

# The Antimicrobial Action of Honey

## 2. Antibacterial Activity of Honey

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### Abstract

Ten samples of honey were studied for the antibiotic equivalent potency. The antibacterial activity of honey was equivalent to 0.675-1.45 units/ml of penicillin against *Staphylococcus aureus* and to <14.7-28.1 mcg/ml of tetracycline against *Escherichia coli*. Five selected samples, having low to high equivalent potency of penicillin against *Staphylococcus aureus*, were determined for MICs against 30 strains of *Staphylococcus aureus* and *Escherichia coli* including standard strains. Most strains were antibiotic-resistant organisms. The MICs against *Staphylococcus aureus* and *Escherichia coli* of honey samples were 0.1-0.3 g/ml and 0.15-0.35 g/ml, respectively. For control solution containing 80% of glucose and fructose in the ratio of 1:1, the growth of test organisms was not inhibited. Our study showed that the various samples of honey had approximately the same antibacterial activity. It was also shown that the honey samples were inhibitory against antibiotic-resistant organisms.

### เรื่องย่อ

ฤทธิ์ต้านจุลชีพของน้ำผึ้ง: 2. ฤทธิ์ต้านแบคทีเรียของน้ำผึ้ง

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การศึกษาฤทธิ์ต้านแบคทีเรียของน้ำผึ้ง 10 ตัวอย่าง โดยเปรียบเทียบความแรงกับยาเพนิซิลลินและเตตราซัยคลิน พบว่าฤทธิ์ต้านเชื้อ *Staphylococcus aureus* ของน้ำผึ้งสมมูลกับเพนิซิลลินความแรง 0.675-1.45 หน่วย/มิลลิลิตร และฤทธิ์ต้านเชื้อ *Escherichia coli* ของน้ำผึ้งสมมูลกับเตตราซัยคลินความแรง <14.7-28.1 ไมโครกรัม/มิลลิลิตร เลือกน้ำผึ้งมา 5 ตัวอย่างที่มีฤทธิ์สมมูลกับความแรงของเพนิซิลลินในช่วงต่าง ๆ ต่อเชื้อ *Staphylococcus aureus* นำมาหาค่าความเข้มข้นต่ำสุดที่ยับยั้งการเจริญของเชื้อ *Staphylococcus aureus* และ *Escherichia coli* ชนิดละ 30 สายพันธุ์ รวมทั้งสายพันธุ์มาตรฐาน เชื้อทดสอบส่วนใหญ่เป็นสายพันธุ์ที่ต้านยาปฏิชีวนะ ความเข้มข้นต่ำสุดของน้ำผึ้งที่ยับยั้งการเจริญของเชื้อ *Staphylococcus aureus* และ *Escherichia coli* มีค่าอยู่ระหว่าง 0.1-0.3 กรัม/มิลลิลิตร และ 0.15-0.35 กรัม/มิลลิลิตร ตามลำดับ สำหรับกลุ่มควบคุมซึ่งประกอบด้วยน้ำตาลร้อยละ 80 คือ กลูโคส:ฟรุกโทส ในอัตราส่วน 1:1 ไม่สามารถยับยั้งการเจริญของแบคทีเรียได้ จากผลการทดลองแสดงให้เห็นว่าน้ำผึ้งตัวอย่างต่าง ๆ มีฤทธิ์ต้านแบคทีเรียใกล้เคียงกัน และมีฤทธิ์ต้านแบคทีเรียสายพันธุ์ที่ดื้อยาปฏิชีวนะ

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## INTRODUCTION

A number of study reported the *in vitro* antibacterial effect of pure honey.<sup>1-3</sup> It was found that many bacteria such as *Staphylococcus aureus*, *Proteus mirabilis* and *Escherichia coli* failed to grow in undiluted and 50% diluted honey. Therefore, honey was used in the postoperative management of patient undergoing radical vulvectomy for vulva carcinoma, and used in the treatment of infantile gastroenteritis.<sup>3,4</sup>

To compare the antibacterial activity of honey with some commonly used antibiotics, we would determine the equivalent potency of antibiotics and minimal inhibitory concentrations (MICs) of honey against clinical isolates.

