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The sensitivity to honey of Gram-positive cocci of clinical significance isolated from wounds

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Aims: To determine the sensitivity to honey of Gram-positive cocci of clinical significance in wounds and demonstrate that inhibition is not exclusively due to osmotic effects.

Methods and Results: Eighteen strains of methicillin-resistant *Staphylococcus aureus* and seven strains of vancomycin-sensitive enterococci were isolated from infected wounds and 20 strains of vancomycin-resistant enterococci were isolated from hospital environmental surfaces. Using an agar incorporation technique to determine the minimum inhibitory concentration (MIC), their sensitivity to two natural honeys of median levels of antibacterial activity was established and compared with an artificial honey solution. For all of the strains tested, the MIC values against manuka and pasture honey were below 10% (v/v), but concentrations of artificial honey at least three times higher were required to achieve equivalent inhibition *in vitro*. Comparison of the MIC values of antibiotic-sensitive strains with their respective antibiotic-resistant strains demonstrated no marked differences in their susceptibilities to honey.

Conclusions: The inhibition of bacteria by honey is not exclusively due to osmolarity. For the Gram-positive cocci tested, antibiotic-sensitive and -resistant strains showed similar sensitivity to honey.

Significance and Impact of the Study: A possible role for honey in the treatment of wounds colonized by antibiotic-resistant bacteria is indicated.

INTRODUCTION

Investigations into the microbial flora of wounds began in the late 19th century. Since then, improvements in techniques have facilitated the recovery, identification and enumeration of a wide variety of microbial species. Most wounds support relatively stable polymicrobial communities (Bowler *et al*. 2001), often without signs of clinical infection (Hansson *et al*. 1995). However, potential pathogens may be present and the delicate balance between a colonized wound and an infected wound depends on the interplay of complex host and microbial influences (Emmerson 1998). The development of wound infection has deleterious effects on patients by causing increased pain, discomfort and inconvenience and can lead to life-threatening illness or even death. Also, it interrupts the healing process, contributing to extended hospital stays, as well as increased treatment costs in terms of antibiotics, dressings and staff time. Both topical antimicrobial agents (O'Meara *et al*. 2001) and appropriately selected antibiotics (Bowler *et al*. 2001) are valuable in the treatment of infected wounds but the routine use of systemic antibiotics for chronic wounds without signs of clinical infection is not recommended (O'Meara *et al*. 2001).

Antimicrobial agents have been applied to wounds for thousands of years (Moellering 1995) but many ancient remedies have been discontinued because the evidence to support their efficacy was anecdotal. Continued use of systemic and topical antimicrobial agents has provided the selective pressure that has led to the emergence of antibiotic-resistant strains which, in turn, has driven the continued search for new agents. Unfortunately, the increased costs of

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searching for such agents and the decreasing rate of their discovery (Moellering 1995) has made the situation increasingly urgent and the prevalence of antibiotic-resistant microbial species now justifies the re-evaluation of former treatments (Anon. 1998).

The medicinal use of honey in wound treatment is derived from diverse ancient civilizations (Jones 2001). The antibacterial properties of honey were recognized more than a century ago and have subsequently been extensively studied (Molan 1992a, 1992b). A wide range of microbial species has been shown to be inhibited by honey but reported susceptibilities are not consistent. Failure to identify the botanical sources of honeys used in many of those studies, or to determine their antibacterial potency, makes comparison of reported sensitivities unreliable. It is remarkable that ancient physicians were selective in the honeys that they utilized in their remedies (Jones 2001), although the underlying principles would have been obscure. Now it is possible to determine quantitatively the antibacterial activity of a honey (Allen *et al*. 1991) and also to discriminate between honeys whose mode of action involves factors beyond their osmolarity in limiting bacterial growth (Allen *et al*. 1991). In most honeys this depends on the enzymic generation of hydrogen peroxide to varying degrees (Molan 1992a) but, in some honeys, there are additional phytochemical antibacterial factors (Molan 1992a). In recent studies, the susceptibility of wound pathogens (Willix *et al*. 1992) and bacteria isolated from infected wounds (Cooper and Molan 1999; Cooper *et al*. 1999) to honeys of known floral source and defined antibacterial activity has been reported. However, the inhibition of antibiotic-resistant bacteria by honey has not been fully explored. Using characterized honeys, this study aims to extend the range of wound pathogens whose susceptibility to honey has been determined and to compare the susceptibilities of antibiotic-sensitive strains with those of antibiotic-resistant strains. Also, to demonstrate unequivocally that inhibition of bacterial species by natural honey in tests *in vitro* is not exclusively due to osmotic effects, an artificial honey (a solution of sugars as in honey) was included in the assays.

