Eur J Clin Microbiol Infect Dis
DOI 10.1007/s10096-008-0630-3

ARTICLE

The in vitro susceptibility of *Campylobacter* spp. to the antibacterial effect of manuka honey

S. M. Lin · P. C. Molan · R. T. Cursons

Received: 5 May 2008 / Accepted: 10 September 2008

Abstract We report the antimicrobial effect of manuka honey against *Campylobacter* spp. isolated by a diagnostic laboratory from specimens from a community in New Zealand. The isolates were differentiated according to species level using multiplex PCR. *C. jejuni* (20 strains) and *C. coli* (7 strains) were identified. The clinical isolates identified and type culture collection strains of these species were subjected to testing to determine the minimum inhibitory concentration (MIC) of manuka honey using a microdilution technique. The MIC of the manuka honey against all of the *Campylobacter* tested was found to be around 1% (v/v) honey. The low MIC values suggest that honey might still inhibit the growth of campylobacteria after dilution by fluid in the gut, but the actual concentration of honey that can be achieved in the intestine is unknown. Therefore, clinical investigation is required to establish the efficacy of honey against *Campylobacter* spp. in the gut environment.

Introduction

Campylobacter spp. is a widespread zoonotic pathogen and has been recognised as a leading cause of gastroenteritis worldwide. The prevalence of campylobacteriosis has been reported to outnumber that of enteritis caused by other common food-borne pathogens such as *Salmonella* spp. or *Escherichia coli* in several developed and developing countries [1, 2]. New Zealand has the highest prevalence of campylobacteriosis in the developed world [3].

Campylobacter spp. is fastidious in respect of nutrition and atmosphere; therefore, strict growth conditions are required for survival, although *Campylobacter* spp. have an extremely low infectious dose of 500 cells [4, 5]. Mostly, campylobacteriosis is self-limited, and it can be treated with antibiotics such as fluoroquinolones. However, deaths have been reported occasionally [6, 7] and its linkage to Guillain-Barré syndrome [8] and abortion [9] is also of great concern. Furthermore, although not reported yet in New Zealand, antibiotic-resistant strains have been reported in developed and developing countries [10–12]. The increasing rate of resistance to antibiotics is thought to be due to the over-use of antibiotics in veterinary treatment [13].

A clinical trial has been conducted in which it was found that administration of honey halved the duration of bacterial diarrhoea [14] and although in that clinical report the function of re-hydration was emphasised, the easing of the symptoms may also have been due to the antibacterial activity of honey, since honey shortened the duration of bacterial diarrhoea, but not that of viral diarrhoea. Honey has been used as a treatment for wound infections since ancient times [15], and has been found to inhibit the growth of a wide range of bacterial species in vitro [16]. However, there have been very few studies testing the efficacy of honey against the widespread *Campylobacter* spp. Although Adebolu reported the effect of two types of African honey on diarrhoea-causing bacteria, including one strain of *C. jejuni* [17], there were several shortcomings in that report that may cast doubt upon the reliability of the results published. From that report it is not known whether or not other strains or species of *Campylobacter* had the same

S. M. Lin (✉) · P. C. Molan
Honey Research Unit, Department of Biological Sciences,
University of Waikato,
Hamilton, New Zealand
e-mail: semin2006@gmail.com

R. T. Cursons
Molecular Genetics Laboratory,
Department of Biological Sciences, University of Waikato,
Hamilton, New Zealand

sensitivity to honey. Also in that report, Adebolu used the agar diffusion method with nutrient media [17], which may not be suitable for testing the sensitivity of slow-growing bacteria like *Campylobacter* spp. against honey, as the honey may have diffused out into the agar to a level below the MIC by the time the organism had grown. But most importantly, in that paper tests were carried out with types of honey whose antimicrobial potency had not yet been determined; yet, the potency of antibacterial activity in honey may in fact vary up to 100-fold [18], and the reported sensitivity of the strain of *C. jejuni* to Adebolu's honey could have been one hundred times higher or lower than the sensitivity to any other randomly chosen honey on the market.

A few types of honey, such as manuka honey from *Leptospermum scoparium* in New Zealand, are reported to have particularly high antimicrobial activity against various bacterial species [19]. Manuka honey is coming into widespread usage for the treatment of infected wounds [20]. Therefore, the objectives of this study were to investigate the antibacterial activity of manuka honey against a number of clinical isolates of *Campylobacter* spp. from clinical patients with diarrhoea using the broth dilution method. The manuka honey we used had its antimicrobial activity standardised against a reference antiseptic, phenol. To distinguish the effect of the antibacterial component of honey from any osmotic effects, artificial honey, which was syrup simulating the sugar composition of honey, was also used for comparison.

