

Antibacterial activity of honey against ESBL -producing strains

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Background

Antibiotic resistance in Gram-positive as well as Gram-negative microorganisms is a growing problem worldwide. The prevalence of Methicillin resistant *Staphylococcus aureus* (MRSA) and Gram negatives producing an Extended Spectrum beta-lactamase (ESBL) is steadily increasing during the past decades. Antibiotic use is generally considered the main risk factor for antibiotic resistance. Efforts to reduce the use of antimicrobial agents are therefore warranted.

One of the possibilities to reduce the use of antimicrobial agents is the use of non-antibiotic compounds for the treatment of infections. Examples of these non-antibiotic agents are the use of cranberries to prevent urinary tract infections or the use of honey in case of wound infections.

In this report, we describe the *in-vitro* antibacterial activity of medical grade honey against clinical isolates of ESBL producing *Escherichia coli* and *Klebsiella pneumoniae*.

Material and methods

Strains:

Clinical isolates of ESBL producing *E. coli* and *K. pneumoniae*, nine and eight of each species respectively were used as well as the following reference strains: *E. coli* producing CTX-M1, *K. pneumoniae* producing SHV1 and *K. pneumoniae* producing CTXM15/SHV11/TEM1.

The production of ESBL was determined according to the method as described by the NVMM. The production of ESBL of the reference strains was genetically characterized.

Material:

Solutions of L-Mesitran Ointment and L-Mesitran Soft gel were tested as follows: one gram of each product was added to Mueller Hinton Broth in 50% w/w resulting in a final concentration of honey in the solutions of 24%w/w and 20% w/w respectively.

Method:

Tubes containing the solution of the L-Mesitran ointment and L-Mesitran Soft gel were inoculated with 100µl of a diluted (10^{-1} and 10^{-3}) overnight culture of the *E. coli* and *K. pneumoniae* isolates, incubated overnight at 37°C and plated onto blood agar plates. The plates were overnight incubated at 37°C and the results (semi)-quantitatively assessed. Tubes without honey solutions inoculated with the same bacterial dilutions were used as control.

All experiments were performed in duplo.

For the semi-quantitative assessment the blood agar plates were inoculated according to the "four streak" method: growth in the first streak only was recorded as 1, in the second streak as 2, in the third streak as 3 and growth up to the last streak as 4.

Results

Characteristics of the clinical isolates:

The antibiotic susceptibility of the tested *E. coli* is presented in table 1. All isolates had different susceptibility to the antibiotics tested.

The antibiotic susceptibility of the tested *K. pneumoniae* is depicted in table 2. All isolates had different susceptibility to the antibiotics tested.

The characteristics of the reference strains are given in table 3.

Effect of L-Mesitran Ointment on clinical isolates

E. coli:

Using an inoculum of $\sim 10^5$ cfu/ml eight out of ten isolates were completely inhibited (=bactericidal) in the presence of honey ointment. No effect was observed in two isolates, i.e. A02 and A06 in the first experiment and A01 and A04 in the second one.

At an higher inoculum ($\sim 10^7$ /ml) three to four out of ten were completely inhibited. A 100-1000 fold reduction in cfu/ml were observed with the other isolates. (table 4a and b)

K. pneumoniae:

Using an inoculum of $\sim 10^5$ cfu/ml a reduction in cfu/ml was observed for strain B05 and B08, whereas the other isolates were completely inhibited in the presence of honey ointment. In the duplo experiment the same results were found.

At an inoculum of $\sim 10^7$ cfu/ml mostly only a reduction in cfu/ml were observed. In the first experiment a reduction was found in all isolates tested, in the second experiment four out of 10 strains were completely inhibited, the others showed a reduction in cfu/ml.

The effect of the ointment on the reference strains were comparable with those obtained with the clinical isolates (table 5a and b)

Table 1. Antibiotic susceptibility of the clinical isolates of *Escherichia coli*

Sample strain#	AMOX	AUG4	TMP	SXT	GEN	NOR	CIP	NIT	TAZ	FUR	MERO	PIP	P/T4	DOX	AMI
A01	>128	16	0.5	1	1	0.5	0.12	16	4	8	<=0.03	>512	4	4	4
A02	>128	32	>64	>64	4	>64	>16	16	16	16	<=0.03	>512	4	64	32
A03	>128	16	16	2	1	0.12	<=0.003	32	8	>128	<=0.03	>512	1	4	2
A04	>128	16	>64	>64	4	>64	>16	16	0.5	>128	<=0.03	>512	2	4	2
A05	>128	8	0.5	0.5	>64	0.12	<=0.5	16	16	128	<=0.03	256	2	1	8
A06	>16	8	>8	>2	<=2	<=2	>2	<=16	16	>16	<=1	>64	<=4		<=8
A07	>16	>16	>8	>2	>8	>8	>2	<=16	16	8	<=1	>64	<=4		<=8
A08	>16	<=4	>8	>2	<=2	>8	>2	<=16	4	>16	<=1	>64	<=4		<=8
A09	>16	16	>8	>2	>8	>8	>2	<=16	>16	>16	<=1	>64	<=4		<=8

