

INTRODUCTION

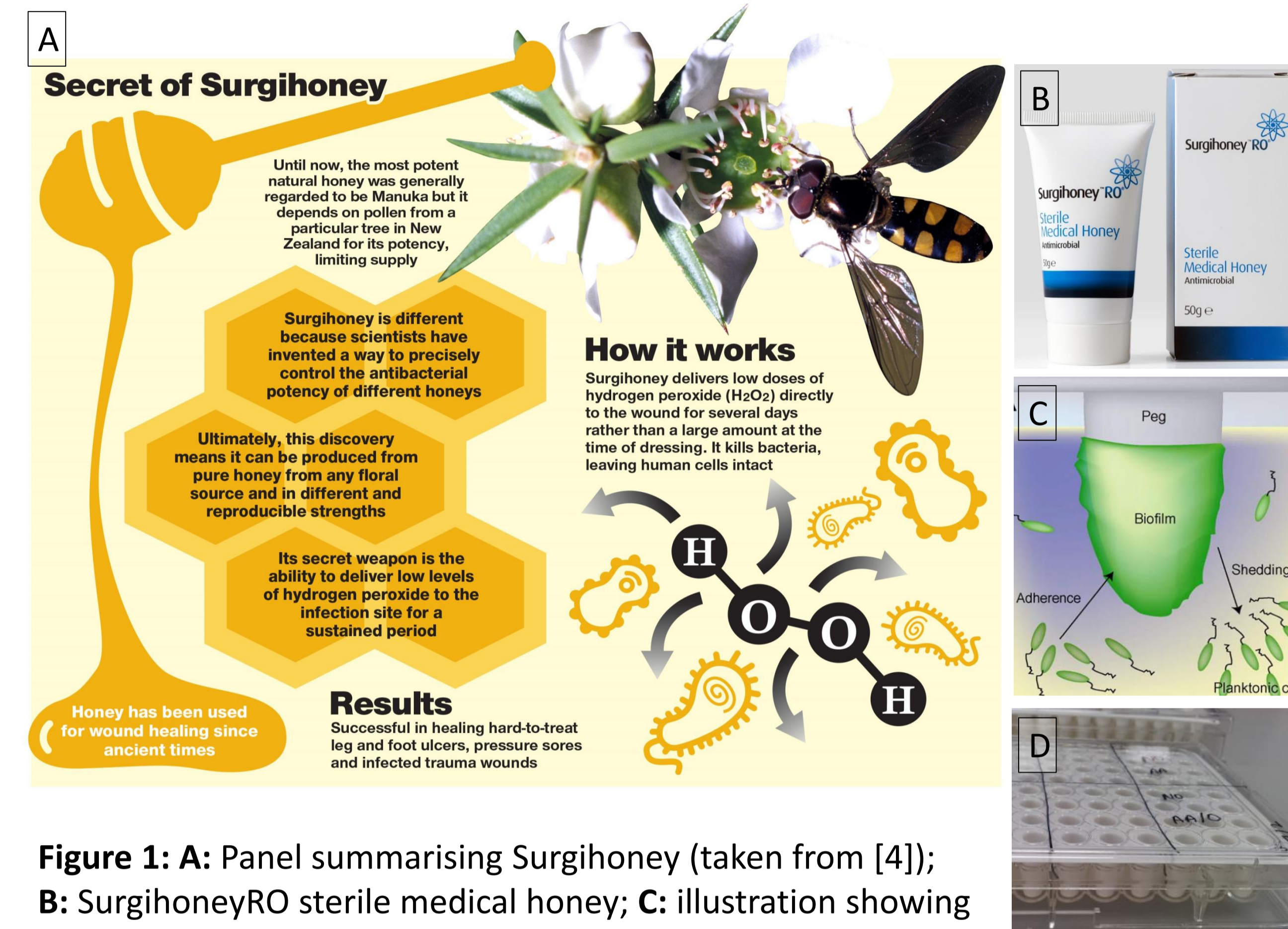
There is a close link between chronicity of wounds, and the presence of biofilms, with biofilms found in >60% of chronic wound infections [1], compared with 6% of acute wounds [2].

Biofilms are hard to treat using conventional antimicrobials [3]. Furthermore, due to antimicrobial resistance, there is increasing interest in 'alternative' agents that can support wound healing and prevent colonisation and infection.