Materials and methods

Honey samples

The manuka honey used in this work had the strength of its antibacterial activity assayed by the method described by Allen et al. with catalase added [19]. This is an agar well diffusion assay that compares the activity of honey with that of a standard antiseptic phenol. The manuka honey used had activity equivalent to that of 29.4% phenol when tested against *Staphylococcus aureus* ATCC 25923. Artificial honey was made up, containing 30.5% glucose, 37.5% fructose and 1.5% sucrose, and was dissolved in distilled water [21]. The two types of honey were stored in the dark at 4°C until used.

Microbiological materials

Campylobacter spp. is widely known as a fastidious pathogen and requires strict control of growth conditions. The National Committee for Clinical Laboratory Standards (NCCLS) has suggested an outline for investigating the susceptibility of *Campylobacter* to antibiotics [22]; nonetheless, a "gold standard" protocol for studying this genus does still not exist [23]. For instance, the agar dilution method using Mueller–Hinton agar supplemented with 5% defibrinated sheep blood is recommended in the outline where the blood is added to the medium to protect *Campylobacter* spp. from damage by oxygen-derived components such as free radicals and hydrogen peroxide [24, 25]. However, it is not applicable in this study because the antibacterial activity of manuka honey may be partially due to hydrogen peroxide [16, 26], which would be inactivated by catalase present in blood. Instead, freshly made Mueller–Hinton broth was used in the susceptibility test. Blood-free *Campylobacter* selective agar (Oxoid) containing amphotericin and cefoperazone (LAB M) as selective agents was used to culture the isolates. Brain heart infusion yeast extract broth (BHIYE, with 0.6% yeast extract) supplemented with FBP (0.025% ferrous sulfate, 0.025% sodium metabisulfite and 0.025% sodium pyruvate) [27, 28] was used for enrichment, and that containing 15% sterile glycerol was used as a cryopreservative agent.

Campylobacter samples

Campylobacter clinical isolates were kindly provided by Chris Picket (Medlab, Hamilton, New Zealand)) and were stored in fastidious anaerobe transport swabs (Copan Italia, Brescia, Italy) when transporting them from Medlab to the Honey Research Unit. The isolates were then streaked on selective agar plates and cultured at 37°C in a micro-aerobic atmosphere generated with the spirits burn method [29] for 2 days. The cultures recovered were enriched in BHIYE-FBP and incubated overnight micro-aerobically as above, then dispensed into small vials containing cryopreservative agent and stored at −70°C. Type culture collection strains *C. jejuni* (ATCC 33560) and *C. coli* (ATCC 33559) were also processed in this way as growth controls.

As Medlab only differentiates the isolates to genus level, extra differentiation work to species level was needed for investigating the effect of manuka honey on different species of *Campylobacter*. In this research the multiplex polymerase chain reaction was used to do this [30].

Campylobacter DNA extraction

Campylobacter DNA was extracted by boiling. A loopful of colony for each isolate was taken from its culture plates, resuspended in 100 µl of distilled water, heated in a boiling water bath for 10 min and chilled on ice for another 10 min, followed by the addition of 100 µl of chloroform and brief centrifuging. The supernatant was stored at −20°C until the PCR test was carried out.

Multiplex PCR

Each PCR mix (20 µl) consisted of 6 µl of DNA templates, 2.4 µl of 20 µmol/l primers mix (Sigma), 8 µl of HotMasterMix (×2.5; Eppendorf) and 3.6 µl of MilliQ water. The primers used in this work are shown in Table 1.

The DNA amplification procedure was carried out in a PTC-100 thermocycler (MJ Research, Waltham, MA, USA). The cycling conditions used were 94°C for 2 min as initial denaturation, followed by 30 cycles of amplification (denaturation at 95°C for 30 s, annealing at 59°C for 20 s, extension at 68°C for 40 s) and 68°C for 6 min for the final extension. The amplified products were electrophoresed in 1.5% agarose gel and analysed using the ScionImage system.

Susceptibility test

Inoculum preparation

Each isolate was recovered by rubbing the surface of the frozen culture with a sterilised cotton swab, then streaking onto blood-free *Campylobacter* selective agar and incubating micro-aerobically for 48 h at 37°C. The colonies recovered were collected with a cotton swab and suspended in Mueller–Hinton broth. The optical density at 625 nm was adjusted to 0.08 with fresh broth and was then further diluted 500-fold. This gave a final culture density of approximately 10^5 cfu/ml after inoculating the honey solution in the microplate wells. The inoculum density was confirmed using the track dilution method [31].

