory action of honey is potentially very important therapeutically, as the consequences of inflammation can be major. Although inflammation is a vital part of the normal response to infection or injury, when it is excessive or prolonged it can prevent healing or even cause further damage. The anti-inflammatory action of honey has been found in a clinical trial to prevent partial-thickness burns from converting to full thickness burns which would have needed plastic surgery (Subrahmanyam, 1998).

The reduction in keloids and scarring that is a feature of the dressing of wounds with honey (Subrahmanyam, 1994; Effem, 1993; Subrahmanyam, 1991), and the cosmetically good results obtained (Dunford et al., 2000), are probably due to the anti-inflammatory action of honey. Thus, there are significant benefits to be derived from therapeutic use of anti-inflammatory substances. However, the pharmaceutical ones have serious limitations: corticosteroids suppress tissue growth and suppress the immune response (Molan, 2001), and the non-steroidal anti-inflammatory drugs are harmful to cells, especially in the stomach. But honey has an anti-inflammatory action free from adverse side effects.

It has been observed clinically that when honey is used as a wound dressing it promotes rapid healing of wounds (Bergman et al., 1983; Blomfield, 1973). It has been reported by many clinicians that honey promotes the formation of clean healthy granulation tissue (the clusters of fibroblasts around new capillary beds that is the regenerating connective tissue) (Effem, 1993). It has also been reported that honey hastens epithelialization of the wound (coverage with a new outer layer of skin), making skin grafting unnecessary (Subrahmanyam, 1998; Hejase et al., 1996; Effem, 1993). It is likely that it is the stimulation of cell growth by honey that is responsible for 'kick-starting' the healing process observed in chronic wounds which have remained non-healing for long periods (Wood et al., 1997; Harris, 1994; Somerfield, 1991).

HARMLESSNESS OF HONEY

Honey has no adverse effects other than a stinging sensation experienced by some people when it is applied to open wounds (Wood et al., 1997; Ndayisaba et al., 1993). A transient stinging sensation and redness of the eye soon after applying honey in the eye, but never enough to stop the treatment, was reported in the 102 cases in a trial of honey for opthalmological use (Emarah, 1982). Over the thousands of years, honey has been used on open wounds and in the eyes. It has not gained any reputation for adverse effects, and this is borne out by histological examination of wound tissues that have been treated with honey (Postmes et al., 1997; Gupta et al., 1992). In papers describing the application of honey to open wounds it is reported to be soothing, to relieve pain (Subrahmanyam, 1993), be non-irritating (Subrahmanyam, 1996), cause no pain on dressing (Mcinerney, 1990), and give no secondary reactions (Ndayisaba et al., 1993). Although allergy to antibiotics is fairly common, allergy to honey is rare and it may be a reaction to either the pollen or the bee proteins in honey (Bauer *et al.*, 1996). There is a hypothetical risk of infection of wounds resulting from the application of honey, as honey sometimes contains viable spores of *Clostridia* (Mossel, 1980). However, in none of the many reports published on the clinical usage of honey on open

wounds (Molan, 1998) there are no reports of any type of infection resulting from the application of honey to wounds. Spores germinate of *Clostridia*, being obligate anaerobes, would be unlikely to survive in the presence of the hydrogen peroxide that is generated in diluted honey. But any concern about risk of infection can be overcome by the use of honey that has been treated by gamma-irradiation, which kills clostridial spores in honey (Molan and Allen 1996) without loss of any of the antibacterial activity.

There is a hypothetical risk of blood glucose levels in diabetics being raised through glucose being absorbed from honey across the bed of large wounds, but in cases where this has been checked there has been no sign of this happening (Akhtar and Khan, 1989). Where honey is taken by mouth by diabetics for treatment of gastrointestinal infections the risk is greater, but research has shown that honey gives a lower peak of blood glucose than table sugar does because the absorption from the gut is slower (Samanta et al., 1985).