Honey is a well-recognised wound care agent, which possesses multifactorial antimicrobial properties. SurgihoneyRO is an engineered honey gel with enhanced antimicrobial properties (owing in part to enhanced production of hydrogen peroxide). The gel provides consistent potency, and can be produced in large quantities as it does not rely on a single floral source.

OBJECTIVES

This study was undertaken to assess whether SurgihoneyRO has antibacterial activity against mature, pre-formed biofilms



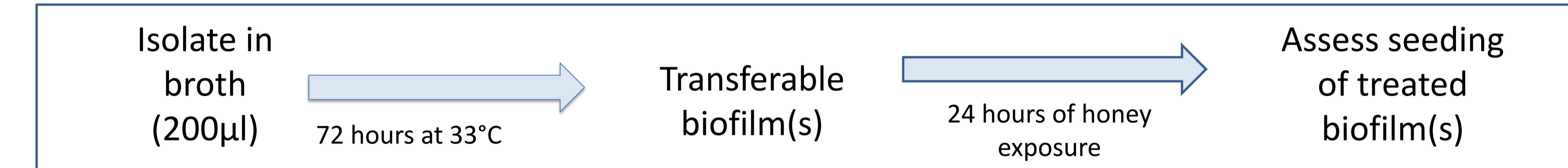
METHODS

16 isolates comprising *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, *Staphylococcus aureus*, *Escherichia coli*, and *Klebsiella pneumoniae* (Table 1) were tested.

Overnight cultures of the isolates were diluted into broth, and added to a 96 well microtitre tray at a concentration of approximately 1×10^5 cfu/ml. A PCR peg plate was added, and this assembly was incubated for 72 hours to form a plate of 'transferable biofilms'.

The biofilms were exposed to SHRO at dilutions (in water, from neat) of 1:3 down to 1:6144 for a period of 24 hours, after which the biofilm seeding was assessed.

The lowest dilution achieving a statistically significant reduction in biofilm seeding of at least 50%, compared to untreated controls, was recorded



RESULTS

All 16 bacterial isolates were susceptible to SurgihoneyRO, with reduced biofilm seeding observed for all, and percentage reductions ranging from 58% (ACI_C59) to 94.3% (MDR_B) for the strongest concentration of honey (1:3). Furthermore at 1:3, biofilm seeding of the test biofilm was reduced by 80-94.3% (when compared to the positive control) for 12/16 isolates.

The MBECs (the minimum concentration of honey that results in a statistically significant reduction in seeding of $\geq 50\%$) ranged from 1:6 to 1:384 (Table 1).

In addition to reducing seeding, we also observed anti-biofilm impacts, with the 24 hour exposure resulting in disruption of the biofilm, and reduced biomass as assessed through a crystal violet assay.

Figure 2: A: The mean average biofilm seeding (optical density) of the *Staphylococcus aureus* isolates following exposure to SurgihoneyRO for 24 hours. B: The mean average biofilm biomass (optical density) for all isolates when exposed to all dilutions of SurgihoneyRO, as measured by a crystal violet assay.