Susceptibility test

A 10% (v/v) solution of manuka honey and 20% (v/v) artificial honey were prepared with Mueller–Hinton broth and filter-sterilised with a 0.2-µm filter (Sartorius) before serial dilution. As the MIC of artificial honey would presumably be higher than that of manuka honey, 20% (v/v) solution of artificial honey was used in this test.

Of the 12 columns in a microplate, the first column was added with 40 µl of manuka honey, the second to the tenth with 40 µl of Mueller–Hinton broth and the last two with a growth control (*Campylobacter* spp. and Mueller–Hinton broth added) and sterility check (plain Mueller–Hinton broth). For serial dilution 160 µl of honey was added into the second column, which was then sequentially transferred to the following wells up to the tenth well. After that, 80 µl of inoculum was added into each well except the last well, in which 80 µl of plain Mueller–Hinton broth was added instead so that the final concentrations of the honey were 3.33%, 2.66%, 2.13%, 1.70%, 1.36%, 1.09%, 0.87%, 0.7%, 0.56% and 0.45% after inoculation. The final concentrations of artificial honey would be twice of those of manuka honey.

The plate was incubated micro-aerobically at 37°C for 48 h. The lowest concentration of honey needed to completely inhibit the growth of the isolate was considered to be its MIC. After this, from each well, 1 µl was subcultured onto blood-free *Campylobacter*-selective agar to see if the honey was bacteriostatic or bacteriocidal to *Campylobacter* spp. The cultures in the growth control wells were also subcultured as positive controls. The susceptibility test for each species was replicated up to five times. The difference between the two types of honey in the results was analysed using the Wilcoxon test in the statistical package R (http://www.r-project.org) [32].

Results

Multiplex PCR identification

According to the multiplex PCR, of the 27 clinical isolates collected from Medlab, 20 were identified as *C. jejuni* and the rest as *C. coli*.

Susceptibility test

The susceptibility test revealed that the growth of all 29 species was largely inhibited by both manuka honey and artificial honey (Table 2). For both *C. jejuni* and *C. coli*, the MIC of manuka honey was significantly lower than that of artificial honey ($P<0.01$). The MIC of manuka honey ranged from 0.8% to 1.1%, whereas that of artificial honey was 3–4 times higher than that of manuka honey (3.1–4.3%).

The subculturing after determining the MIC showed that growth occurred when subculturing from concentrations of honey below the MIC, whereas there was no growth from

Table 1 Oligonucleotide primers and their amplicon sizes used in this study [30]

Species	Target gene	Sequence (5′-3′)	GeneBank accession no.	Amplicon size (bp)
C. jejuni	*C. jejuni* hipO	Forward: ACTTCTTTATTGCTTGCTGC Reverse: GCCACAACAAGTAAAGAAGC	Z36940	323
C. coli	*C. coli* glyA	Forward: GTAAAACCAAAGCTTATCGTG Reverse: TCCAGCAATGTGTGCAATG	AF136494	126

Table 2 Minimum inhibitory concentration (percentage v/v) of manuka honey and artificial honey for each strain

Strains	Manuka honey	Artificial honey
C. jejuni 1	0.84±0.08	3.13±0.39
C. jejuni 2	1±0.12	3.58±0.38
C. jejuni 3	1±0.12	3.44±0.55
C. jejuni 4	0.8±0.09	3.13±0.39
C. jejuni 5	0.88±0.14	3.58±0.38
C. jejuni 6	1.05±0.1	3.27±0.32
C. jejuni 7	0.92±0.17	3.75±0.47
C. jejuni 8	0.96±0.12	3.75±0.47
C. jejuni 9	1±0.12	3.92±0.47
C. jejuni 10	0.8±0.09	3.58±0.38
C. jejuni 11	0.91±0.1	3.58±0.38
C. jejuni 12	1.05±0.1	3.92±0.47
C. jejuni 13	0.96±0.12	3.92±0.47
C. jejuni 14	0.88±0.14	3.61±0.66
C. jejuni 15	0.96±0.12	3.75±0.47
C. jejuni 16	1.05±0.1	3.75±0.47
C. jejuni 17	0.8±0.09	3.92±0.47
C. jejuni 18	1±0.12	3.92±0.47
C. jejuni 19	0.96±0.12	3.58±0.38
C. jejuni 20	0.92±0.17	3.61±0.66
C. jejuni ATCC 33560	1±0.12	3.58±0.38
C. coli 1	1.05±0.1	4.09±0.38
C. coli 2	1±0.12	4.3±0.68
C. coli 3	1.14±0.12	4.09±0.38
C. coli 4	1.1±0.17	3.92±0.47
C. coli 5	1.2±0.15	3.92±0.47
C. coli 6	1.14±0.12	4.09±0.38
C. coli 7	1.1±0.17	3.92±0.47
C. coli ATCC 33559	1.05±0.1	4.09±0.38
Mean of *C. jejuni* (n=21)	0.94±0.08	3.63±0.24
Mean of *C. coli* (n=8)	1.1±0.06	4.05±0.13

