MAJOR ARTICLE

# Medical-Grade Honey Kills Antibiotic-Resistant Bacteria In Vitro and Eradicates Skin Colonization

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***Background.*** Antibiotic resistance among microbes urgently necessitates the development of novel antimicrobial agents. Since ancient times, honey has been used successfully for treatment of infected wounds, because of its antibacterial activity. However, large variations in the in vitro antibacterial activity of various honeys have been reported and hamper its acceptance in modern medicine.

***Methods.*** We assessed the in vitro bactericidal activity of Revamil (Bfactory), a medical-grade honey produced under controlled conditions, and assessed its efficacy for reduction of forearm skin colonization in healthy volunteers in a within-subject–controlled trial.

***Results.*** With *Bacillus subtilis* as a test strain, we demonstrated that the variation in bactericidal activity of 11 batches of medical-grade honey was <2-fold. Antibiotic-susceptible and -resistant isolates of *Staphylococcus aureus, Staphylococcus epidermidis, Enterococcus faecium, Escherichia coli, Pseudomonas aeruginosa, Enterobacter cloacae,* and *Klebsiella oxytoca* were killed within 24 h by 10%–40% (vol/vol) honey. After 2 days of application of honey, the extent of forearm skin colonization in healthy volunteers was reduced 100-fold ($P < .001$), and the numbers of positive skin cultures were reduced by 76% ($P < .001$).

***Conclusions.*** Revamil is a promising topical antimicrobial agent for prevention or treatment of infections, including those caused by multidrug-resistant bacteria.

Antibiotic-resistant bacteria pose a very serious threat to public health [1, 2]. For all kinds of antibiotics, including the major last-resort drugs, the frequencies of bacterial resistance are increasing worldwide [1, 2]. Even more alarming, very few new antibiotics are being developed, because many large pharmaceutical companies have abandoned the field of antibiotic drug discovery [3]. Therefore, alternative antimicrobial strategies are urgently needed.

Since ancient times, honey has been known to possess antimicrobial properties, as well as wound-healing activity [4–6]. Microbial resistance to honey has never been reported, which makes it a very promising topical antimicrobial agent. Indeed, the in vitro activity of honey against antibiotic-resistant bacteria [7–9] and the reported successful application of honey in the treatment of chronic wound infections that were not responding to antibiotic therapy [5] have attracted considerable attention [10–12].

At present, the evidence that honey is an effective antimicrobial agent is limited. Although the efficacy of honey for treatment of burns and postoperative wounds was demonstrated in randomized, controlled trials, these studies concerned small numbers of patients and/or lacked well-defined end points [13, 14], or honey was used as a last-resort medication because standard treatments had failed [5]. Furthermore, there is large variation in the antimicrobial activity of honeys collected from natural environments [15, 16], which is a concern from the view of clinical applications [14].

Revamil medical-grade honey (Bfactory) is produced by bees in closed greenhouses. We assessed the batch-to-batch reproducibility and the bactericidal spectrum of this medical-grade honey in vitro and tested its ef-

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ficacy in reducing microbial skin colonization in healthy human volunteers.

## MATERIALS AND METHODS

### Honey

Revamil, a CE-marked, standardized, medical-grade honey, was used in all experiments. Concentrations of honey are expressed as the percentage of honey volume per total volume (vol/vol). In the study involving volunteers, graduated $\gamma$-sterilized syringes with Revamil honey were used.

### In Vitro Studies

***Microorganisms.*** Bactericidal activity of medical-grade honey was assessed for the following microorganisms: *Escherichia coli* ML-35 [17], *Pseudomonas aeruginosa* PAO1 (American Type Culture Collection 15692), clinical isolates of *Enterobacter cloacae* and *Klebsiella oxytoca,* extended-spectrum $\beta$-lactamase (ESBL)–producing strains of all the species mentioned above, gentamicin-resistant *E. coli,* methicillin-susceptible and -resistant strains of *Staphylococcus epidermidis* and *Staphylococcus aureus,* and vancomycin-susceptible and -resistant strains of *Enterococcus faecium.*

Oxacillin susceptibility of *S. aureus* and *S. epidermidis* strains and vancomycin susceptibility of *E. faecium* strains were determined by Etest (AB Biodisk; Solna) in accordance with the manufacturer's instructions. ESBLs were identified as described elsewhere [18].

