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Honeydew honey as a potent antibacterial agent in eradication of multi-drug resistant *Stenotrophomonas maltophilia* isolates from cancer patients

Short title: Honey in eradication of multi-drug resistant *S. maltophilia*

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Abstract

Multi-drug resistance in nosocomial pathogens is a continually evolving and alarming problem in health care units. Since ancient times, honey has been used successfully for treatment of broad spectrum of infections with no risk of resistance development.

In this study, we investigated the antibacterial activity of two natural honeys, namely honeydew and manuka, against 20 nosocomial multi-drug resistant *Stenotrophomonas maltophilia* (*S. maltophilia*) isolates from cancer patients. Antibiotic susceptibility test was carried out using disk diffusion method with 20 antibiotic disks. The antibacterial activity of honey was determined using a broth dilution method. The concentration of honey used in study was within the range of 3.75% to 25% (w/v). All 20 clinical isolates were multi-drug resistant against 11 to 19 antibiotics. The MICs for honeydew honey ranged from 6.25% to 17.5%, while those for active manuka honey ranged from 7.5% to 22.5%. Honeydew honey had the lower MICs than manuka honey against 16 of tested isolates.

This study showed that Slovak honeydew honey has exceptional antibacterial activity against multi-drug resistant *S. maltophilia* isolates and was more efficient than manuka honey (UMF 15+). Honeydew honey with strong antibacterial activity could be used as a potential agent to eradicate of multi-drug resistance clinical isolates.

Keywords: honey, *Stenotrophomonas maltophilia*, multi-drug resistance, antibacterial, manuka, honeydew

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Introduction

Nowadays, *Stenotrophomonas maltophilia* has been considered, as an important opportunistic nosocomial pathogen in immune-competent individuals associated with different diseases that include both acute and chronic infections. Especially susceptible to *S. maltophilia* are immune-compromised patients, for example, patients with cancer (Khardori *et al.*, 1990; Paez and Costa, 2008). *S. maltophilia* infection in hospitalized patients with cancer has significantly increased over the past two decades (Safdar and Rolston, 2007).

Very recent studies have revealed that *S. maltophilia* originating from external environment, the enteric tract or respiratory secretion may also participate in pathogenesis of persistent infections, particularly in chronic mucosal of skin surface infections (Dowd *et al.*, 2008; Guggenheim *et al.*, 2009; Tan *et al.*, 2009). It has been found that incidence of *S. maltophilia* in burn wounds markedly increased in last decade (Guggenheim *et al.*, 2009). In addition, Dowd et al. (2008) showed that *S. maltophilia* was a predominant bacterial type in biofilm associated with venous leg ulcers.

A characteristic feature of *S. maltophilia* is its low susceptibility to most agents currently used for treatment of human infections (Looney *et al.*, 2009). Moreover, *S. maltophilia* exhibits high-level resistance to a variety of structurally unrelated antibiotics, including β-lactams, quinolones, aminoglycosides, tetracycline, disinfectants and heavy metals (Zhang *et al.*, 2000). The treatment of choice for *S. maltophilia* infections is still co-trimoxazole alone, or in combination with other agents. However, isolates resistant to this agent have already been reported (Nicodemo and Paez, 2007; Vartivarian *et al.*, 1994). The increasing lack of antimicrobials with activity against *S. maltophilia* is of concern and there is an urgent need for new drugs.

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With increasing frequency modern medicine directs attention to natural products with antimicrobial activity and their use in clinical practice. The major arguments for implementing natural products such as honey are the low cost and absence of antimicrobial resistance risk. Honey can inhibit the growth wide range o bacteria, fungi, protozoa and viruses (Blair and Carter, 2005; Molan, 1992). Manuka honey derived from the floral source of *Leptospermum scoparium* in New Zealand has been claimed to have particularly high antimicrobial activity against various bacterial species (Allen *et al.*, 1991; Lin *et al.*, 2009; Lusby *et al.*, 2005; Molan, 2006).

