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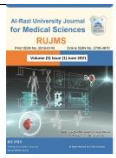

Antibacterial Susceptibility and Sider Honey Activity against Isolated Bacteria from Wound Patients attending at Al-Gmohori hospital in Hajja City, Yemen

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Abstract:

Background: The emergence increase of infections caused by resistance bacteria become a real threat to patients and healthcare workers. **Aims:** The present study was designed to assess the antibacterial susceptibility and Sider honey effect against isolated bacteria from wound infection of patients attending at Al-Gmohori hospital in Hajja City, Yemen. **Methods:** One hundred and seventy-four swabs specimens were collected from wound patients from July to September 2021. The associated factors were gathered by the intended questionnaire. The identification of bacteria isolates was performed by using standard microbiological methods. Also, the antibacterial activity of some antibiotics and Sider honey were assessed by the disc diffusion method. **Results:** Out of 176 examined specimens, 124(70.45%) were contaminated by bacteria. The higher rate of bacterial infection was recorded among the age group of 16-25 years (75%), having wound infection in the leg (81.25%), wound period for four weeks (82.35%), from hospital inpatient (82.35%), with abscess (82.61%), injured previously, and not using antibiotics. The most isolated bacteria species from wound infection was *Staphylococcus aureus* (49.01%) followed by *Pseudomonas aeruginosa* (23.18%), *Escherichia coli* (8.61%), *Streptococcus pyogenes* (4.64%), and *Citrobacter* sp., and *Bacillus* sp. (3.97% in each), *Klebsiella pneumonia* (3.31%), *Klebsiella* sp. (1.99%), and *Streptococcus pneumonia* (1.32%). The *S. aureus, S. pyogenes, K. pneumonia,* and *S. pneumonia* were sensitive to piperacillin/tazobactam and resistant to cefepime. Also, *P. aeruginosa* showed high resistance to all used antibiotics. While *E. coli* was totally sensitive to cefepime and resistant to piperacillin/ tazobactam. Also, Sider honey revealed had highly antimicrobial against *P. aeruginosa* and *S. pyogenes, K. pneumonia* bacteria and moderate against *S. aureus, E. coli, Bacillus* sp. isolates. **Conclusion:** The obtained results consider the alarming for serious problem for the health system in upcoming years might be resulting from antibiotic-resistant bacteria. Therefore, further experimental are required to evaluate the effectiveness of honey on bacterial resistance.

Keywords: Antibacterial, Wound Infections, Sider Honey, Hajjah City, Yemen

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Introduction

A wound is any physical injury involving a break in the integrity of the skin or tissues and exposed subcutaneous tissues provide a favourable substratum for a wide diversity of microbial colonization and proliferation [1,2].

Infection of the wound occurred by the entry of the microorganisms through broken skin which leads to stop healing and produce signs and symptoms[3]. Wound infections could be caused by a diversity of microbes ranging from bacteria to fungi and some parasites, and viruses [4].

Wound infections by bacteria are the most common nosocomial infections causing more than 80% of the mortality [5]. Several reports documented that the most isolated bacterial genera infecting wounds are *Staphylococcus*, *Pseudomonas, Escherichia*, *Enterobacter, Klebsiella, Enterococci, Streptococcus, Proteus,* and *Acinetobacter* [6,7,8].

Patients with wounds are contaminated by numerous bacteria from resident bacteria in the skin and whole body. Also, hospital-acquired infection is becoming a major concern affecting patients during the hospitalization period[9]. Most bacterial infections of hospital-acquired are resistant to most common antibiotics which can slow down the healing process and prolonged hospital stay and increase the cost of treatment [10].

The high occurrence of resistant bacteria to common antibiotics has become a major threat among patients and healthcare workers. Uncontrolled and rapidly increasing bacterial colonization has turned the controlling of wound infections into a serious challenge [11,12].

So, an alternative therapy is urgently needed to address the issue. Honey exhibits excellent antimicrobial properties that have been well known and have been used from earlier as a method of hastening wound healing[13]. A possible reason behind its activity relies on its ability to generate hydrogen peroxide by the bee-derived enzyme glucose oxidase[14,15]. Also, one of the most important properties of honey remains its broad-spectrum antibacterial activity[16].