***Microbroth dilution assay.*** Bactericidal activity of honey was quantified with a microbroth dilution assay. Bacteria from logarithmic-phase cultures in trypticase soy broth (Difco) were washed twice with incubation buffer (10 mmol/L phosphate buffer [pH 7.0] and 0.03% wt/vol trypticase soy broth) and were diluted to $5 \times 10^6$ cfu/mL in this buffer on the basis of optical density. Before each experiment, a 50% vol/vol solution of honey (density, 1.4 g/mL) in incubation buffer was freshly prepared. Twenty-microliter aliquots of bacterial suspensions were mixed with 80-$\mu$L aliquots of various concentrations of honey and were incubated on a rotary shaker at 150 rpm at 37°C. At indicated time points, duplicate 10-$\mu$L aliquots of undiluted and 10-fold serially diluted incubation mixtures were spotted on blood agar. The plates were incubated at 37°C and were inspected for growth after 24 h.

### Study of Honey for Treatment of Skin Colonization in Healthy Volunteers

***Study design.*** We investigated the potential of medical-grade honey to decrease microbial skin colonization on the forearms of healthy volunteers. The study protocol was approved by the Academic Medical Center Amsterdam Research Ethics Committee. Forty-two healthy adult volunteers were recruited for the study in September and October 2006. Written informed consent was obtained from all volunteers before inclusion. Exclusion criteria were infectious skin diseases or other skin diseases that can be considered to influence colonization.

Two patches of skin (diameter, 2 cm) on the left forearm were sampled with cotton swabs moistened with 0.9% NaCl (saline). A volume of 0.5 mL of honey was applied to 1 of these patches, covering an area of ~2 cm in diameter. Transparent polyurethane dressing (Tegaderm; 3M Health Care) was applied to the honey-covered and control skin patches. After 2 days, the honey was collected using dry cotton swabs, and the skin patches were sampled using moistened cotton swabs. Skin colonization was defined as the presence of a total of >5 cfu in the collected honey and skin swab.

***Identification of bacterial skin microflora.*** Identification of bacterial isolates from honey-treated skin patches was performed using 16S rRNA gene sequencing. Single colonies were suspended in 100 $\mu$L of demineralized water, and the suspension was boiled for 10 min and was centrifuged at 20,000 *g* for 3 min. Five microliters of the supernatant served as the template for PCR amplification of ~900 bp of the rRNA gene by use of primers P515F and P13B [19], for 25 cycles at 95°C for 1 min, 62°C for 1 min, and 72°C for 2 min, with a final extension for 10 min at 72°C. The PCR products were sequenced with primers P515F and P13B by use of the ABI Prism Big Dye Terminator cycle-sequencing kit, version 2.0 (Applied Biosystems), and overlapping sequences from both strands were aligned and inspected using the software package CodonCode 1.5.2 (CodonCode Corporation). The Ribosomal Database Project II from the Center of Microbial Ecology [20] was used for identification of the bacteria at the genus level. The coagulase tube test was used to discriminate between *S. aureus* and coagulase-negative staphylococci.

***Statistics.*** The percentages of culture-positive skin swabs of honey-treated and control skin patches in the volunteer study

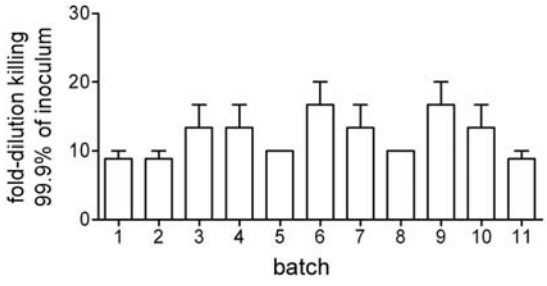


**Figure 1.** Bactericidal activity of 11 batches of Revamil medical-grade honey against *Bacillus subtilis. B. subtilis* was incubated for 2 h in 0%–40% vol/vol honey with 5% incremental steps. The highest dilutions of honey causing a 1000-fold reduction in the number of cfu are indicated as mean values for independent triplicate incubations. Error bars represent SEMs.