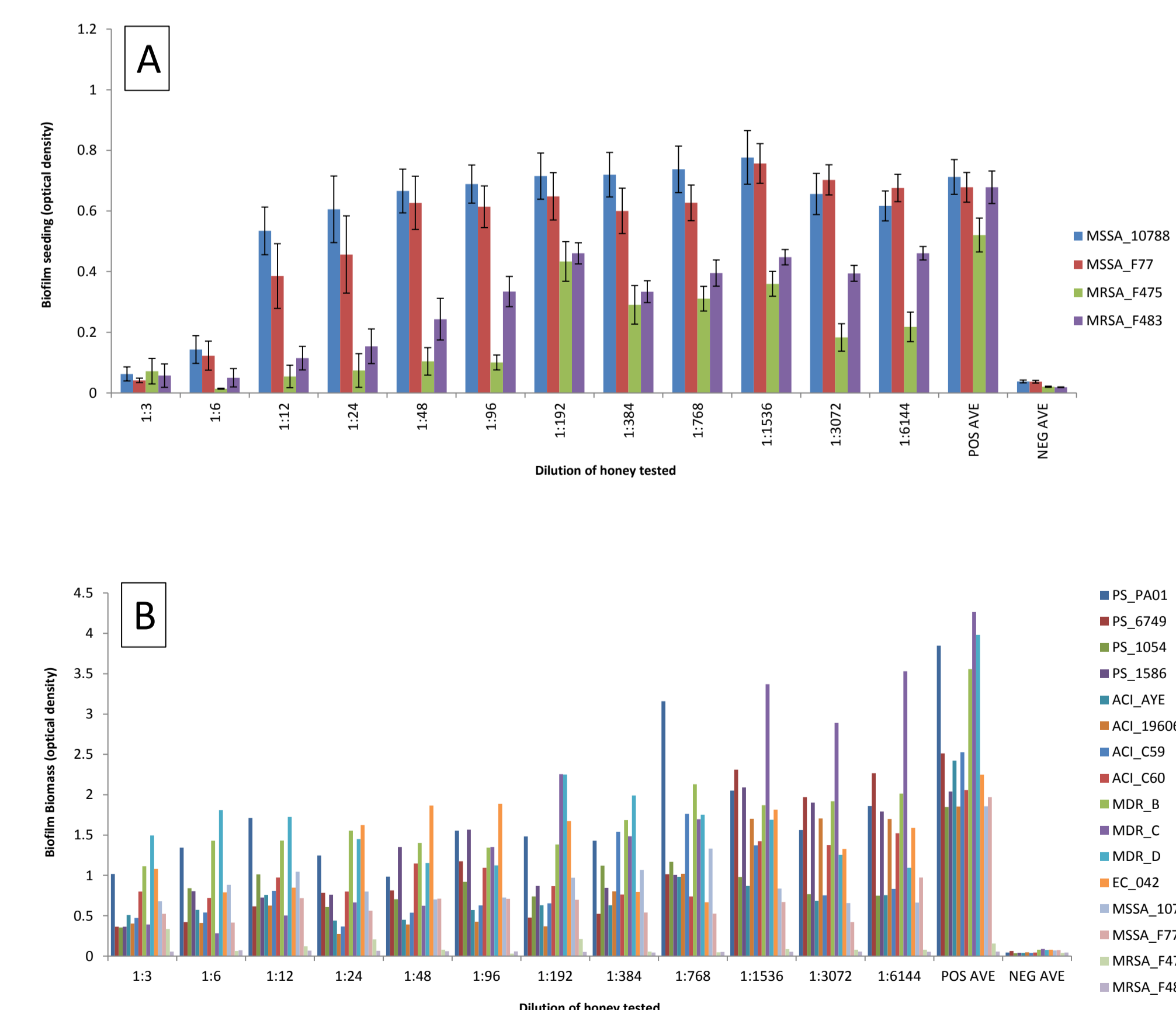


Table 1: Showing the MBEC values for all the isolates, the % reduction in seeding (average) and the p-value.

	Isolate	MBEC	% reduction in seeding	p-value
<i>P. aeruginosa</i>	PS_PA01	1:48	-88.7	<0.001
	PS_6749	1:6	-86.8	<0.001
	PS_1054	1:48	-79.8	<0.001
	PS_1586	1:96	-53.8	<0.001
<i>A. baumannii</i>	ACI_AYE	1:96	-67.9	<0.001
	ACI_19606	1:192	-91.7	<0.001
	ACI_C59	1:384	-53.5	<0.001
Comparator Gram negatives	ACI_C60	1:6	-71.2	<0.001
	MDR_B	1:6	-90.1	<0.001
	MDR_C	1:6	-90.2	<0.001
	MDR_D	1:48	-59.7	<0.001
<i>S. aureus</i>	EC_042	1:6	-93.8	<0.001
	MSSA_10788	1:6	-79.9	<0.001
	MSSA_F77	1:6	-81.9	<0.001
	MRSA_F475	1:96	-80.7	<0.001
	MRSA_F483	1:96	-50.7	<0.001

Some limitations of the work:

- In vitro* testing only, and on abiotic surfaces which are unlikely to represent complex *in vivo* biofilms
- Monomicrobial biofilms (*in vivo* biofilms are polymicrobial)

CONCLUSIONS

SurgihoneyRO is effective at reducing seeding of pre-formed biofilms of clinically important wound pathogens *in vitro*.

We also have evidence that SurgihoneyRO also has anti-biofilm activity, since biofilm biomass was reduced (in a dose-dependent manner) following exposure.

Our *in vitro* data supports the existing clinical data for antibiofilm efficacy, and furthermore supports the use of SurgihoneyRO as a promising topical wound care agent.