Beside two well-characterized major antibacterial factors in honey, hydrogen peroxide and high osmolarity; methylglyoxal (Adams *et al.*, 2008; Mavric *et al.*, 2008) and bee defensin-1 (Kwakman *et al.*, 2010) also act as antibacterial substances. In addition, phenolic compounds found in dark honeys are partially responsible for antibacterial activity (Aljadi and Yusoff, 2003; Estevinho *et al.*, 2008; Wahdan, 1998).

In our study, we investigate the antibacterial effect of Slovak honeydew honey against 20 nosocomial multi-drug *S. maltophilia* isolates from cancer patients and compare it with the activity of manuka honey.

Materials and Methods

Bacterial isolates

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S. maltophilia clinical isolates (n = 20) from cancer patients were received from Department of Clinical Microbiology of National Institute for Oncology (Bratislava, Slovakia). These clinical isolates were collected during the period from December 2003 to March 2004.

Honey samples

The antibacterial activity of Slovak honeydew honey against 20 nosocomial clinical isolates of *S. maltophilia* was determined by comparison to the commercially available active manuka honey imported from New Zealand (Natur's Nectar, UMF 15+). Honeydew honey used in this study is monofloral honey derived from the floral source of *Abies spp.* localized in Bardejov (Slovakia) and was obtained from an apiary of Mr. Jozef Volansky (Medar s.r.o., Bardejov, Slovakia).

To distinguish the effect of the antibacterial components of honey from any osmotic effect, artificial honey, a control solution with sugar content similar to that of natural honey, was also used for comparison. The artificial honey was prepared by dissolving 39 g D-fructose, 31 g D-glucose, 8 g maltose, 3 g sucrose and 19 g distilled water. All honey samples were stored in darkness at 2-5°C when not in use.

Fifty percent (w/v) stock solution of each type of honey was prepared by weighing 20 g of honey and bringing the volume up to 40 ml of Trypton soya broth (TSB). Further dilutions of stock solution of natural honeys were done to obtain honey concentrations of 25%, 22.5%, 20%, 17.5%, 15%, 12.5%, 11.25%, 10%, 8.75%, 7.5%, 6.25%, 5% and 3.75%. Dilution range of 50%, 25% and 12.5% of artificial honey was used.

Antibiotic susceptibility test

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Antibiotic susceptibility was determined by the disk diffusion method with 20 antibiotic disks according to the criteria of the Clinical and Laboratory Standards Institute (CLSI) (Clinical and Laboratory Standards Institute, 2005) on Mueller-Hinton (MH) agar (Oxoid, Hampshire, UK). The following antimicrobial agents (disk content indicated in parentheses) were tested: amoxicillin-clavulanic acid (20/10 µg), ampicillin (10 µg), aztreonam (30 µg), cefepime (30 µg), cefotaxime (30 µg), cefoxitin (30 µg), cefpodoxime (10 µg), ceftazidime (30 µg), ceftriaxone (30 µg), chloramphenicol (30 µg), ciprofloxacin (5 µg), cloxacillin (5 µg), gentamicin (10 µg), nalidixic acid (30 µg), piperacillin-tazobactam (100/10 µg), streptomycin (10 µg), tetracycline (30 µg), trimethoprim (5 µg), sulfisoxazole (250 µg) trimethoprim-sulfamethoxazole (1.25/23.75 µg), *E. coli* ATCC 25922 was used as the control.

Minimum inhibitory concentration assay

Determination of MIC was conducted according to the recommendation of the CLSI (Clinical and Laboratory Standards Institute, 2003) with minor modification of using TSB instead of MH broth. Briefly, one bacterial colony was suspended in PBS buffer, pH 7.2 and the turbidity of suspension was adjusted to 10^8 colony forming unit (cfu)/mL and diluted with medium to final concentration of 10^7 cfu/mL. Ten µl aliquots of suspension were inoculated to each well of sterile 96-well polystyrene plates (Sarstetd, Germany). The final volume in each well was 100 µL, consisting of 90 µL of sterile medium or diluted honey and 10 µL of bacterial suspension. After 18 h of incubation at 37°C, bacterial growth inhibition was determined by monitoring the

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optical density at 490 nm. The MIC was defined as the lowest concentration of antibiotic inhibiting bacterial growth. All tests were performed in triplicate and were repeated three times.