Amox = amoxicilline
 Aug = co- amoxyclav
 Tmp = trimethoprim
 Sxt = trimethoprim-sulfamethoxazole

Gen = gentamicine
 Cip = ciprofloxacin
 Nit = nitrofurantoin

Taz = ceftazidime
 Fur = Cefuroxime
 Mero = Meropenem
 Pip = piperacillin

P/t4 = piperacillin - tazobactam
 Dox = doxycycline
 Ami = amikacin

Table 2. Antibiotic susceptibility of the clinical isolates of *Klebsiella pneumoniae*

Sample strain#	AMOX	AUG4	TMP	SXT	GEN	NOR	CIP	NIT	TAZ	FUR	MERO	PIP	P/T4	DOX	AMI
B01	>128	16	1	1	4	0.12	<=0.003	32	4	16	<=0.03	256	4	4	1
B02	>128	16	4	1	4	0.5	0.12	32	4	16	<=0.03	256	8	16	4
B03	>128	64	4	2	8	32	2	128	128	>128	<=0.03	32	16	8	8
B04	>128	128	1	1	32	>64	>16	4	0.5	>128	0.25	512	8	32	8
B06	>16	16	>8	>2	8	8	>2	16	16	>16	<=1	>64	>64		<=8
B07	>16	8	>8	>2	<=2	<=2	>2	>16	16	>16	<=1	>64	<=4		<=8
B08	>16	16	>8	>2	>8	4	>2	16	4	>16	<=1	>64	32		<=8
B09	>16	16	<=1	<=0.5	>8	<=2	>2	>16	>16	>16	<=1	>64	<=4		<=8

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 Dox = doxycycline
 Ami = amikacin

Table 3. Characteristics of the reference strains

Species	ESBL type
<i>E. Coli</i>	CTX-M*
<i>K.pneumoniae</i>	SHV*
<i>K.pneumoniae</i>	CTX-M15/SHV/TEM*

Table 4a. Antibacterial effect of L-Mesitran ointment *Escherichia coli*

Sample strain#	Initial Inoculum	Bacterial count (log cfu.ml) after overnight incubation with honey ointment
A01	1,2 [±] +07	2,6 [±] +05(3) ¹
A02	1,3 [±] +07	5,8 [±] +06(3)
A03	8,2 [±] +06	-
A04	1,8 [±] +07	1,9 [±] +04(2)
A05	5,5 [±] +06	4,5 [±] +06(3)
A06	1,3 [±] +07	1,4 [±] +06(3)
A07	6,8 [±] +06	2,7 [±] +03(2)
A08	1,5 [±] +07	-
A09	3,8 [±] +06	-
A10 ²	5,3 [±] +07	2,3 [±] +05(3)

¹Semi quantitative result
²Reference strain

Table 4b. Antibacterial effect of L-Mesitran ointment *Escherichia coli*

Sample strain#	Initial Inoculum	Bacterial count (log cfu.ml) after overnight incubation with honey ointment
A01	1,4 [±] +07	6,4 [±] +04(2) ¹
A02	6,0 [±] +06	1,3 [±] +06(3)
A03	3,8 [±] +06	1,3 [±] +0,4(2)
A04	5,8 [±] +06	2,7 [±] +06(3)
A05	5,5 [±] +06	-
A06	1,5 [±] +07	2,9 [±] +04(2)
A07	2,6 [±] +07	-
A08	2,0 [±] +07	2,6 [±] +06(3)
A09	7,6 [±] +06	-
A10 ²	6,4 [±] +06	-