Different types of honey vary in their therapeutic effectiveness. Abd-El Aal *et al*.[17] observed that honey had a higher noticeable inhibitory effect on *Pseudomonas aeruginosa*, *Enterobacter* sp., *Klebsiella* sp. bacteria. Complete inhibition was documented among infected cases by methicillin-resistant *Staphylococcus aureus* when compared to the use of antibiotics alone. Also, the higher effect was recorded when applied honey together with the antimicrobial agents as a synergistic in both Gram-negative and positive bacteria.

The present study was designed to assess the antibacterial susceptibility and Sider honey effect against isolated bacteria from wound infection of patients attending at Al-Gmohori hospital in Hajja City, Yemen.

Materials and Methods

Study area and period

The current study was conducted throughout the period from July to September 2021 among wound patients who were hospitalized at Al-Gmohori hospital in Hajja City. Yemen.

Specimens collection

One hundred and seventy-four (174) swab specimens were randomly sampled from wound patients attending at Al-Gmohori hospital in Hajja City, Yemen. The swab specimens were sampled by surgical physicians from the infected area by using a sterile cotton swab and directly transported to the microbiology laboratory for microbiological examination.

Ethical approval

The ethical declaration of this study was permitted by the Ethics Research Committee of the Applied Sciences College, Hajjah University.

Data collection

A structured questionnaire was applied to collect the required information from infected wounds patients. The questionnaire included the age, duration of hospitalization, location of a patient, wound location, type of wound, injured previously, and antibacterial using were gathered from each patient via face-to-face interview.

Bacterial cultivation and identification

The collected specimens were independently inoculated into the surface of McConkey agar, Blood agar, and Chocolate agar and incubated aerobically for 24 hrs at 37°C. The bacteria isolates were identified according to standard procedures such as morphological, microscopy, and biochemical tests [18].

Honey sample

The Sider honey sample was purchased from Bani-Qais, Hajjah governorate.

Preparation of honey discs

Honey discs (6 mm diameter) were prepared by using filter paper (Watman No.1) and impregnated with the undiluted Sider Honey small glass bottle **.**

Antibacterial susceptibility testing

Antimicrobial susceptibility testing of bacterial isolates was done by the Kirby-Bauer disks diffusion technique on Mueller-Hinton agar. Five antibiotic discs have been used that include; cefepime (30µg), gentamycin (10µg), kanamycin (30µg), vancomycin (30µg), and piperacillin/ tazobactam (100/10µg) discs (HiMedia Comp., India). The antibiotic and the prepared honey discs were placed on the Mueller Hinton agar and incubated for 24 h. The bacterial inhibition zone was measured and interpreted according to the Clinical and Laboratory Standard Institute [19].

Results

Socio-demographic characteristics

The highest specimens of wounds infection were collected of age group 16-25 years, wound period for four weeks, and inpatients. Also, it was observed the most collected specimens from the patients had wound infections in the leg, abscess form, didn't injure previously, and used antibiotics (Table 1).

Table 1: Socio-demographic characteristics of enrolled patients

Variables	Examined No. (%)	Variables	Examined No. (%)
Age group (in years)		**Wound location**	
≤15	36(20.46)	Leg	64(36.36)
16-25	64(36.36)	Abdomen	28(15.91)
26-35	44(25)	Hand	32(18.18)
36-45	20(11.36)	Head neck	20(11.36)
≥46	12(6.82)	Foot	32(18.18)
Wound period		**Type of wound**	
1 week	36(20.46)	Trauma	28(15.91)
2 week	40(22.72)	Postoperative wound	56(31.82)
3 week	32(18.81)	Abscess	92(52.27)
4 week	68(38.64)	**Injured previously**	
Location of patient		Yes	80(45.45)
Outpatient	32(18.18)	No	96(54.55)
Preadmission	16(9.09)	**Antibacterial use**	
Inpatient	68(38.64)	Yes	120(68.18)
Operating Room	60(34.09)	No	56(31.82)

Bacterial growth result

In the present result, it was 124(70.45%) of specimens recorded positive for bacterial growth while 52(29.55%) of specimens didn't grow in the culture media (Figure 1).

Table (2) shows that the higher frequency of infection was reported among the age group of 16-25 years whereas the lower rate was noticed among the age group of 36-45 years at 60%.