MATERIALS AND METHODS

Bacterial strains

One strain of methicillin-resistant *Staphylococcus aureus* (MRSA) and seven strains of vancomycin-sensitive enterococci (VSE) were isolated from outpatients attending the Wound Healing Research Unit (WHRU, Cardiff Medicentre, Cardiff, UK). Twenty strains of vancomycin-resistant enterococci (VRE) were isolated from the environment in the intensive care unit, haematology and cardiology wards at University Hospital of Wales (UHW), Cardiff. All other strains were isolated from wound swabs being routinely processed at the Department of Medical Microbiology and Public Health at the UHW. Cultures of VRE and isolates from the wound swabs processed at UHW were kindly provided by Mr Alan Paull, together with information on their identities and antibiotic sensitivities (Table 1). Strains isolated from the WHRU patients were identified using API 20 Strep and API Staph according to the manufacturer's instructions (bioMérieux, Basingstoke, UK).

Antibiotic susceptibilities

Antibiotic resistance profiles were determined using comparative (BSAC 91) methodology (Anon. 1991).

Honey samples

A manuka honey (M109), with non-peroxide activity equivalent to 18% (w/v) phenol (Allen *et al*. 1991), and a pasture honey (Lorimer's pasture), with hydrogen peroxide activity equivalent to 13·7% (w/v) phenol (Allen *et al*. 1991), were used in this study. Artificial honey (100 g) was prepared by dissolving 1·5 g sucrose, 7·5 g maltose, 40·5 g

Table 1 Characteristics of clinical isolates used in this study

	% Strains resistant								
Isolates	*n*	P	AMP	TE	E	W	FD	Met	VA
MRSA	18	100			82		0*	100	
VSE-*Enterococcus faecalis*	7	100		43	14	100			0
VRE-*Enterococcus avium*	1								100
VRE-*Enterococcus faecalis*	3		30	100	100	100			100
VRE-*Enterococcus faecium*	15		90	70	80	80			100
VRE-*Enterococcus raffinosus*	1		100	100	100	0			100

P, Penicillin; AMP, ampicillin; TE, tetracycline; E, erythromycin; W, trimethoprim; FD, fusidic acid; Met, methicillin; VA, vancomycin; MRSA, methicillin-resistant *Staphylococcus aureus*; VSE, vancomycin-sensitive enterococci; VRE, vancomycin-resistant enterococci; *n*, number of strains.
*One strain was moderately sensitive.

fructose and 33·5 g glucose in 17 ml sterile deionized water. This solution represents the proportions of the four predominant sugars in natural honey samples.

Minimum inhibitory concentration of honey

Assuming a density of honey as 1·37 g ml^{-1}, honey was weighed out and dissolved in sterile deionized water to prepare a stock solution of 20% (v/v) honey immediately before use. Further dilutions were prepared by adding honey and sterile deionized water to sterile 10-ml volumes of molten double-strength nutrient agar (Oxoid) at 50°C and pouring immediately to produce a range of plates containing honey at 1% (v/v) intervals between 0 and 10% (v/v). Plates were dried at 37°C for 15 min before use. Undiluted overnight broth cultures of MRSA (in nutrient broth; Oxoid; 37°C) and enterococci (in Todd Hewitt broth; Oxoid; 37°C) were inoculated onto dried honey-containing plates as 0·3-μl spots using a multipoint inoculator (Mast Diagnostics, Bootle, UK). Plates were incubated at 37°C for 24 h before visual assessment. Reference strains *Staph. aureus* NCTC 6571 and *Escherichia coli* NCTC 10418 were used to assure consistency. For artificial honey, a range between 12 and 30% (v/v) in nutrient agar was similarly prepared. Two or three replicate plates were used at each concentration of honey and the experiment was repeated at least twice.