¹Semi quantitative result
²Reference strain

Table 5a. Antibacterial effect of L-Mesitran Ointment *K. pneumoniae*

Sample strain#	Initial Inoculum	Bacterial count (log cfu.ml) after overnight incubation with honey ointment
B01	1,2 ^E +07	-
B02	3,9 ^E +06	2,3 ^E +05(2) ¹
B03	5,3 ^E +06	-
B04	7,7 ^E +06	-
B05 ²	1,2 ^E +07	2,3 ^E +06(2)
B06	4,7 ^E +06	3,9+05(3)
B07	7,4 ^E +06	6,3+05(3)
B08	1,4 ^E +07	-
B09	4,9 ^E +06	6,1 ^E +06(3)
B10 ²	1,1 ^E +07	3,9 ^E +05(1)

¹ Semi quantitative result
² Reference strain

Table 5b. Antibacterial effect of L-Mesitran Ointment *K. pneumoniae*

Sample strain#	Initial Inoculum	Bacterial count (log cfu.ml) after overnight incubation with honey ointment
B01	7,2 ^E +06	- (3) ¹
B02	2,9 ^E +06	2,2 ^E +0,5(3)
B03	3,0 ^E +06	4,0 ^E +04(2)
B04	2,3 ^E +07	7,9 ^E +04(3)
B05 ²	2,0 ^E +07	-(3)
B06	2,1 ^E +07	-(2)
B07	1,3 ^E +07	-(3)
B08	1,1 ^E +07	-(3)
B09	3,5 ^E +06	1,2 ^E +05(2)
B10 ²	1,6 ^E +07	1,8 ^E +05(2)

¹ Semi quantitative result
² Reference strain

Effect of L-Mesitran Soft gel on clinical isolates

Both *E. coli* and *K. pneumoniae* isolates were completely inhibited in the presence of honey gel, irrespective of the inoculum size. After incubation of the *E. coli* and *K. pneumoniae* strains with solutions of the honey gel no growth at all was observed.

The effect of honey gel on the reference strains were similar as those obtained with the clinical isolates. The reference stains were completely inhibited.

Discussion

The antibacterial activity of the L-Mesitran Soft gel against ESBL producing strains (*E. coli* and *K. pneumoniae*) was better than that of the L-Mesitran Ointment. Similar results were also obtained in previous experiments (Stobberingh, Vandersanden: December 2010).

The variable results obtained with the Ointment are probably due to difficulties to obtain a homogenous solution of the Ointment in the broth, a problem which we did not encounter with the Soft gel.

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The anti-bacterial activity of honey-based ointments against antibiotic resistant *Staph. aureus* and *Ps. aeruginosa* (*in-vitro*, clinical isolates)

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Key words

Wound infections, antibiotics, burns, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, honey, dilution method, medical microbiology, post-operative surgical wounds

Abstract

Four commercial available honey based products and irradiated honey were *in-vitro* compared for antibacterial activity against clinical isolates and reference strains of *Staphylococcus aureus* and *Pseudomonas aeruginosa* with known antibiotic resistance. Different amounts were diluted with Mueller Hinton Broth (MHB) or NaCl and incubated at 37°C for 18-24 hours on a mixing plate. After incubation 100 µl was plated on to a blood agar plate and after incubation for another 18-24 hours at 37°C the bacterial growth was semi-quantitative assessed. As a control 80 µl MHB + 20 µl overnight culture of *S. aureus* and *P. aeruginosa* was used.

L-Mesitran Soft showed the highest anti-bacterial properties after 24 hrs at the lowest dilution. Honey has as an added benefit that it can speed up wound healing and therefore (apart from lower material costs) can provide a cost effective therapy. The tested products provide an attractive alternative for the use of (topical) antibiotics in the management of wound infections, L-Mesitran Soft in particular.

Introduction

The golden standard for treatment of infected wounds is still the use of systemic or topical antibiotics (1). There is however a growing concern worldwide about the rise in antibiotic resistance (2, 3). Data collected by the European Antimicrobial Resistance Surveillance System (EARSS) reported "an unpleasant, but important message: antimicrobial resistance is becoming a larger public health problem" (4). This European concern is shared worldwide (5). Finding effective alternatives for antibiotics to reduce the use and therefore reduce the emerging antibiotic resistance is therefore of the utmost importance.

Recently Cooper *et al.* described the antibacterial efficacy of different types of honey, varying from table honey to specific floral types of honey (6). All *in vitro* research shows that most honeys do have an antibacterial effect, albeit with a variation in effectivity (7). However, not all pure unprocessed honey can be used for

wound care, because honey may contain pesticides, herbicides, heavy metals, antibiotics (used for the treatment of diseases in bees) and spores of *Clostridium botulinum* which can lead to wound botulism. Killing of these spores without affecting the antibacterial activity of the honey is only possible using gamma irradiation (8). The other pollutants are absent in honeys harvested in carefully selected areas without e.g. industrial pollution. Honey free from all these pollutants is called a medical grade honey (MGH).