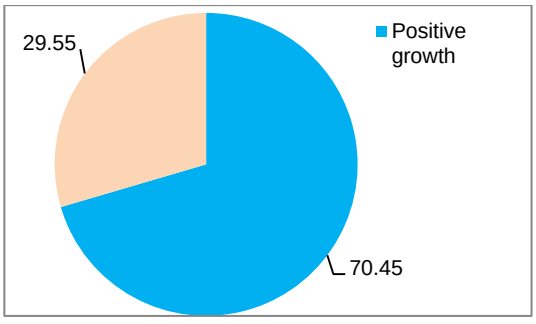

Figure 1: Rate of bacterial contamination

Table 2: Prevalence rate of wound infection concerning age

Age group (in years)	No. examined (%)	Infected (%)	Non-infected (%)
≤15	36(20.46)	24(66.67)	12(33.33)
16-25	64(36.36)	48(75)	16(25)
26-35	44(25)	32(72.73)	12(27.27)
36-45	20(11.36)	12(60)	8(40)
≥46	12(6.82)	8(66.67)	4(33.33)
Total	**176**	**124(70.45)**	**52(29.55)**

Table 3 reveals that the wound specimens collected were from the leg that had a higher rate of bacterial infection while the abdomen specimens had a lower rate of bacterial infection.

Table 3: Frequency rate of wound infection concerning wound location

Wound location	No. examined (%)	Infected (%)	Non-infected (%)
Leg	64(36.36)	52(81.25)	12(18.75)
Abdomen	28(15.91)	16(57.14)	12(42.86)
Hand	32(18.18)	20(62.5)	12(37.5)
Head neck	20(11.36)	12(60)	8(40)
Foot	32(18.18)	24(75)	8(25)
Total	**176**	**124(70.45)**	**52(29.55)**

Table 4 shows the highest rate of bacterial contamination was detected among the patients with a period of four weeks (82.35%) and the lowest rate was found among patients with a period of the first week (55.55%).

Table 4: Frequency rate of wound infection regarding wound period

Wound period	No. examined (%)	Infected (%)	Non-infected (%)
1 week	36(20.46)	20(55.55)	16(44.45)
2 week	40(22.72)	28(70)	12(30)
3 week	32(18.81)	20(62.5)	12(37.5)
4 week	68(38.64)	52(82.35)	12(17.65)
Total	**176**	**124(70.45)**	**52(29.55)**

The current result reports that the higher rate of wound infection was recorded among inpatients followed by operating room, outpatient, and preadmission participants (Table 5).

Table 5: Frequency rate of wound infection regarding patient location

Location of patient	No. examined	Infected (%)	Non-infected (%)
Outpatient	32(18.18)	16(50)	16(50)
Preadmission	16(9.09)	8(50)	8(50)
Inpatient	68(38.64)	52(82.35)	12(17.65)
Operating room	60(34.09)	44(73.33)	16(26.67)
Total	**176**	**124(70.45)**	**52(29.55)**

Figure 2 shows the injured previously had a higher rate of bacterial infection when compared to patients who didn't. Similarly, the patients who didn't administrate antibiotics had a higher rate of bacterial infection.

Identification of isolated bacteria

This result shows that the most isolated bacteria species from wound infection was *S. aureus* 74(49.01%) followed by *P. aeruginosa* 35(23.18%), *E. coli* 13(8.61%), *S. pyogenes* 7(4.64%), and 6(3.97%) for each of *Citrobacter* sp., and *Bacillus* sp. Also, it was 5(3.31%) recorded for *Klebsiella pneumonia*, 3(1.99%) for *Klebsiella* sp. and 2(1.32%) for *S. pneumonia* as figured in Figure (3).