RESULTS

Characteristics of clinical isolates

The identities and antibiotic sensitivities of the bacteria utilized in this study are presented in Table 1.

Susceptibility of methicillin-resistant *Staphylococcus aureus* to honey

The minimum inhibitory concentration (MIC) values for the MRSA strains were found to be remarkably consistent (Table 2). None of the strains were inhibited by 30% (v/v) artificial honey in nutrient agar, which is the highest concentration achievable in this assay. The MIC values of both manuka and pasture honey were between 2·7 and 4% (v/v) and most were 3% (v/v).

Susceptibility of vancomycin-sensitive enterococci to honey

The MICs of the honeys tested against VSE showed very little variation between strains (Table 3). Unlike the MRSA, a mean MIC value for artificial honey against all strains of VSE was obtained and manuka honey gave lower MIC values than pasture honey.

Table 2 Minimum inhibitory concentration (% v/v)* of honey for methicillin-resistant *Staphylococcus aureus* (MRSA) isolated from infected wounds

Strain	Artificial honey	Manuka honey	Pasture honey
MRSA 1	>30 (8)	3 ± 0 (9)	3 ± 0 (6)
MRSA 2	>30 (8)	3 ± 0 (9)	3 ± 0 (6)
MRSA 3	>30 (8)	3 ± 0 (9)	3 ± 0 (6)
MRSA 4	>30 (8)	3 ± 0 (9)	3·2 ± 0·4 (6)
MRSA 5	>30 (8)	3 ± 0 (9)	3 ± 0 (6)
MRSA 6	>30 (8)	3 ± 0 (9)	3 ± 0 (6)
MRSA 7	>30 (8)	3 ± 0 (9)	3 ± 0 (6)
MRSA 8	>30 (8)	3 ± 0 (9)	3 ± 0 (6)
MRSA 9	>30 (8)	3 ± 0 (9)	3 ± 0 (6)
MRSA 10	>30 (8)	3 ± 0 (9)	3 ± 0 (6)
MRSA 11	>30 (8)	3 ± 0 (9)	3 ± 0 (6)
MRSA 12	>30 (8)	3 ± 0 (9)	3 ± 0 (6)
MRSA 13	>30 (8)	3 ± 0 (9)	4 ± 0 (6)
MRSA 14	>30 (8)	3 ± 0 (9)	3 ± 0 (6)
MRSA 15	>30 (8)	3 ± 0 (9)	3 ± 0 (6)
MRSA 16	>30 (8)	3 ± 0 (9)	3 ± 0 (6)
MRSA 17	>30 (8)	2·7 ± 0·5 (9)	3 ± 0 (6)
MRSA 18	>30 (8)	3 ± 0 (9)	3·2 ± 0·4 (6)
Mean for all strains†	>30 (144)	2·98 ± 0·14 (162)	3·07 ± 0·26 (108)

*The number in parentheses is the number of determinations totalled for all strains.

†The values shown are means of replicate determinations ± S.D. with the number of replicate assays given in parentheses.