ANTIMICROBIAL ACTIVITY OF BOVINE MILK

Milk is an established and healthy food source for energy, proteins, vitamins, and minerals. In addition to its value as a nutrient source, interest has arisen in the ability of milk to kill bacteria and how this knowledge can be applied to human health. A number of proteins like immunoglobulins found in milk under various conditions exhibit antimicrobial activity and can confer passive immunity from mother to the neonate. The young of many mammalian species are not born with an effective immune system. The immunoglobulins protect the neonate from infection until their own immune system is developed. Immunoglobulins are found in high concentrations in colostrums, the first milk, and in low concentrations in milk. In addition to the immunoglobulins, other proteins found in milk are thought to have antimicrobial activities. Four of these proteins are lactoferrin, lactoperoxidase, lysozyme and N acetyle-eta-D-glucosaminidase; (NAGase).

Lactoferrin, an iron-binding glycoprotein, was first isolated from cow's milk and subsequently from human milk. Lactoferrin is present in large quantities in mammalian secretions such as milk, tears, saliva and seminal fluid, as well as in some white blood cells. Lactoferrin is one of the minor proteins naturally occurring in cow's milk at an average concentration of about 0.2 g/L (Kai et al., 2002). In colostrums, the lactoferrin content can be as high as 0.5 to 1 g/L. Lactoferrin concentration in mammary secretions from dry cows increases until about 30 days after drying off. The highest lactoferrin concentration found in cow mammary secretions is about 50 to 100 g/L. In human milk and colostrum, the reported concentrations of lactoferrin are 2 to 4 g/L and 6 to 8 g/L, respectively. In its natural state, lactoferrin is only partly saturated with iron (5 to 30%). Lactoferrin has many proposed biological functions, including antibacterial/anti-inflammatory activities, defense against gastro-intestinal infections, participation in local secretory immune systems in synergism with some immunoglobulins and other protective proteins (Joslin et al., 2002). Other functions include provision of iron binding antioxidant protein in tissues, and possibly promotion of growth of animal cells such as lymphocytes and intestinal cells.

Most microorganisms need iron for growth and lactoferrin has the potential to inhibit the growth of bacteria, and even kill them by depriving them of iron. The effectiveness of the antibacterial activity of lactoferrin depends on the iron requirement of the organism, the availability of exogenous iron, and the concentration and degree of iron-saturation of lactoferrin (Nagy et al., 1976). It has been shown that 'natural' lactoferrin is bacterio-static against a wide range of microorganisms, including Gram negative bacteria with high iron requirements (coliforms, which are major mastitis pathogens) and also against some Gram positive organisms such as *Staphylococcus aureus* (also a major mastitis pathogen), *Bacillus* species, and *Listeria monocytogenes*. Lactic acid bacteria in the stomach and intestine have low iron requirements and are generally not affected.

Diarra et al. (2002) conducted a study to evaluate the therapeutic potential of bovine lactoferrin or lactoferricin in combination with penicillin G against *S. aureus*. Minimal inhibitory concentrations of lactoferrin, lactoferri-cin, penicillin, and combinations of lactoferrin or lactoferri-cin with penicillin were determined for fifteen *S. aureus* strains including several strains resistant to eta-lactam antibiotics. The fractional inhibitory concentration index indicated a synergistic effect between lactoferrin and penicillin. Combination of lactoferrin with penicillin increased the inhibitory activity of penicillin by 2 to 4 fold and reduced the growth rate of *S. aureus* strains tested, whereas the increase in the inhibitory activity of lactofer-rin by penicillin was 16 to 64 fold. The addition of iron to the medium containing a combination of penicillin and lactoferrin had no effect on growth inhibition. Electron microscopy revealed that concentrations below the minimal inhibitory concentrations of penicillin induced important ultra-structure alterations, which were further enhanced by the presence of lactoferrin. When *S. aureus* is grown in the presence of a combination of penicillin and lactoferrin, changes in the protein profile of the bacteria, including the disappearance of several protein bands due to the presence of lactoferrin, were observed. These data suggested that bovine lactoferrin in combination with eta- lactam antibiotics can increase the antibacterial activity of these antibiotics against *S. aureus* resistant to antibiotics.