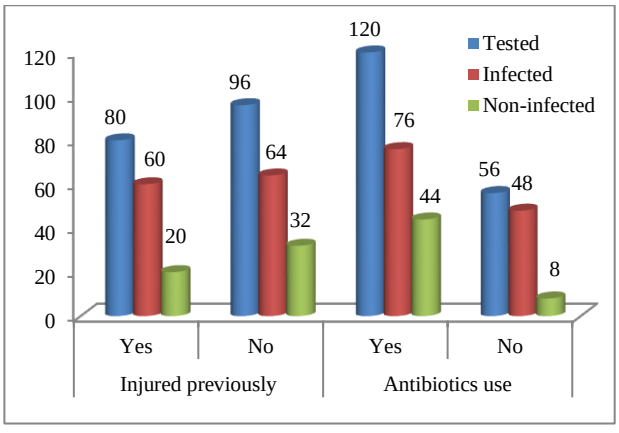

Figure 2: Wound infection in relation to injured previously and antibiotics use

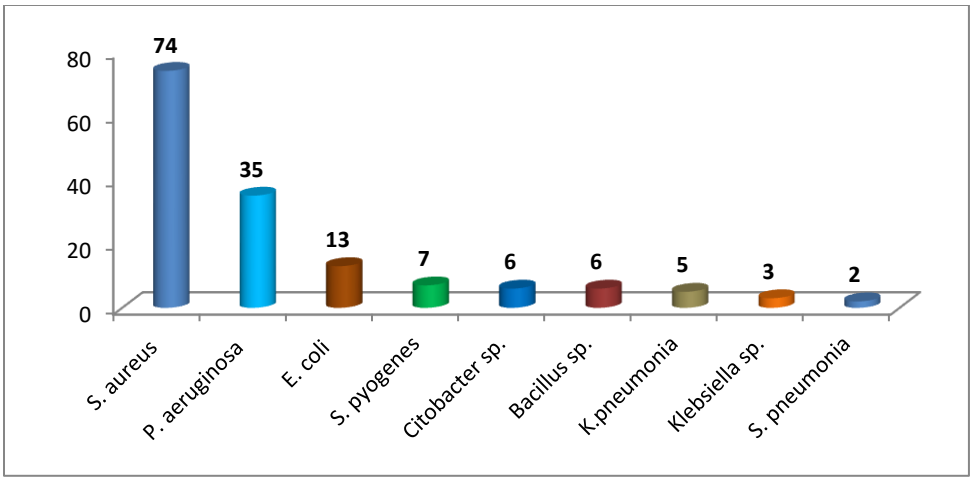

Figure 3: Frequency of bacteria isolates from wound infection

Antibacterial activity results

Table 7 illustrates the antibacterial susceptibility and activity of Sider honey results of bacteria isolated from the infected wounds. *S. aureus* revealed high sensitivity to piperacillin/tazobactam and high resistance to cefepime (81.08%). Also, *P. aeruginosa* showed high resistance to all used antibiotics. *E. coli* showed complete sensitivity to cefepime (100%) and resistance to piperacillin/ tazobactam (92.31%). The vancomycin and piperacillin/tazobactam showed high effectiveness against *S. pyogenes* at 100% and 71.43%, respectively.

However, *Citrobacter* sp. was sensitive to gentamicin and resistant to kanamycin and cefepime. Also, *Bacillus* sp. was sensitive to gentamicin and kanamycin and resistant to cefepime. *K. pneumonia* showed sensitivity to piperacillin/tazobactam and resistance to gentamicin and kanamycin. *S. pneumonia* was sensitive to gentamicin and piperacillin/tazobactam and resistant to kanamycin and cefepime (Table 7).

The antibacterial activity of Sider honey results revealed that the *S. aureus* isolates showed complete resistance at 55.41% while the *P. aeruginosa* and *S. pyogenes* showed highly sensitive at 82.85% and 85.71%, respectively. In different, the Sider honey had moderate activity against *E. coli*.

Table 7: Antibacterial activity of antibiotics and Sider honey

Bacterial species		VA	GEN	PIT	K	CPM	H
S. aureus	S(%)	51(68.92)	38(51.35)	65(87.84)	40(54.05)	14(18.92)	33(44.59)
	R(%)	23(27.38)	36(48.65)	9(12.16)	34(45.95)	60(81.08)	41(55.41)
P. aeruginosa	S(%)	0(0)	12(34.29)	7(20)	10(28.57)	8(22.86)	29(82.85)
	R(%)	35 (100)	23(65.71)	28 (80)	25(71.43)	27(77.14)	6(17.15)
E. coli	S(%)	0 (0)	7(53.85)	1(7.69)	6(46.15)	13 (100)	7(53.85)
	R(%)	13(100)	6(46.15)	12(92.31)	7(53.85)	0 (0)	6(46.15)
S. pyogenes	S(%)	7(100)	4(57.14)	5(71.43)	4(57.14)	1(14.29)	6(85.71)
	R(%)	0 (0)	3(42.86)	2(28.57)	3(42.86)	6(85.71)	1(14.29)
Citrobacter sp.	S(%)	0 (0)	5(83.33)	3(50)	0(0)	0(0)	0 (0)
	R(%)	6(100)	1(16.17)	3(50)	6(100)	6 (100)	6(100)
Bacillus sp.	S(%)	3(50)	6(100)	4(66.67)	6(100)	0 (0)	3(50)
	R(%)	3(50)	0(0)	2(33.33)	0 (0)	6(100)	3(50)
Klebsiella pneumonia	S(%)	0 (0)	1(20)	5(100)	0 (0)	2(40)	1(20)
	R(%)	5(100)	4(80)	0 (0)	5(100)	3(60)	4(80)
S. pneumonia	S(%)	1 (50)	2(100)	2(100)	0 (0)	0(0)	2(100)
	R(%)	1 (50)	0 (0)	0 (0)	2(100)	2(100)	0(0)