In recent years MGH products have been approved by the EU for the use in wound care. These products are used with success in wound care management and are considered to have antibacterial activity. They all use different types of MGH and are either 100% MGH or contain added (natural) ingredients.

Aim

The aim of this *in-vitro* study was to determine the antibacterial activity of commercially available MGH and MGH-based products against clinical isolates and reference strains of antibiotic resistant and susceptible *Staphylococcus aureus* and *Pseudomonas aeruginosa*. These species were selected because of their important role in dermal infections (*Staphylococcus aureus*) and burns (*Pseudomonas aeruginosa*). A test method was developed that could provide comparable results of all the tested products.

Materials

Nine clinical isolates of *Staphylococcus aureus*, (numbered 1-9), 6 methicillin susceptible and 3 methicillin resistant were selected with different characteristics i.e. Pantone Valentine Leucocidine (PVL), and Toxic Shock Staphylococcal Toxine (TSST), positive and negative strains were selected from different genetic background. The PVL and TSST positive strains were chosen because these are commonly found in severe wound infections, including MRSA-strains.

Five clinical isolates of *Pseudomonas aeruginosa* (n=5), numbered A-E also varied in susceptibility to antibiotics. The antibiotic susceptibility of the isolates have been described previously: reference strains *Pseudomonas aeruginosa* ATCC 27853 and *Staphylococcus aureus* ATCC 29213 were used (table 1).

Table 1

	PVL	TSST	MSSA	MRSA	SPA-type
1	-	-	+	-	t051 (CC211)
2	+	+	+	-	t211 (CC221)
3	-	-	-	+	t786 (no founder)
4	-	-	+	-	t346 (CC084)
5	-	-	-	+	t008 (CC064)
6	-	-	-	+	t008 (CC064)
7	+	-	+	-	t659 (CC659)
8	+	+	+	-	t127 (CC127-ST-1)
9	-	+	+	-	t012 (CC021-ST30)

The honey (based) products used were:

- "Regular Honey": this pure honey, it is the main ingredient of products C + D, listed here under. This honey was gamma irradiated;
- pure honey, Revamil® (B-factory, NL);
- pure Manuka honey (Activon, UK);
- L-Mesitran® Soft (Tricum, NL): this gel contains 40% "Regular Honey" (see a.), hypoallergenic medical grade lanolin (Medilan®), propylene glycol, PEG 4000 and vitamin C & E.
- L-Mesitran® Ointment (Triticum, NL): this ointment contains 48% "Regular Honey" (see a.), hypoallergenic medical grade lanolin (Medilan®), sunflower oil, cod liver oil, Calendula Officinalis, Aloe Barbadosensis, zinc oxide and vitamin C & E.

Method

Different amounts of "Regular Honey" and Manuka were diluted with 0,9 % NaCl to obtain the following concentrations i.e. 96%, 80%, 64%, 48%, 32% . One ml of each concentration was added to 1 ml of Mueller Hinton Broth (MHB) resulting in final concentrations of honey of 48%, 40%, 32%, 24%, 16% (w/w).

L-Mesitran Ointment and Soft contain fatty elements and dilution with 0,9 % NaCl to obtain different concentrations was not possible. Therefore these products, as well as Revamil, were tested in one dilution only: L-Mesitran Ointment 24% w/w/ honey equivalent, L-Mesitran Soft 20% w/w honey equivalent and Revamil 50% w/w honey.

All tubes were inoculated with different inoculum sizes of the strains of *Staphylococcus aureus* from 1.5X10e6-1.5x10e8 cfu/ml and *Pseudomonas aeruginosa* 1.5X10e6-1.5x10e8 cfu/ml. The tubes were incubated at 37°C for 18-24hours on a mixing plate.

After incubation 100 µl was plated on to a blood agar plate(OXOID CM) and after incubation for another 18-24 hours at 37C the bacterial growth were semi-quantitative assessed as +,++,+++ and +++++. As a control

80µl MHB + 20µl overnight culture of *S. aureus* and *P. aeruginosa* was used.

Results

Compared to 'Regular Honey', Manuka honey proved to be more effective against *Staphylococcus aureus*. Manuka was more effective against the clinical isolates than against the reference strain ATCC29213, i.e. clinical isolates were killed at 24% w/w, the reference strain needed at least 40% w/w. No difference in activity of both products were observed against the different MRSA strains and MSSA.