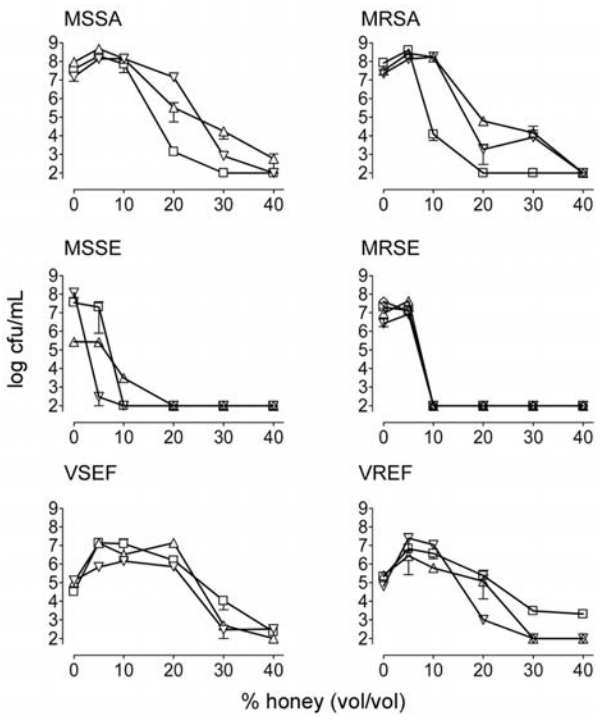


**Figure 2.** Susceptibility of gram-positive bacteria to honey. Methicillin-susceptible *Staphylococcus aureus* (MSSA), methicillin-resistant *S. aureus* (MRSA), methicillin-susceptible *Staphylococcus epidermidis* (MSSE), methicillin-resistant *S. epidermidis* (MRSE), vancomycin-susceptible *Enterococcus faecium* (VSEF), and vancomycin-resistant *E. faecium* (VREF) isolates were exposed to the indicated concentrations of honey for 24 h, after which survival was determined quantitatively as cfu/mL.

were analyzed with McNemar's test for matched case-control studies. Univariate analyses by paired Wilcoxon rank-sum test were performed to investigate the significance of differences in numbers of cfu (GraphPad Prism, version 4.03) [21].

## RESULTS

***Batch-to-batch reproducibility of bactericidal activity of honey.*** We compared the activity of 11 batches of Revamil medical-grade honey in a microdilution assay with *Bacillus subtilis* as the target microorganism. Bactericidal activity varied <2-fold between all batches (figure 1).

***Activity of honey against gram-positive bacteria.*** Antibiotic-susceptible and -resistant strains of *S. aureus, S. epidermidis,* and *E. faecium* were exposed to dilutions of honey (figure 2). Methicillin-susceptible and -resistant *S. aureus* isolates were equally susceptible to honey (figure 2). At 40% vol/vol, honey completely killed the inocula of all isolates tested, and 20% vol/vol honey had potent bactericidal activity against all methicillin-resistant *S. aureus* isolates and 2 of 3 methicillin-susceptible *S. aureus* isolates (figure 2).

*S. epidermidis* was even more susceptible; all isolates tested were completely killed by 10% vol/vol honey. As for *S. aureus* isolates, there were no marked differences in susceptibility between methicillin-susceptible and -resistant *S. epidermidis* isolates (figure 2). For *E. faecium,* incubation in 30% vol/vol honey resulted in a marked reduction in the numbers of cfu for all isolates tested, including antibiotic-susceptible isolates and antibiotic-resistant isolates (figure 2).

***Activity of honey against gram-negative bacteria.*** Nearly all gram-negative bacteria tested, including the ESBL-producing strains, were killed after 24 h of incubation in 20% vol/vol honey (figure 3). For antibiotic-susceptible *E. cloacae* and antibiotic-resistant *K. oxytoca* (ESBL-producing) isolates only, at least 30% vol/vol honey was required to kill the entire inocula (figure 3).

***Reduction of forearm skin colonization by application of honey.*** To determine the potential of honey for topical applications in vivo, we quantitatively determined the capacity of honey to reduce microbial colonization of forearm skin of healthy volunteers. Forty-eight h after application of honey, the median level of skin colonization was reduced 100-fold, compared with control incubations, from 110 cfu/swab in the control group to 1 cfu/swab in the honey-treated group ($P < .001$) (figure 4*A*). The percentage of positive skin cultures, defined as cultures with >5 cfu, was reduced from 79% in the control group to 19% in the honey-treated group ($P < .001$) (figure 4*A*). Within the honey-treated group, median colonization was reduced from 26.5 cfu to 1 cfu ($P < .001$) 48 h after application, whereas, in the control group, the median had increased from 21.5 cfu to 110 cfu ($P < .001$) after a similar incubation period (figure 4*A*). There was a large variation in baseline skin colonization among subjects, ranging from 0 to >1000 cfu/swab, but colonization of control and honey-treated skin patches on the same arm were similar for most of the subjects at the start of the experiment (figure 4*B,* left panel). Forty-eight h after application (figure 4*B,* right panel), 39 of 42 honey-treated patches showed less colonization than did the corresponding untreated control patches on the same arm. After treatment with honey, skin colonization was reduced for 38 of 42 patches, compared with colonization before treatment (figure 4*C,* right panel), whereas, for control skin patches, increased colonization was observed for 30 of 42 patches at 48 h after application of a dressing (figure 4*C,* left panel).