Peroxidase enzymes can kill bacteria by oxidative mechanisms. Peroxidase activity occurs in various exocrine gland secretions including saliva, tears,

bronchial, nasal, and intestinal secretions, as well as milk (Duran et al., 2002). Milk peroxide is known as lactoperoxidase, which is one of the non immunoglobulin protective proteins and a prominent enzyme that plays a role in protection against microbial invasion of the mammary gland. Each lactoperoxidase molecule contains one iron atom. Bovine milk contains concentrations of about 0.03 g/L of lactoperoxidase (Losnedahl et al., 1996). In bovine colostrum, the lactoperoxidase content is very low but increases rapidly after 4 to 5 days postpartum. Lactoperoxidase itself has no antibacterial activity. However, together with hydrogen peroxide and thiocyanate, lactoperoxidase forms a potent natural antibacterial system, the so-called lactoperoxidase system. Both hydrogen peroxide and thiocyanate are naturally distributed in animal and human tissues, although they are generally in very low concentrations.

Lysozyme is an enzyme present in the milk of some species, especially human milk. There are two types of lysozyme: one type is found in the hen egg-white and is known as chicken-type or C-lysozyme and the other type is found in the goose egg-white and is known as goose type or G-lysozyme. Human lysozymes are considered to be the C-lysozyme type. However, cow milk may contain both C- and G-lysozymes because both types are found in various other body fluids and in the stomach tissue of the cow. Lysozyme is believed to kill bacteria by distributing the formation of a glycosidic bond between the two components of peptidoglycan, a constituent of the bacterial cell wall (Losnedahl et al., 1996).

Lysozyme activity is nearly undetectable in cow's milk, but very high in human milk (0.12 g/L) (Musser et al., 2002; Losnedahl et al., 1996). The concentration of lysozyme is highest in human colostrum and pre-colostral milk. The limited lysozyme activity in cow milk increases due to mastitis and high somatic cell counts. Heating cow milk at 75°C for 15 minutes destroys 25% percent of the activity of this enzyme. However, human milk lysozyme is more heat stable than cow milk lysozyme. Lysozyme possesses antibacterial activity against a number of bacteria. This enzyme usually functions in association with lactoferrin or immunoglobulin-A. Lysozyme is effective against *Escherichia coli* in concert with immunoglobulin-A. It causes lysis of some species of salmonellae in association with ascorbate and peroxide both of which are present in low concentrations in milk. The enzyme can limit the migration of neutrophils into damaged tissue and might function as an anti-inflammatory agent (Saraswathi et al., 2002).

COMBINATIONS OF ANTIBACTERIAL AGENTS

The combination of two or more antibacterial agents has been long accepted in the treatment of tuberculosis, which limits the selection of mutants resistant to the individual components. The use of eta-lactamase antibiotics with an aminoglycoside in the treatment of streptococcal endocarditis is well established, since the mixture is more bactericidal than the individual components. A combination of a eta-lactamase antibiotic with a eta-lactamase inhibitor may prevent destruction of the antibiotic. Thus, the enzyme inhibitor clavulanic acid in combination with amoxicillin (co-amoxiclav) restores the activity of the antibiotic against many eta-lactamase-producing bacteria.

Potentiation of the antibacterial effect by antibiotics combinations is referred to as synergy. Some combinations exhibit a lesser effect than the individual components and this is called antagonism. These interactions are generally displayed *in vitro*, and may be difficult to establish evidence of advantages or disadvantages *in vivo*. Thus, the combination of trimethoprim and sulphamethoxazole (cotrimoxazole) can be shown to be synergistic in the test tube but it has been difficult to demonstrate clinical benefits, occasionally trimethoprim is now often used on its own to avoid the chance of toxic reactions to sulphonamides (Greenwood et al., 2002).

Recently, the emergence of multi-drug resistant organisms has created a lot of concern in the medical field, hence there is a need to find an alternative to counter these multi-drug resistant organisms. In one study, honey was used at a concentration of 30 to 50% and it was found to be superior to cephaloridine, ampicillin, gentamicin, nitrofurantoin, nalidixic acid and cotrimoxazole in inhibiting growth of nine types of pathogenic organisms isolated from urine samples of 149 patients with confirmed urinary tract infection (Ibrahim, 1981; Karayil et al., 1998).