## MATERIALS AND METHODS

**1. Honey** Nine out of 18 honey samples from various provinces in Thailand and 1 out of 2 samples of imported honey for commercial consumption from U.S.A., passing the test for invert sugar substitute by the method specified in the Pharmaceutical Codex 11<sup>th</sup> ed,<sup>5</sup> were determined for the equivalent potency of antibiotics. Five selected samples, having low to high equivalent potency of penicillin on *S. aureus*, were determined for minimal inhibitory concentrations.

**2. Control solution** Control solution containing 80% of glucose and fructose in the ratio of 1:1 was used in this study.

**3. Antibiotic working standard** Penicillin potency 1,664.0 units/mg, tetracycline potency 100.0%.

### 4. Antibiotic sensitivity discs

Ampicillin	10 mcg (BBL, Lot. No.908636)
Cefotaxime	30 mcg (BBL, Lot. No.812534)
Colistin	10 mcg (BBL, Lot. No.806517)
Erythromycin	15 mcg (BBL, Lot. No.907532)
Neomycin	30 mcg (BBL, Lot. No.812519)
Penicillin	10 u (BBL, Lot. No.903617)
Tetracycline	30 mcg (BBL, Lot. No.904556)
Trimethoprim-sulfamethoxazole	1.25/23.75 mcg (BBL, Lot. No.909579)

**5. Test organisms** *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922.

*Staphylococcus aureus*: 30 clinical isolates from Department of Microbiology, Faculty of Medicine, Siriraj Hospital, Mahidol University.

*Escherichia coli*: 30 clinical isolates from Department of Microbiology, Faculty of Medicine, Chulalongkorn University.

**6. Medium** Mueller Hinton agar (Difco).

### 7. Preparations of inoculum

**7.1 For determination of the equivalent potency of antibiotics.** Each standard organism was grown on Mueller

Hinton agar slant at 37°C overnight. The inoculum was obtained from the surface growth of each organism in sterile saline and diluted to give 50% light transmission in 1 cm layer cuvette at a wavelength of 650 nm.

**7.2 For determination of disc susceptibility.** The culture suspension was prepared as described in 7.1, except that it was diluted to obtain a turbidity comparable to the 0.5 McFarland turbidity standard.

**7.3 For determination of MICs.** The culture suspension was prepared as described in 7.2. In addition, a 1:20 dilution of the culture suspension was prepared in sterile saline for inoculation.

## 8. Determination of the equivalent potency of antibiotic by cylinder-plate method<sup>6</sup>

**8.1** 1% potassium phosphate buffer pH6 was used to dissolve and dilute penicillin to obtain concentrations of 0.51, 0.64, 0.8, 1.0 and 1.25 units/ml. For tetracycline, 0.1 M potassium phosphate buffer pH 4.5 was used to dissolve and dilute the antibiotic to obtain concentrations of 12.8, 16, 20, 25 and 31.2 mcg/ml.

**8.2** The assay plates were prepared using petri dishes 100x20 mm. Twenty-one ml of Mueller Hinton agar was placed in each plates which then were allowed to harden. Four ml of seed layer containing 1% of inoculum in Mueller Hinton agar were added and was allowed to harden. Twelve plates were used for the establishment of the standard curve, and 3 more plates were used for the assay of each sample of honey.

**8.3** Plates containing *S. aureus* ATCC 25923, or *E. coli* ATCC 25922 were used for penicillin and tetracycline assay, respectively.

**8.4** In order to derive the standard curve, 6 stainless steel cylinders (6 mm internal diameter and 10 mm height) were placed on the surface of each of 3 inoculated plates. They were then alternately filled with the medium antibiotic dilution and each of the remaining cylinders with 1 out of the other 4 antibiotic concentrations. Repeated the process for the remaining 3 antibiotic concentrations.

**8.5** To determine the antibiotic equivalent potency of honey, the alternate cylinders were filled, on each of 3 plates, with the median antibiotic solution, and the remaining 9 cylinders with 1 sample of honey. After maintaining at room temperature of 1 hr, the plates were incubated at 37°C overnight. The diameters of inhibition zone were measured.