Results

Antibiotic susceptibility testing

All 20 clinical isolates were multi-drug resistant against 11 to 19 antibiotics. Among all isolates, four strains (20%) were resistant against co-trimoxazole (trimethoprim-sulfamethoxazole). The resistance against co-trimoxazole was associated with isolates resistant at least to 15 antibiotics. Ciprofloxacin resistance was found in two isolates (10%). Antimicrobial resistance profiles of all isolates are summarized in Table 1.

Determination of MIC of honey

The MIC values of the two natural honeys and artificial honey are shown in Table 2. The MICs for honeydew honey ranged from 6.25% to 17.5%, while those for active manuka honey ranged from 7.5% to 22.5%. Honeydew honey had the lower MICs than manuka honey against 16 of tested *S. maltophilia* isolates, where the MIC values of honeydew honey were below 10% in 14 isolates. No difference was observed in the antibacterial activity of both natural honeys against two isolates. Artificial honey inhibited the growth of all isolates at concentration higher than 25%.

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Discussion

S. maltophilia has emerged as an important pathogen, whose eradication is often problematic due to resistance to broad spectrum of antibiotics. When the administration of co-trimoxazole for the treatment of patients with *S. maltophilia* infections is not possible, there may be other effective alternative treatments using ciprofloxacin as a monotherapy or in combination with other antibiotics, or ticarcillin-clavulanate alone or in combination with other agents. An increasing trend of resistance to ciprofloxacin and emerging co-trimoxazole resistance in *S. maltophilia* was reported in last decade (Gales *et al.*, 2001; San Gabriel *et al.*, 2004). We found that 20% of isolates used in this study were resistant to co-trimoxazole, an agent still recommended for treatment of *S. maltophilia* infections. In addition, 10% of isolates exhibited the resistance to ciprofloxacin, as a second therapeutic option. Novel or re-emerged antibacterial agents are urgently needed for treatment of infections caused by multi-drug resistant *S. maltophilia*. Honey could be a potential natural candidate to help eradicate of *S. maltophilia* infections.

It has been demonstrated that medical-grade manuka honey is effective against broad range of microorganisms including multi-resistant clinical strains (George and Cutting, 2007). Until now, it has not been shown that honey is able to inhibit the growth of multi-drug clinical *S. maltophilia* isolates. However, Tan *et al.* (2009) demonstrated that Malaysian tualang honey together with manuka honey (UMF 10+) with MIC of 10% (w/v) showed good antibacterial activity against *S. maltophilia* clinical strain with unknown antibiotic susceptibility profile.

Both Medihoney, a medical grade manuka honey and Revamil, a medical grade buckwheat honey are well-characterized honeys with pronounced healing properties. It has been documented that antibacterial activity of manuka honey directly originates from the

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methylglyoxal (Adams *et al.*, 2008; Mavric *et al.*, 2008). On the other hand, antibacterial bee defensin-1 has very recently been identified in Revamil and it substantially contributes to the antibacterial activity of honey (Kwakman *et al.*, 2010). It remains to establish whether methylglyoxal and antibacterial bee peptide are also present in other natural honeys.

Along with methylglyoxal and defensin-1, the phenolic compounds in honey have been considered as potential antimicrobial agents (Gomez-Caravaca *et al.*, 2006; Taormina *et al.*, 2001). The honey phenolic compounds composition and consequent antibacterial and antioxidant capacity of honey depends on their floral sources. In present study, dark honeydew honey derived from the floral source of *Abies spp.* was used to investigate its bactericidal activity against multi-drug resistant *S. maltophilia* isolates. According to recent studies (Estevinho *et al.*, 2008; Kwakman *et al.*, 2010), we speculate that several non-peroxide factors, namely defensin-1 and phenolic compounds of honeydew honey participate in its strong antibacterial properties. Further detailed studies are needed to identification of components responsible for antibacterial properties of honeydew honey.