In our investigation, two types of tests were done to evaluate the interaction between honey and gentamicin by testing for synergy and their killing activities. Synergy is known to exist between penicillin and streptomycin and between sulphamethoxazole and trimethoprim but that between honey and gentamicin has not been reported. The agar well diffusion method recommended by (Collee et al., 1996) was used in the synergy experiment. The method measures both the bacteriostatic and bactericidal interactions among antibiotics. It measures the combined interaction at concentrations that are normally below the minimum inhibitory concentration for each individual drug. This test can be quantified and we can establish whether drugs interact to give a response that is synergistic, additive, indifferent, or antagonistic (Koneman et al., 1997). From the well diffusion method in our experiment, it was observed that the inhibition zone of gentamicin was bigger than that of honey and at the point where both zones meet there was no increase in zone size suggesting that no synergy existed between them. For synergy to exist, the combined zone size would be bigger than any zone produced by either alone.

We have also investigated the reduction rate in viable counts of *S. aureus* using honey, gentamicin and honey with gentamicin (Al-Jabri et al., 2005a). Honey killed 34,

Table 1. Zones of inhibition (mm) of the various dilutions of honey and four different milk samples.

Substance	Zone of inhibition (mm) using different dilutions of milk/honey				
	100%	50%	25%	12.5%	6.3%
Honey	42	33	27	22	<10
Al-Marai Milk	20	11	00	00	00
Al-Rawabi milk	00	00	00	00	00
A'Safwa milk	00	00	00	00	00
Sohar milk	18	<10	00	00	00

Table 2. The *Staphylococcal aureus* growth inhibition (%) by honey, milk and combinations of honey with milk.

Substance	% of *S. aureus* growth reductions at different times (h)													
	0	1	2	4	6	8	10	12	14	16	18	20	22	24
Honey alone (50%, v/v)	0	41	50	63	71	79	83	91	91	93	97	99	100	100
Al-Marai milk	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Honey and Al-Marai milk	0	45	66	59	91	93	89	96	98	100	100	100	100	100
Al-Rawabi milk	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Honey and Al-Rawabi milk	0	58	69	70	91	95	96	98	100	100	100	100	100	100

Al-Rawabi milk and AL-Marai milk on their own did not demonstrate any killing activities, instead they actually promoted the growth of *S. aureus* bacteria.

84 and 100% of test organisms (*S. aureus*) within half an hour, in six hours and 24 hours, respectively, while gentamicin on its own killed 79% in 30 minutes and achieved 100% killing in six hours (Al- Jabri et al., 2005a). The combination of gentamicin and honey killed 90% within 30 minutes and 99% in six hours (Al-Jabri et al., 2005a). Though the combination of honey and gentamicin showed some enhancement, the level of enhancement was not high enough (1000 fold) to describe it as synergy (Greenwood et al., 2002).

Honey in combination with milk provides excellent nutritional value and it is recommended for use for children especially for the newborns as the main source of nutrition (Garanis-Papadatos and Katsas*,* 1999; Klain and Massimo, 1969). Milk is generally accepted as having antimicrobial activities. Many bioactivities in milk are encrypted within the primary structure of milk proteins, requiring proteolysis for their release from precursors. Proteolysis may release these biogenic peptides during gastrointestinal transit or during food processing (Minervini et al., 2003; Meisel, 2001). These biological activities include opioid, agonist and antagonist peptides, hypotensive peptides which inhibit angiotensin-I-converting enzyme (ACE), and mineral binding, immunomodulatory, antibacterial, and antithrombotic peptides (Minervini et al., 2003; Florisa et al., 2003; Meisel, 2001).

Although more potent antibiotics are available, antimicrobial peptides show the advantages of being able to kill target cells rapidly and having a broad spectrum of activity, including activity for some of the more serious antibiotic -resistant pathogens in clinics. Since the rate of killing is higher than the rate of bacterial multiplication, this enhances the potential to overcome drug resistance. The main site of action of amphipathic or hydrophobic antimicrobial peptides is the cytoplasmic membrane where they tend to assemble to form channels. Some known antimicrobial fragments from bovine milk proteins are isracidin, casocidin-I, and lactoferricin. Bovine milk obtained by pepsin digestion was shown to be antimicrobial against Gram-positive and Gram-negative bacteria (Minervini et al., 2003; Florisa et al., 2003; Meisel, 2001).