Table 3 Minimum inhibitory concentration (% v/v)* of honey for vancomycin-sensitive enterococci isolated from infected wounds

Strain	Artificial honey	Manuka honey	Pasture honey
Enterococcus faecalis 1	29·5 ± 0·5 (2)	5 ± 0 (7)	9·66 ± 0·46 (6)
Enterococcus faecalis 2	29·6 ± 0·55 (5)	5 ± 0 (7)	9·66 ± 0·46 (6)
Enterococcus faecalis 3	29·75 ± 0·5 (4)	4·66 ± 0·49 (7)	9·66 ± 0·46 (6)
Enterococcus faecalis 4	29·75 ± 0·5 (4)	4·66 ± 0·49 (7)	9·66 ± 0·46 (6)
Enterococcus faecalis 5	29·75 ± 0·5 (4)	5 ± 0 (7)	9·66 ± 0·46 (6)
Enterococcus faecalis 6	29·75 ± 0·5 (4)	5 ± 0 (7)	9·66 ± 0·46 (6)
Enterococcus faecalis 7	29·75 ± 0·5 (4)	5 ± 0 (7)	9·66 ± 0·46 (6)
Mean for all strains†	29·7 ± 0·47 (27)	4·92 ± 0·28 (49)	9·66 ± 0·46 (42)

*The number in parentheses is the number of determinations totalled for all strains.
†The values shown are means of replicate determinations ± S.D. with the number of replicate assays given in parentheses.

Susceptibility of vancomycin-resistant enterococci to honey

The sensitivity of strains of VRE to honey (Table 4) was similar to that of VSE. The mean MIC for artificial honey against all strains of VRE was 28·75% (v/v), whereas the mean MIC values for manuka and pasture honey were 4·61 and 8·25% (v/v), respectively.

DISCUSSION

Several authors are of the opinion that the sugar content of honey is exclusively responsible for its antibacterial effect (Seymour and West 1951; White *et al*. 1963; Keast-Butler 1980; Mossel 1980; Bose 1982; Chirife *et al*. 1983; Green 1988; Somerfield 1991; Tovey 1991; Condon 1993) but the MIC values obtained in this study demonstrate that two natural honeys of median levels of potency were significantly more effective in inhibiting MRSA, VSE and VRE in *in vitro* tests than an artificial honey solution. *Staphylococcus aureus* is the most osmotolerant bacterium capable of causing wound infection (Chirife *et al*. 1983), with 29% (v/v) sugar solutions required to prevent growth (Molan 1992a). Here, 30% (v/v) artificial honey incorporated into nutrient agar failed to prevent the growth of 18 strains of MRSA, whereas manuka and pasture honey at least 10 times more dilute than

Table 4 Minimum inhibitory concentration (% v/v)* of honey for vancomycin-resistant enterococci isolated from hospital environmental surfaces