The values are represented as means of the replicates ± standard deviation. The numbers of the replicates are given in parentheses. The determination of the MIC values for each isolate was replicated five times

concentrations at and above the MIC. This revealed that the MIC of either manuka honey or artificial honey was also the minimum bacteriocidal concentration for all of the *Campylobacter* isolates in this study.

Discussion

Although the manuka honey used in the present study had a level of activity twice as high as that of the manuka honey used in other studies published, overall, the average concentration of manuka honey required to inhibit the growth of all the *Campylobacter* spp. tested was still far lower than that required to inhibit most other micro-organisms with manuka honey [33–38]. Although the data obtained from this study cannot fully represent the profile of the genus *Campylobacter*, our results establish that the species tested are susceptible to both the antibacterial components and the osmolarity of manuka honey. Manuka honey has been reported to be highly effective against various pathogens, including methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant enterococci (VRE) [34], and its low pH, low water activity, slowly released hydrogen peroxide and phytochemical antimicrobial components are collectively thought to be responsible for its high efficacy against bacteria [26]. The result in this work revealed that the efficacy is also observable on *Campylobacter*, regardless of strain.

In this study we also observed that even a low concentration sugar solution was effective against the isolates, which may suggest that *Campylobacter* spp. would be highly susceptible to osmolarity. Doyle [39] reported that *C. jejuni* could grow in brucella broth containing 1.5% of NaCl, but failed in 2.0% NaCl or greater, and although a large amount of *C. jejuni* (10^5–10^6 cfu/ml) may increase the tolerance in 6.5% salt at 4°C, the viable cells significantly decreased in 4.5% salt at room temperature. In that report Doyle showed that nalidixic acid-resistant thermophilic *Campylobacter* (NARTC) was generally tolerant to salt concentration; yet, it was still unable to grow in the presence of 2.5% NaCl. Doyle also noted that a few strains would adapt to up to 6.5% NaCl after frequent subculturing and claimed that osmolarity might not be ideal for inhibiting the growth of *Campylobacter* spp., but this increasing tolerance against osmotic solution was not observed in our studies. Interestingly, Reezal et al. [40] noted that the osmotic effect on *Campylobacter* was seen regardless of whether the osmolyte in the medium was glucose or salts. Accordingly, the high susceptibility of *Campylobacter* spp. to honey solutions observed in this study may be due in part to the osmotic effect of the sugar content as well as to other antimicrobial factors.

The high susceptibility of *Campylobacter* spp. to osmolarity, however, may not be of practical consequence from an antimicrobial viewpoint. The concentration of sugar in the gut would decline rapidly down below the effective dosage through absorption and may not inhibit the growth of *Campylobacter* spp. in the gut. Sugar is usually used for oral rehydration therapy or as immediate treatment for hypoglycaemia due to its rapid absorption through intestinal villi [41]. Therefore, dietary sugar is unlikely to contribute to the inhibition of campylobacteriosis. At this stage it is not known whether the phytochemical antibacterial component of manuka honey [42, 43] would be absorbed in a short time or would remain in the gut to inhibit bacterial growth after honey has been ingested. It

would be of interest to investigate in the future whether or not this component is absorbed in the gut.

In short, of the *Campylobacter* spp. isolates most were identified as *C. jejuni* and *C. coli*, and these were found to be sensitive to the types of honey used in this work. An unspecified type of honey with unknown antibacterial potency [14] has been reported to ease the symptoms of bacterial diarrhoea, and the findings in the present study on the susceptibility of *Campylobacter* spp. to manuka honey also suggest that honey might be useful for treating bacterial diarrhoea.