The anti *S. aureus* activity of the screened bovine milk samples varied considerably; some had good activity while others did not have any. Our recent studies (Al-Jabri et al., 2005b), confirms the previous observation that not all types of bovine milk possess antibacterial activity and that their antibacterial activities depended on the season of the year when they are collected. In one study we have demonstrated the killing activity of bovine milk and its mixture with honey (Al-Jabri et al., 2005b). Two milk samples were selected; one having an antibacterial activity by well diffusion method (Al- Marai), while the other did not (Al-Rawabi). When each of the milk was tested for antibacterial activity by growth reduction method of viable counts, it was observed that none of the milk samples killed the test organism but instead allowed the organism to grow (Tables 1 and 2). The reason why this happens is unknown but the availability of nutrients in the milk may be one reason. Most microorganisms need iron for growth and lactoferrin content of milk has the potential to inhibit the growth of bacteria and in most cases deprive them of iron. It has been observed that the effectiveness of the antibacterial activity of lactoferrin depends on the iron requirements

of the organism, the availability of exogenous iron and the degree of iron saturation of lactoferrin (Nagy et al., 1976). The presence of exogenous iron ions in the milk may be responsible for lack of inhibition of growth of *S. aureus* by the individual milk samples. Our results agree with that of Diarra et al. (2002) who found that bovine milk containing lactoferrin when mixed with penicillin increased the inhibitory activity of penicillin and lactoferrin by 16 to 64 fold and reduced the growth rate of *S. aureus* strains tested. Though we used honey instead of penicillin and did not test the level of lactoferrin content of our milk, Al-Marai and Al-Rawabi milk used in our experiments are known to contain lactoferrin (Al-Abri, 2003; Al-Jabri et al., 2005b).

The killing rate of honey for *S. aureus* was shown to be 40% in one hour, 82% in eight hours and 100% in twenty-four hours. While the killing rate of Al-Marai and Al-Rwabi milk in separate combinations with honey was 92 and 94%, respectively, in 8 hours. The result indicates that milk mixed with honey killed *S. aureus* faster than either alone. However, the improvement in this killing rate is better described as mild enhancement rather than synergy where the level of differences in antibacterial activity should be 1000 fold more than the individual activities of either milk or honey alone.

From our experience, we have shown that not all milk samples possessed antibacterial activity against *S. aureus* (Table 1). Two of four milk samples (Al-Rawabi and A'Safwa) failed to demonstrate any activity against *S. aureus,* rather allowed the organisms to grow (Al Hosni, 2005). At 2 hours, honey alone demonstrated 50% killing, while the combination of honey with either Al-Marai or Al-Rawabi milk demonstrated 66% and 69% killing, respectively. At 6 hours, honey killed 71% while the combination with either milk samples killed more than 90% (Table 2). The combination of honey and milk showed higher growth reduction, enhancing the killing activity by approximately 20%. Honey alone requires more than 20 hours for killing all bacteria. However, when milk was combined with honey, 100% killing was achieved in 16 hours.

The chemical composition of each honey sample is attributable to the nectar of each flower from where the bees produced honey. Honey is known to contain, phenol, fatty acids, lipids, amylases, ascorbic acid, peroxidases and fructose and has high osmolarity and low pH. These elements acting alone or synergistically may contribute significantly to the antibacterial activity of honey (Oka et al., 1987, Wahdan, 1988). Although honey is known to have high antibacterial actions on different bacteria including those that are highly resistant to antibiotics (Nzeako and Hamdi, 2000), the exact mode of action of honey against many micro-organisms is still not clear at present. Although we do not know the exact mechanisms by which honey in combination with milk kills *S. aureus* faster than either alone, it is possible that honey may release some of the biogenic peptides in milk and this may lead to the observed faster killing activity.

This obviously requires more research in this important area. In addition to the antibacterial activity of honey and milk, both have excellent nutritional values and would be an additional enhancer of immunity in aid to the treatments of bacterial infections. Honey in combination with milk may prolongs or improves the shelf life of each other.

In conclusion, honey when mixed with an antibiotic had best killing effect within half an hour of exposure to bacteria than either an antibiotic or honey used alone. Honey in combination with bovine milk reduced the survival of bacteria faster than either honey or milk alone. Though honey on its own is used as medicine, antiseptic and food, we recommend that honey when used with milk may offer even faster killing rate of bacteria than either used alone. Combining honey and milk may well prove to be an inexpensive way to fight infection and reduce the widespread use of antibiotics.

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