Manuka honey was more effective against *Pseudomonas aeruginosa* than 'Regular Honey'. This effect is less than the difference between the two for *Staphylococcus aureus*. The reference strain of *Pseudomonas aeruginosa* was more inhibited in the presence of honey than the clinical isolates, this effect is similar for Manuka honey and 'Regular honey'. The clinical isolates of *Pseudomonas aeruginosa* were completely killed at concentrations of 32 % w/w Manuka honey. The 'Regular honey' showed the same effect from 40 % w/w.

Revamil is bactericidal against *Pseudomonas aeruginosa*, but not effective against *Staphylococcus aureus*. At an inoculum of 10e7 CFU/ml of the different *Staphylococcus aureus* strains, bacterial growth was rather inhibited.

L-Mesitran Ointment is bactericidal against the reference strain of *Staphylococcus aureus*, the antibacterial effect against the other isolates showed varying results. Against *Pseudomonas aeruginosa* the product was bactericidal at concentrations of 10e7 CFU/ml. However, at higher bacterial concentrations (10e9 CFU/ml) no growth inhibition was observed, both for the reference strain as for the clinical isolates.

L-Mesitran Soft was bactericidal at a honey concentration of 20% w/w against all *Staphylococcus aureus* and *Pseudomonas aeruginosa* strains tested.

The tested honey products also have an antimicrobial effect, but their efficacy was variable. (table 2).

L-Mesitran Soft was by far the most effective product against *Staphylococcus aureus* isolates both methicillin susceptible and resistant, and *Pseudomonas aeruginosa*. Moreover, the least amount of the product was needed for optimal antibacterial effectivity. To obtain a similar level of antibacterial activity one would need relatively more material of the other honey products, with the exception of the Revamil product, which does not seem to have any significant activity against *Staphylococcus aureus*.

Table 2

General interpretation of the results of antibacterial effectiveness against two bacteria and their clinical isolates

		<i>Staph. aureus</i>	<i>Ps. aeruginosa</i>
Antibacterial activity	↑ High	LM Soft LM Ointment Manuka	LM Soft Revamil
	Moderate	Regular honey	LM Ointment Manuka Regular honey
	↓ None	Revamil	

Discussion

This *in vitro* study shows that medical grade honey products have the potential to kill bacteria commonly found as causative agents such as *Staphylococcus aureus* and *Pseudomonas aeruginosa* within 24 hours. L-Mesitran Soft was the most effective compound tested in terms of antibacterial activity against *Staphylococcus aureus* and *Pseudomonas aeruginosa* i.e. antibacterial (killing) activity was observed at the lowest concentration compared to the other compounds tested.

It was not the aim of the study to (re)define the mode of action of the products. The antimicrobial activity has been described in literature as being primarily caused by the high osmolarity of honey (due to the high sugar content), the production of hydrogen peroxide and its low acidity (typically between 3-4) and the presence of other components such as antioxidants (9).

The *in-vitro* antibacterial activity of honey has also been demonstrated in the study of Noori: Gram-positive bacteria were killed after 1 hour of exposure to 50% honey, with complete elimination after 3-24 hours. Killing of Gram-negative bacteria started after 4-6 hours, and were completed after 48 hours (10). Concentrations needed for an optimal effect were as low as 5%, but the highest inhibition was seen at 20% (7).

The worldwide growing antibiotic resistance against *Staphylococcus aureus* (3) and *Pseudomonas aeruginosa* (2) is a cause for concern, taken into account the continued use of antibiotics. To date no resistance against honey has been reported (11, 12) and the findings in this study support the clinical use of honey for the treatment of infected wounds caused by *Staphylococcus aureus* and/or *Pseudomonas aeruginosa*. Several clinical studies described promising results.

In patients with wounds infected with antibiotic-resistant strains of bacteria, not responding to antibiotic therapy, good results have been achieved within a couple of weeks after application of honey. The bacteria infect-

ing the wounds were found to be resistant to ampicillin, oxytetracycline, gentamicin, chloramphenicol and cephadine (13).

Honey was used on nine infants with large infected surgical wounds that failed to heal with intravenous antibiotics, cleaning the wound with aqueous 0.05% chlorhexidine solution and application of fusidic acid ointment. Marked clinical improvement was seen in all cases after five days of treatment with honey, and all wounds were closed, clean and free of infection after 21 days of application of honey (14).

The use of honey was compared with gentamicin, ofloxacin, chloramphenicol in animal models (10). Honey had a comparable result to that of local antibiotics for infections of surgical wounds and conjunctiva.