VA= Vancomycin; **GEN**= Gentamicin; **PIT**= Piperacillin/Tazobactam; **K**=Kanamycin; **CPM**=Cefepime; **H**=Honey.

Discussion

The prevalence of resistant microorganisms to common antimicrobial drugs in Yemen has been documented well [20]. The present result found that only 70.45% of cases showed positive for bacterial growth in culture media while 29.55% of cases didn't. This finding is lower than the report by Alghalibi *et al*.[21] observed about 83.5% of wound specimens collected from wound patients at Sana'a city had bacterial growth. In contrast, this finding is higher than the study by Alhlale *et al*.[22] who found 56.67% of wound specimens had bacterial contamination. Similarly, it was documented that 62.95% of collected specimens showed positive bacterial growth[23].

This disparity could result from the differences in the method of swab collection, experiences practices of a collector as well as the needed cleaning of the wound surface before specimens collection.

The current work reported that a higher rate of wound infection was recorded among the age group of 16-25 years old. These results are in disagreement with different reports that documented that a higher rate of wound infection was recorded among the age group of 45-59 years by Mama *et al*.[24], more than 50 years by Alghalibi *et al*.[21] and age group of 41-50 years by Edrees and Banafa[23].

In the present result, it was noticed that a high rate of bacterial infection was recorded in specimens obtained from the patient's leg. This finding in agreement with a report of Mama *et al*.[24] revealed that collected wound specimens of the leg were most parts contaminated by bacteria. The leg is the lowest part of the body which is more likely to be susceptible to bacterial contamination from the surrounded environment during movement and activities.

In this work, it was reported that a high frequency of bacterial contamination was observed among patients having a wound for four weeks. This finding is similar to the finding by Edrees and Banafa[22].

The present result showed that the collected specimens from inpatients had a higher rate of bacterial infection than other patients. This finding is similar to the reports by Yakha *et al*.[2]. The high frequency of infection prevalent among hospitalized patients might be resulting from the acquisition of nosocomial pathogens in patients with frequent long-term hospitalization, prior administration of antimicrobial agents, and complicating illness.

This result showed that a higher frequency of wound infection was recorded among specimens collected of patients with abscess followed by postoperative and trauma patients. A similar result reported that the patient wounds with an abscess had a higher rate of bacterial infection[23].

This result showed that the most isolated bacteria species from wound infection was *S. aureus* (49.01%) followed by *P. aeruginosa* (23.18%), *E. coli* (8.61%), *S. pyogenes* (4.64%), and *Citrobacter* sp., and *Bacillus* sp. (3.97% in each), *Klebsiella pneumonia* (3.31%), *Klebsiella* sp. (1.99%), and *S. pneumonia*(1.32%).

A similar study by Edrees and Banafa[23] found that the most predominant bacteria isolated from wound infection were *S. aureus,* followed by *P. aeruginosa*, *E. coli*, *S. pyogenes*, *Klebsiella* sp., *Enterobacter* sp., *Acinetobacter* sp., and *P. mirabilis*.

An investigation by Alghalibi *et al*.[21] revealed that the *S. aureus* was the most bacteria isolates followed by *P. aeruginosa*, *E. coli*, *S. plymuthica*, *P. mirabilis*, *Salmonella* sp., *Acinetobacter* sp., *S. faecalis*, and *Bacillus* sp., *Citrobacter freundii*, *Klebsiella* sp., and *S. pyogenes*. A similar study performed by Alhlale *et al*.[7] observed that the *S. aureus* was the predominant isolated bacteria followed by *E. coli*, *P. aeruginosa*, and *P. mirabilis*.