In conclusion, our results document that Slovak honeydew honey has exceptional antibacterial activity against multi-drug resistant *S. maltophilia* isolates from cancer patients. In addition, in the most cases it exhibited considerably stronger antibacterial activity than manuka honey and it could potentially be used as an alternative therapeutic agent against *S. maltophilia* infections.

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Table 1. Incidence of antibiotic resistance in 20 clinical isolates of *S. maltophilia*

Isolate No. (n=20)	Antibiogram
57/03	A AMC ATM CPD CRO CTX FEP FOX OB TMP TZP
58/03	A AMC ATM C CAZ CPD CRO CTX FEP FOX NAL OB S Su SXT TMP TZP
122/03	A AMC ATM CPD CRO CTX FEP FOX NAL OB S Su TMP TZP
241/03	A AMC ATM CAZ CPD CRO CTX FEP FOX OB TMP TZP
255/03	A AMC ATM C CIP CPD CRO CTX FEP FOX G NAL OB S Su SXT T TMP TZP
269/03	A AMC ATM CPD CRO CTX FEP FOX OB TMP TZP
325/03	A AMC ATM C CAZ CIP CPD CRO CTX FEP FOX G NAL OB S Su SXT T TMP
368/03	A AMC ATM CPD CRO CTX FEP FOX OB Su TMP TZP
388/03	A AMC ATM C CPD CRO CTX FEP FOX OB TMP TZP
389/03	A AMC ATM CAZ CPD CRO CTX FEP FOX OB TMP TZP
509/03	A AMC ATM CPD CRO CTX FEP FOX OB TMP TZP
996/03	A AMC ATM CPD CRO CTX FEP FOX NAL OB TMP TZP
1444/03	AMC ATM CAZ CPD CRO CTX FEP FOX OB Su TMP TZP
3354/03	A AMC ATM CPD CRO CTX FEP FOX OB TMP TZP
47/04	A AMC ATM CPD CRO FEP FOX OB S Su TMP TZP
287/04	A AMC ATM CAZ CPD CRO CTX FEP FOX OB S Su TMP TZP
300/04	A AMC ATM C CAZ CPD CRO CTX FEP FOX OB Su SXT TMP TZP
846/04	A AMC ATM CPD CRO CTX FEP FOX OB Su TMP TZP
993/04	A AMC ATM CPD CRO CTX FEP FOX OB Su TMP TZP

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999/04	A AMC ATM CPD CRO CTX FEP FOX OB TMP TZP

A, ampicillin; AMC, amoxicillin-clavulanic acid; ATM; aztreonam; C, chloramphenicol; CAZ, ceftazidime; CIP, ciprofloxacin; CPD, cefpodoxime; CRO, ceftriaxone; CTX, cefotaxime; FEP, cefepime; FOX, cefoxitin; G, gentamicin; OB, cloxacillin; NAL, nalidixic acid; S, streptomycin; Su, sulfisoxazole; SXT, trimethoprim-sulfamethoxazole; T, tetracycline; TMP, trimethoprim; TZP, piperacillin-tazobactam

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Table 2. Minimum inhibitory concentration (%) of different honeys towards *S. maltophilia* clinical isolates

***S. maltophilia* strain** (n=20)	**Type of honey**		
	Honeydew	**Manuka (UMF 15+)**	**Artificial**
57/03	7.5	15	50
58/03	7.5	15	25
122/03	7.5	11.25	50
241/03	7.5	15	25
255/03	10	20	50
269/03	7.5	15	50
325/03	8.75	17.5	50
368/03	7.5	15	50
388/03	7.5	11.25	25
389/03	6.25	11.25	25
509/03	7.5	15	25
996/03	7.5	15	25
1444/03	7.5	15	50
3354/03	7.5	15	50
47/04	12.5	11.25	50
287/04	8.75	11.25	25
300/04	11.25	7.5	25

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846/04	15	10	50
993/04	17.5	17.5	50
994/04	17.5	17.5	50

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