Acknowledgements We thank Chris Pickett and the staff of Medlab, Hamilton, New Zealand, for advice and for the provision of the campylobacteria cultures.

References

1. Allos BM (2001) *Campylobacter jejuni* infections: update on emerging issues and trends. Clin Infect Dis 32(8):1201–1206 doi:10.1086/319760
2. Meldrum RJ, Smith RM, Wilson IG (2006) Three-year surveillance program examining the prevalence of *Campylobacter* and *Salmonella* in whole retail raw chicken. J Food Prot 69(4):928–931
3. Institute of Environmental Science and Research (2006) Notifiable and other diseases in New Zealand—annual report 2005. Population and Environmental Health Group, Institute of Environmental Science and Research, pp 1–56
4. Robinson DA (1981) Infective dose of *Campylobacter jejuni* in milk. Br Med J (Clin Res Ed) 282:1584
5. Wallis MR (1994) The pathogenesis of *Campylobacter jejuni*. Br J Biomed Sci 51(1):57–64
6. Meyer A, Stallmach T, Goldenberger D, Altwegg M (1997) Lethal maternal sepsis caused by *Campylobacter jejuni*: pathogen preserved in placenta and identified by molecular methods. Mod Pathol 10(12):1253–1256
7. Peetermans WE, De Man F, Moerman P, van de Werf F (2000) Fatal prosthetic valve endocarditis due to *Campylobacter fetus*. J Infect 41(2):180–182 doi:10.1053/jinf.2000.0699
8. Ang CW, Jacobs BC, Laman JD (2004) The Guillain-Barré syndrome: a true case of molecular mimicry. Trends Immunol 25 (2):61–66 doi:10.1016/j.it.2003.12.004
9. Smith JL (2002) *Campylobacter jejuni* infection during pregnancy: long-term consequences of associated bacteremia, Guillain-Barré syndrome, and reactive arthritis. J Food Prot 65(4):696–708
10. Delsol AA, Sunderland J, Woodwared MJ, Pumbwe L, Piddock LJV, Roe JM (2004) Emergence of fluoroquinolone resistance in the native *Campylobacter coli* population of pigs exposed to enrofloxacin. J Antimicrob Chemother 53:872–874 doi:10.1093/jac/dkh150
11. Padungton P, Kaneene JB (2003) *Campylobacter* spp in human, chickens, pigs and their antimicrobial resistance. J Vet Med Sci 65 (2):161–170 doi:10.1292/jvms.65.161
12. Takayama S, Satake S, Ishihara K (2005) Antimicrobial susceptibility of *Campylobacter jejuni* and *Campylobacter coli* isolated from human diarrheic samples. Kansenshogaku Zasshi 79(3):169–175
13. van Boven M, Veldman KT, de Jong MCM, Mevius DJ (2003) Rapid selection of quinolone resistance in *Campylobacter jejuni* but not in *Escherichia coli* in individually housed broilers. J Antimicrob Chemother 52(4):719–723 doi:10.1093/jac/dkg402
14. Haffejee IE, Moosa A (1985) Honey in the treatment of infantile gastroenteritis. Br Med J (Clin Res Ed) 290 (6485):1866–1867
15. Rolfe RD (2000) The role of probiotic cultures in the control of gastrointestinal health. J Nutr 130:396S–402S
16. Molan PC (1992) The antibacterial activity of honey. I. The nature of the antibacterial activity. Bee World 73(1):5–28
17. Adebolu TT (2005) Effect of natural honey on local isolates of diarrhea-causing bacteria in southwestern Nigeria. Afr J Biotechnol 4(10):1172–1174
18. Molan PC (1992) The antibacterial activity of honey. II. Variation in the potency of the antibacterial activity. Bee World 73(2):59–76
19. Allen KL, Molan PC, Reid GM (1991) A survey of the antibacterial activity of some New Zealand honeys. J Pharm Pharmacol 43(12):817–822
20. Molan PC, Betts JA (2004) Clinical usage of honey as a wound dressing: an update. J Wound Care 13(9):353–356
21. Shannon IL, Edmonds EJ, Madsen KO (1979) Honey: sugar content and cariogenicity. ASDC J Dent Child 46(1):29–33
22. National Committee for Clinical Laboratory Standards (2002) Performance standards for antimicrobial disk and dilution susceptibility tests for bacteria isolated from animals: approved standard (M31-A2). NCCLS, Wayne, PA
23. Silley P (2003) *Campylobacter* and fluoroquinolones: a bias data set? Environ Microbiol 5(4):219–230 doi:10.1046/j.1462-2920.2003.00425.x
24. Bolton FJ, Coates D, Hutchinson DN (1984) The ability of *Campylobacter* media supplements to neutralize photochemically induced toxicity and hydrogen peroxide. J Appl Bacteriol 56 (1):151–157
25. Corry JEL, Post DE, Laisney MJ (1995) Culture media for the isolation of Campylobacters. Int J Food Microbiol 26:43–76 doi:10.1016/0168-1605(95)00044-K
26. Allen KL, Molan PC, Reid GM (1991) The variability of the antibacterial activity of honey. Apiacta XXVI:114–121
27. George HA, Hoffman PS, Smibert RM, Krieg NR (1978) Improved media for growth and aerotolerance of *Campylobacter fetus*. J Clin Microbiol 8(1):36–41
28. Gorman R, Adley CC (2004) An evaluation of five preservation techniques and conventional freezing temperatures of –20 degrees C and −85 degrees C for long-term preservation of *Campylobacter jejuni*. Lett Appl Microbiol 38:306–310 doi:10.1111/j.1472-765X.2004.01490.x
29. Ribeiro CD, Marks J, Grimshaw AD (1985) Economic cultivation of "thermophilic" *Campylobacter* spp. J Clin Pathol 38(11):1311–1312 doi:10.1136/jcp.38.11.1311
30. Wang G, Clark CG, Taylor TM, Pucknell C, Barton C, Price L et al (2002) Colony multiplex PCR assay for identification and differentiation of *Campylobacter jejuni*, *C. coli*, *C. lari*, *C. upsaliensis*, and *C. fetus* subsp. *fetus*. J Clin Microbiol 40 (12):4744–4747 doi:10.1128/JCM.40.12.4744-4747.2002
31. Jett BD, Hatter KL, Huycke MM, Gilmore MS (1997) Simplified agar plate method for quantifying viable bacteria. Biotechniques 23(4):648–650
32. R Development Core Team (2007) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna
33. Cooper RA, Halas E, Molan PC (2002) The efficacy of honey in inhibiting strains of *Pseudomonas aeruginosa* from infected burns. J Burn Care Rehabil 23(6):366–370 doi:10.1097/00004630-200211000-00002