Strain	Artificial honey	Manuka honey	Pasture honey
Enterococcus avium	27·7 ± 1·5 (7)	3·83 ± 0·4 (12)	5·60 ± 0·5 (10)
Enterococcus faecalis 1	29·2 ± 0·45 (5)	4·77 ± 0·4 (9)	9·38 ± 0·5 (8)
Enterococcus faecalis 2	29·8 ± 0·45 (5)	4·00 ± 0 (12)	9·25 ± 0·9 (8)
Enterococcus faecalis 3	29·5 ± 0·7 (2)	5·00 ± 0 (7)	9·66 ± 0·5 (6)
Enterococcus faecium 1	29·2 ± 0·45 (5)	4·88 ± 0·3 (9)	8·00 ± 0 (8)
Enterococcus faecium 2	29·4 ± 0·54 (5)	4·86 ± 0·4 (7)	8·50 ± 0·5 (6)
Enterococcus faecium 3	29·2 ± 0·40 (6)	4·77 ± 0·4 (9)	8·50 ± 0·5 (8)
Enterococcus faecium 4	28·4 ± 1·13 (7)	4·50 ± 0·5 (12)	7·50 ± 0·5 (10)
Enterococcus faecium 5	29·2 ± 0·45 (5)	4·77 ± 0·4 (9)	8·88 ± 0·4 (8)
Enterococcus faecium 6	29·3 ± 0·5 (4)	4·86 ± 0·4 (7)	8·0 ± 0 (6)
Enterococcus faecium 7	29·0 ± 0 (2)	5·00 ± 0 (7)	8·33 ± 0·8 (6)
Enterococcus faecium 8	28·2 ± 1·6 (6)	4·66 ± 0·5 (9)	9·00 ± 0 (8)
Enterococcus faecium 9	29·3 ± 0·57 (3)	4·77 ± 0·4 (9)	8·38 ± 0·5 (8)
Enterococcus faecium 10	29·5 ± 0·55 (6)	4·88 ± 0·3 (9)	8·00 ± 0 (8)
Enterococcus faecium 11	29·3 ± 0·52 (6)	4·86 ± 0·4 (7)	9·16 ± 0·4 (6)
Enterococcus faecium 12	28·2 ± 0·95 (7)	4·42 ± 0·5 (12)	7·70 ± 0·5 (10)
Enterococcus faecium 13	28.3 ± 0·95 (7)	4·66 ± 0·5 (12)	7·70 ± 0·7 (10)
Enterococcus faecium 14	29·4 ± 0·54 (5)	4·83 ± 0·4 (12)	8·88 ± 0·4 (8)
Enterococcus faecium 15	28·6 ± 1·5 (5)	4·08 ± 0·3 (12)	7·80 ± 0·4 (10)
Enterococcus raffinosus	29·2 ± 0·45 (5)	4·86 ± 0·4 (7)	9·00 ± 0 (6)
Mean for all strains†	28·9 ± 0·99 (103)	4·61 ± 0·51 (189)	8·25 ± 1·03 (158)

*The number in parentheses is the number of determinations totalled for all strains.
†The values shown are means of replicate determinations ± S.D. with the number of replicate assays given in parentheses.

artificial honey prevented growth (Table 2). Similarly, a mean concentration of artificial honey above 28% (v/v) was required to inhibit enterococci, whereas manuka and pasture honeys achieved equivalent inhibitory effects at concentrations six and three times more dilute, respectively (Tables 3 and 4). The antibacterial activity of these natural honeys was, therefore, undoubtedly not attributable to sugar content alone. Variability in the composition of honey is expected (White 1979), but the osmolarity of the honeys used in this study were shown to be similar using a freezing-point osmometer.

The mode of action of honey has not yet been fully elucidated, but osmolarity, acidity, hydrogen peroxide generation and phytochemical components are considered important (Molan 1992a). In undiluted honey, the osmolarity and acidity undoubtedly limit bacterial growth. When many honeys are diluted, a bee-derived enzyme (glucose oxidase) present in the honey is activated and catalyses the slow generation of hydrogen peroxide which inhibits bacterial growth (White *et al*. 1963). This activity varies markedly from honey to honey (Molan 1992b). Generally, the phytochemical components make only a minor contribution to the antibacterial activity of honey but, for a few honeys (e.g. manuka honey), unidentified phytochemical compounds make a major contribution (Molan 1992b). In the present study, MRSA was found to be equally sensitive to a hydrogen peroxide honey (pasture honey) and a non-peroxide honey (manuka honey); enterococci were more sensitive to manuka than pasture honey. Because hydrogen peroxide may be degraded by catalase, an enzyme present in both body tissues and serum, manuka honey has been preferred for clinical use. In practice when undiluted honey is applied to wounds, it is diluted by exudate and its antimicrobial activity at low concentrations is, therefore, crucial. For clinical use, the selection of honeys with high levels of antibacterial activity is indicated to maximize therapeutic effects.

Comparisons between the sensitivity to honey of VSE and VRE showed no substantial differences: mean MIC values with manuka honey were 4·9 and 4·7% (v/v) and with pasture honey 9·7 and 8·4% (v/v), respectively (Tables 3 and 4). The emergence of enterococci as significant human pathogens (Morrison *et al*. 1997), their increased prevalence in nosocomial infections and the development of vancomycin-resistant strains increase the necessity to limit their presence in wounds. Furthermore, the possibility that vancomycin resistance may be transferred to MRSA cannot be ignored.