The high frequency of *S. aureus* and *P. aeruginosa* found among wound infection in the present study refer to these types of bacteria being resident in the human body and easily infecting the opened wound. Also, they are frequently widespread in hospital environments and the occurrence of diseases associated with nosocomial infections[24,25].

E. coli normally lives in the intestinal tract of humans and frequently causes wound infections during defecation. The contamination a wound by this bacterium indicates is known due to poor hospital hygiene [26].

The results of antibacterial susceptibility profiles revealed that the bacterial isolates varied in their sensitivity to all the used antibacterial. It was reported that the *S. aureus* was highly sensitive to piperacillin/tazobactam and moderate to gentamicin and vancomycin antibiotics. Also, this bacterium was high resistance to cefepime.

A similar study by Edrees and Banafa[23] reported that most *S. aureus* isolates showed highly sensitivity to imipenem and erythromycin and resistance to ceftriaxone. Also, Al-Khawlany *et al*.[27] documented that the vancomycin and gentamicin antibiotics had a higher effect against *S. aureus*. Similarly, Mama *et al*.[24] showed that *S. aureus* was high sensitivity to vancomycin and gentamicin antibiotics. In contrast, Edrees and Anbar[12] reported that 80% of isolates *S. aureus* was sensitive to cefepime.

However, this result revealed that most *P. aeruginosa* isolates showed high resistance to gentamicin, piperacillin/tazobactam, kanamycin, and cefepime antibiotics. This result is in agreement with some reports carried out in some regions in Yemen. A different study by Mama *et al*.[24] revealed that gentamicin had high effectiveness on *P. aeruginosa*. Also, it was recorded that the piperacillin/ tazobactam was effective against *P. aeruginosa* [11].

In the current result, it was found that the *E. coli* showed complete sensitivity to cefepime and highly resistant to piperacillin/ tazobactam. This result is similar to the report by Nigussie *et al*. [28]. A similar report by Roy *et al*.[9] detected that *E. coli* was sensitive to ceftriaxone and resistant to ciprofloxacin.

The present work showed that the vancomycin and piperacillin/tazobactam antibiotics were effective against *S. pyogenes*. Similar results documented that the *S. pyogenes* showed sensitivity to vancomycin and high resistant to penicillin and ampicillin [29].

However, the obtained result showed that *Citrobacter* sp. and *Bacillus* sp. were sensitive to gentamicin and resistant to cefepime. Similarly, *K. pneumonia* and *S. pneumonia* showed sensitivity to piperacillin/tazobactam and resistance to kanamycin. In similar study, it was found that the *Klebsiella* sp. was high sensitivity to ceftazidime, amikacin, and tetracycline and moderate resistance to erythromycin and ciprofloxacin.

This result revealed that the Sider honey had moderate antibacterial against *S. aureus, E. coli, Bacillus* sp. isolates. It was shown highly antimicrobial against *P. aeruginosa* and *S. pyogenes, K. pneumonia* bacteria. Whereas, *Citrobacter* sp. showed totally resistance to Sider honey activity. A similar study by Al-Haik *et al*.[30] noticed that the different types of Hadhrami honey (Somur, Sidr, and Meria) have the antibacterial effect against *P. aeruginosa, Proteus vulgaris, E. coli, Enterobacter sp., S. aureus*, and *Klebsiella* sp. bacteria.

Previous reports documented that the high prevalence of resistant bacteria to antimicrobial in some regions of Yemen resulting from the availability of antibiotics as over-the-counter-drugs which anybody can purchase without physicians prescription as well as the quality of antibiotics and drug misuse. In addition, most doctors describe the antibiotic for a patient without referring to antimicrobial susceptibility results or they didn't recommend him to make the antimicrobial susceptibility test [29,31,32].

Conclusion

The obtained findings indicate the high prevalence of antibiotic-resistance bacteria is an alarming increase of diseases due to antibiotic-resistant bacteria. The absence of official antibiotic policies for using antibiotics maybe develop resistant bacteria strains. The resistance of nosocomial bacteria to third and fourth-generation antibiotics is an actual threat to control hospital-acquired infection. So, the honey products as natural are a promising future for remedy the wound infection because they contain a large amount of substance acting as antimicrobial.

Conflict of interest

No conflict of interest is associated with this work.

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