34. Cooper RA, Molan PC, Harding KG (2002) The sensitivity to honey of Gram-positive cocci of clinical significance isolated from wounds. J Appl Microbiol 93:857–863 doi:10.1046/j.1365-2672.2002.01761.x
35. French VM, Cooper RA, Molan PC (2005) The antibacterial activity of honey against coagulase-negative staphylococci. J Antimicrob Chemother 56(1):228–231 doi:10.1093/jac/dki193
36. Natarajan S, Williamson D, Grey J, Harding KG, Cooper RA (2001) Healing of an MRSA-colonized, hydroxyurea-induced leg ulcer with honey. J Dermatolog Treat 12:33–36 doi:10.1080/095466301750163563
37. Al Somal N, Coley KE, Molan PC, Hancock BM (1994) Susceptibility of *Helicobacter pylori* to the antibacterial activity of manuka honey. J R Soc Med 87:9–12
38. Cooper RA, Molan PC, Harding KG (1999) Antibacterial activity of honey against strains of *Staphylococcus aureus* from infected wounds. J R Soc Med 92(6):283–285
39. Doyle MP, Roman DJ (1982) Response of *Campylobacter jejuni* to sodium chloride. Appl Environ Microbiol 43(3):561–565
40. Reezal A, McNeil B, Anderson JG (1998) Effect of low-osmolality nutrient media on growth and culturability of *Campylobacter* species. Appl Environ Microbiol 64(12):4643–4649
41. Cuccurullo SJ (2004) Physical medicine and rehabilitation board review. Demos Medical Publishing, New York
42. Adams CJ, Boult CH, Deadman BJ, Farr JM, Grainger MN, Manley-Harris M et al (2008) Isolation by HPLC and characterisation of the bioactive fraction of New Zealand manuka (*Leptospermum scoparium*) honey. Carbohydr Res 343(4):651–659 doi:10.1016/j.carres.2007.12.011
43. Mavric E, Wittmann S, Barth G, Henle T (2008) Identification and quantification of methylglyoxal as the dominant antibacterial constituent of Manuka (*Leptospermum scoparium*) honeys from New Zealand. Mol Nutr Food Res 52(4):483–489 doi:10.1002/mnfr.200700282