The MRSA strains were more sensitive to manuka and pasture honeys than were either VSE or VRE. The mean MIC values of manuka and pasture honey against MRSA (2·98 and 3·1% v/v, respectively; Table 2) were close to those previously determined for *Staph. aureus* (2·88 and 3·79% v/v, respectively) using honeys of similar potency (Cooper *et al*. 1999). In the previous study, the methicillin sensitivity of *Staph. aureus* strains was not reported (Cooper *et al*. 1999) but a recent review of those isolates has revealed that 56 of the 58 strains were methicillin-sensitive strains (MSSA). Although the honey samples used in the two studies were not identical, they were similar in their level of antibacterial activity against *Staph. aureus* ATCC 25923. (The manuka honey had non-peroxide antibacterial activity equivalent to 18% phenol in the present study compared with 13·2% phenol in the previous study; the pasture honey had antibacterial activity due to hydrogen peroxide equivalent to 13·7% phenol compared with 14·8% in the previous study.) Thus, the MIC values determined with the MRSA strains in this study and those reported for the MSSA strains of our former study (Cooper *et al*. 1999) indicate that there is not much difference in sensitivity to honey between methicillin-sensitive and methicillin-resistant staphylocoocci. Hence, honey has potential in the decontamination of wounds colonized by antibiotic-resistant strains of bacteria.

Generally, *in vitro* tests provide only an indication of the dilution capacity of an antimicrobial agent and do not assure that such potency will persist *in vivo*. Daily topical application of honey to infected wounds, however, has been reported to achieve wound sterility within 7–10 d (Armon 1980; Efem 1988). Eradication of MRSA from colonized wounds of two patients has recently been reported (Dunford *et al*. 2000; Natarajan *et al*. 2001) and the MRSA strain no. 18 used in this study was isolated from one of those cases (Natarajan *et al*. 2001). Hence, for one strain of MRSA *in vitro* sensitivity to active manuka honey did reflect effective inhibition *in vivo*. It is imperative that this single observation be validated by testing the effectiveness of manuka honey in a much larger cohort of MRSA-colonized patients and that this treatment be compared with the effectiveness of conventional topical antimicrobial agents in blinded randomized clinical trials.

The presence of MRSA in a wound is always a matter of concern and MRSA-colonized wounds are an increasingly urgent problem in hospitals (Morgan *et al*. 2000), nursing homes (Fraise *et al*. 1997) and in the community (Cookson 2000). Their management consumes significant NHS materials and staff time and often erodes patients' morale and relatives' patience. Unsuccessful attempts to eradicate MRSA may lead to increased long-term carriage in patients, with increased risk of cross-infection and hospital-acquired infection (MacKinnon and Allen 2000). The continued emergence of strains with patterns of multiple resistance to systemic and topical antibiotics, or even to disinfectants and antiseptics (Suller and Russell 1999), exacerbates these difficulties. The potential of some unconventional remedies, such as tea tree oil, has been explored by *in vitro* (Carson *et al*. 1995) and *in vivo* (Caelli *et al*. 2000) studies. Any

possible remedy that is cheap, non-toxic and unlikely to select for further antibiotic-resistant strains merits investigation, and honey seems to be in this category.

The findings of this study, together with two previous studies (Cooper and Molan 1999; Cooper *et al*. 1999), show that honey offers promise as an effective wound antiseptic, with broad spectrum antimicrobial activity. Unlike the use of antibiotics in treating wounds, laboratory evaluation of susceptibility to honey would not be necessary before the commencement of treatment. Also, honey does not adversely affect human tissue (Molan 1998), unlike other topical antimicrobial agents (Ward and Saffle 1995). Not only has it the potential to limit the growth of wound pathogens, but there is evidence that honey has the potential to promote healing (Molan 1999; Tonks *et al*. 2001). No other antimicrobial agent possesses these characteristics.

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