For every subject, all phenotypically distinct bacteria that survived on honey-treated skin patches were isolated and characterized. Of the total 18 isolates, 14 (78%) were identified as coagulase-negative staphylococci, and *Bacillus, Micrococcus, Brevundimonas,* and *Corynebacterium* species were each identified once. In an assessment of their susceptibility to honey in vitro, inocula of all these isolates were killed by ≤30% vol/vol

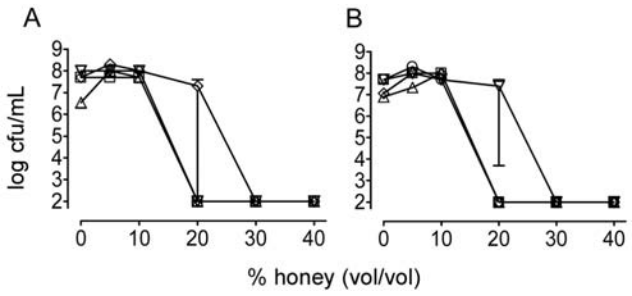


**Figure 3.** Susceptibility of various gram-negative bacteria to honey. Antibiotic-susceptible isolates *(A)* and extended-spectrum β-lactamase–producing isolates *(B)* of *Escherichia coli (squares), Pseudomonas aeruginosa (upward-pointing triangles), Enterobacter cloacae (diamonds), Klebsiella oxytoca (downward-pointing triangles),* and gentamicin-resistant *E. coli (circles)* were exposed to the indicated concentrations of honey for 24 h, after which survival was determined quantitatively as cfu/mL.

honey after 24 h of incubation, which demonstrates that these bacteria were not intrinsically resistant to honey.

## DISCUSSION

We demonstrate that Revamil medical-grade honey had reproducible bactericidal activity in vitro and was equally active against antibiotic-resistant and -susceptible isolates for all species tested. Application of Revamil for 2 days on forearm skin of healthy volunteers was highly effective in reducing both the frequency of positive skin cultures and the numbers of cfu cultured (both $P < .001$, for comparison with controls).

Honey has several well-known properties responsible for its antimicrobial activity. These include a high osmolarity due to the high concentration of sugars (~80% wt/vol) [22], a low pH (3.2–4.5 for undiluted honey), and the production of hydrogen peroxide, which, after dilution of honey, is produced by glucose oxidase originating from the bees [6, 23]. In addition, unknown floral or bee components contribute to the activity [24, 25]. Unfortunately, large variation in antimicrobial activity exists among honeys collected from different environments [13, 16], possibly related to spatial and temporal variation in sources of nectar [15]. Even honey collected from a single location can have significant batch-to-batch variation in antibacterial activity [15]. The unpredictable antibacterial activity of such nonstandardized honey preparations hampers its introduction as an antimicrobial agent.

Revamil is produced in greenhouses under standardized conditions. With <2-fold variation in activity for 11 different batches, this honey showed good batch-to-batch reproducibility