A randomized controlled trial (101 patients) demonstrated that regular, thrice-weekly, topical exit-site application of standardized antibacterial honey was safe and cost-effective and resulted in a comparable rate of catheter-associated infection to that obtained with topical mupirocin exit-site application in patients with tunnelled, cuffed hemodialysis catheters (15).

In a study with 102 patients with infected open fracture wounds and unresponsive to conventional antibiotic treatment (cephalexin and cefazolin), honey treatment caused wound discharge to end and promoted healthy granulation tissue within two weeks of treatment (16). A non-insulin-dependent diabetic female patient with an MRSA infected post-operative surgical wound in the groin was successfully debrided and cleansed within nine days of treatment with honey ointment. Wound malodour was eliminated and a swab culture for MRSA was negative (17).

The same ointment was used to treat a male patient with pathogenic obesity (BMI 96.5) with a *Pseudomonas aeruginosa* infected excised wound on his right leg. The infection and the malodour were cleared within three days without the use of any additional considered antibiotics (18).

In a large number of randomized controlled clinical trials with (acute and chronic) infected wounds, honey treatment has been successfully used in comparison to conventional therapies such as povidone iodine treatment, silver sulfadiazine, saline, eusol, sugar, ampicillin ointment and antibiotics (19).

For the management of infections the clinician might also choose an appropriate silver impregnated dressing. Silver dressings have been used with success in clinical practice to reduce infection. *In-vitro* results confirm the capability of these dressings to effectively kill bacteriae in 24hrs (20). However, research shows that silver can be cytotoxic to fibroblasts and keratinocytes and therefore inhibitory for wound healing (21). In an evaluation of the epidermal cell proliferation, silver dressings significantly

delayed re-epithelialization (22) and a recent study identified two silver-resistant bacteria (23).

Moreover: in a direct comparison *in vitro* of honey and silver dressings it was demonstrated that the silver interfered with epidermal cell proliferation and migration, implying that it contains cytotoxic material. On the other hand honey significantly stimulates the growth of new cells (keratinocytes and fibroblasts), results in a good new cell structure without abundant scar formation and is not cytotoxic (24).

Although effective, the use of silver as an alternative to antibiotic therapy for the management of infected wounds is obviously not without drawbacks.

This *in-vitro* research did not focus on toxicity or the influence of the tested honey products on cell proliferation and therefore wound healing. However, no reports of toxicity of honey have been reported to date. On the contrary, honey has been shown to stimulate wound healing as discussed here above.

Honey also can provide a cost effective solution. In a comparison study between honey and hydrogel for the healing of shallow wounds and abrasions, honey was extremely cost effective in material use, 24 times cheaper (25). Honey speeds up the healing of wounds significantly compared to sugar (26) and e.g. silver sulphadiazine, povidone iodine, eusol and paraffin gauze/hydrocolloid (19). This resulted in reduced hospital stay and nursing time, therefore reducing significantly costs related to wound care, not forgetting the patient comfort realized with the fast and efficient recovery from a wound.

All these studies strongly support the use of honey in the clinical setting. However, without standardized products certified for clinical use in hospital and home care setting they are somewhat flawed from a practical point of view. European (CE), American (FDA), Australian (TGA) and other government authorities, have broken ground by allowing certain products to be sold and prescribed by physicians, but only after rigorous investigations into product safety and reproducible quality. Clinicians would be placing their patients and their own careers at risk, by using non authorised honey products. This *in-vitro* study should be followed up by *in-vivo* research on L-Mesitran Soft, because it tested as the best choice as an antibacterial agent *in-vitro*. The AZM academic hospital in Maastricht is currently devising a randomized *in-vivo* comparison model with topical antibiotics to that extend.

Conclusion

Medical grade honey based products are antibacterial, but vary in activity. From the *in-vitro* tested honey products available on the European market, L-Mesitran Soft showed the highest anti-bacterial properties when tested against clinical isolates and reference strains of *Staphylococcus aureus* and *Pseudomonas aeruginosa* with known antibiotic resistance.

Compared to antibiotics or the use of silver, the use of honey is safe and without adverse effects for the treatment of infected wounds. The tested honeys can achieve the same goal as antibiotics and silver: reducing the bacterial burden. Honey has as an added benefit that it can speed up wound healing and therefore (apart from lower material costs) can provide a cost effective therapy. Honey, and in particular the tested products, does therefore provide an attractive alternative for the use of (topical) antibiotics in the management of wound infections.

Potential conflicts of interest

None

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