## 9. Determination of antibiotic disc susceptibility

**9.1** The inoculated plates were prepared by inoculating each test organism on the surface of plates (100 mm diameter) containing 25 ml of Mueller Hinton agar by streak method.<sup>7</sup>

**9.2** The antibiotic susceptibility discs were placed on the surface of inoculated plates. The discs tested with *S. aureus* were cefotaxime, erythromycin, penicillin, tetracycline and trimethoprim-sulfamethoxazole.

The discs tested with *E. coli* were ampicillin, colistin, neomycin, tetracycline and trimethoprim-sulfamethoxazole.

9.3 After maintaining at room temperature for 15 minutes, the plates were incubated at 37°C overnight. The diameters of inhibition zone were measured. Each determination was carried out in duplicate.

#### 10. Determination of MICs of honey by agar dilution method<sup>8</sup>

10.1 In order to prepare plates containing honey, each sample of honey was added to melted Mueller Hinton agar to give final honey concentration of 50, 45, 40, 35, 30, 25, 20, 15, 10 and 5%. Twenty-five ml of each medium containing honey was pipetted into plate (100 mm diameter) and then allowed to harden.

10.2 The test organisms were inoculated onto the plates containing honey, control solution and control plate (no honey and no control solution) by using the inoculum replicating device which delivered 1 µl of inoculum, containing approximately  $1 \times 10^4$  viable cells per spot, onto the agar surface. The inoculum plates were allowed to stand undisturbed until the spots of inoculum were absorbed completely. The plates were then incubated at 37°C overnight and observed for growth of the organisms. Each determination was carried out in triplicate.

## RESULTS

**The equivalent potency** The antibacterial activities of honey were equivalent to 0.675-1.45 units/ml of penicillin against *S. aureus* and to <14.7-28.1 mcg/ml of tetracycline against *E. coli*.

**Table 1** Susceptibility of clinical isolated strains of *S. aureus*.

No. of Strains	Pattern of Susceptibility				
	P	T	E	Cef	T-S
1	+	+	+	+	+
16	-	+	+	+	+
10	-	-	+	+	+
1	-	±	+	+	+
1	-	-	-	-	+
1	-	-	-	-	-

P = Penicillin  
 T = Tetracycline  
 E = Erythromycin  
 Cef = Cefotaxime  
 T-S = Trimethoprim + sulfamethoxazole  
 + = Susceptible  
 - = Resistant  
 ± = Intermediate

**Table 2** Susceptibility of clinical isolated strains of *E. coli*.

No. of Strains	Pattern of Susceptibility				
	A	Co	N	T	T-S
8	-	+	-	-	-
6	-	+	±	-	-
3	-	+	±	-	+
3	+	+	±	-	+
2	-	+	+	-	-
2	+	+	±	+	+
1	-	+	-	±	-
1	-	+	±	±	-
1	-	+	-	+	-
1	+	+	+	-	-
1	-	+	-	+	+
1	+	+	+	-	+

A = Ampicillin  
 Co = Colistin  
 N = Neomycin  
 T = Tetracycline  
 T-S = Trimethoprim + sulfamethoxazole  
 + = Susceptible  
 - = Resistant  
 ± = Intermediate

**Antibiotic disc susceptibility** The standard strains of *S. aureus* and *E. coli* were susceptible to all test antibiotics. The strains of *S. aureus* and *E. coli* isolated from clinical specimens could be grouped into 6 and 12 groups according to the patterns of susceptibility as shown in Table 1 and 2, respectively.

**Minimal inhibitory concentrations (MICs)** The MICs of honey samples for *S. aureus* and *E. coli* were 0.1-0.3 g/ml and 0.15-0.35 g/ml, respectively. For some strains of *S. aureus*, the MICs of some samples were below 0.1 g/ml. However, most of them were 0.1 g/ml. There was only 1 sample with the MIC of 0.3 g/ml and this sample also had the highest MIC for *E. coli*. For plates containing control solution, the growth of test organisms was not inhibited.

## DISCUSSIONS AND CONCLUSIONS

The antibiotic equivalent potencies of various honey samples differed about 2 fold. Most strains of both test organisms were susceptible to the same concentration of honey. The results from our study showed that the antibacterial activity of various samples of honey were similar. Therefore, the honey susceptibility of bacteria did not correlate to antibiotic susceptibility of those test organisms.

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