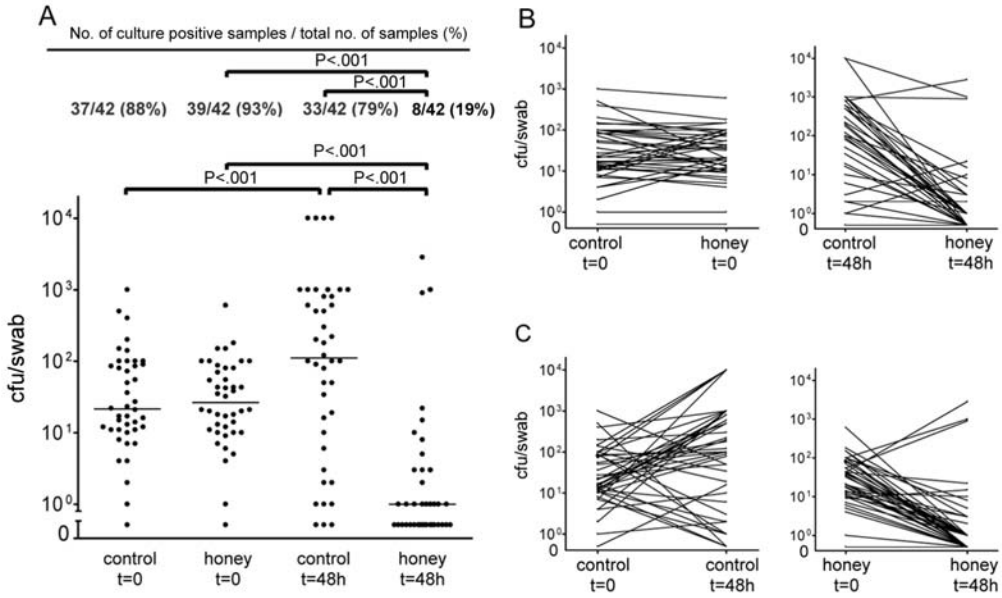


**Figure 4.** Efficacy of honey in reducing forearm skin colonization. *A,* Number of culture-positive samples and number of cfu cultured from control and honey-treated skin patches. An aliquot of 0.5 mL of honey was applied on 1 forearm skin patch, and a second patch on the same arm served as a no-treatment control. Both patches were covered with polyurethane dressing. Before and after 2 days of application, the skin patches were sampled to assess quantitatively the level of microbial skin colonization. *B,* Colonization of control and honey-treated skin patches on the same arm of individual subjects at the start of the experiment *(left panel)* and after 48 h *(right panel). C,* Colonization of individual skin patches at the start of the experiment and after 48 h for control patches *(left panel)* and honey-treated patches *(right panel).* The percentages of culture-positive skin swabs of honey-treated and control skin patches were analyzed with McNemar's test for matched case-control studies, and univariate analyses by the paired Wilcoxon rank-sum test were performed to investigate the statistical significance of the differences in numbers of cfu.

of activity, which is an important criterion for clinical application.

As many infections become more and more difficult to treat, because of increased antibiotic resistance among pathogens [26–28], new antimicrobial agents are needed. Revamil had potent in vitro bactericidal activity against antibiotic-resistant gram-positive and gram-negative bacteria. Inocula of all *S. epidermidis* strains tested were completely killed after 24 h of incubation in 20% vol/vol honey, and 40% vol/vol honey killed all *S. aureus* and *E. faecium* strains tested. Antibiotic-susceptible and -resistant strains of all gram-negative bacteria tested were killed by 20%–30% vol/vol honey. Therefore, medical-grade honey has the potential to be a topical antibacterial prophylaxis or to be a treatment for topical infections caused by antibiotic-resistant bacteria.

To the best of our knowledge, our study is the first to assess quantitatively the efficacy of honey to reduce microbial skin colonization in a controlled trial. Unfortunately, it is very difficult to evaluate honey in a blind trial, because no suitable control substance resembling honey exists. Therefore, we performed an open-label, within-subject trial to assess the efficacy of honey to reduce skin colonization, compared with no-treatment controls. The mean numbers of cfu cultured from skin were reduced 100-fold after application of honey for 2 days, and 81% of the honey-treated skin patches yielded negative culture results, compared with 21% for control patches. In 3 subjects, honey was not effective, but we showed that the bacteria isolated from these subjects were susceptible to honey in vitro. Possibly, the isolated bacteria had emerged from deeper sites within the skin during the sampling, and had not been reached by the honey applied on the skin surface.

Two major indications for application of honey could be treatment of wound infections and topical prophylaxis at sites where microorganisms may give rise to infection, such as catheter-insertion sites [29]. Small, randomized, controlled trials reported the efficacy of honey to reduce the time needed for healing and to reduce the time to negative culture results for burn wounds [30–34] and postoperative wound infections [35]. Another relatively small study showed that there was a comparably bacteremia-free period in the use of tunneled, cuffed hemodialysis catheters after application of honey, compared with after mupirocin treatment [36]. This indicates that honey could be a valuable alternative to mupirocin, but larger, randomized trials using well-defined honey are required to assess the efficacy of honey as an antimicrobial agent.

In summary, we showed that Revamil medical-grade honey has batch-to-batch reproducible and broad-spectrum bactericidal activity and is a good disinfectant for human skin. Thus, this honey has excellent potential as an anti-infective agent for topical prophylaxis or for topical treatment of skin infections caused by antibiotic-susceptible or -resistant bacteria.

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***Potential conflicts of interest.*** All authors: no conflicts.

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