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Original Article

ANTIBACTERIAL ACTIVITY OF HONEY AGAINST ESBL PRODUCING *KLEBSIELLA PNEUMONIAE* FROM BURN WOUND INFECTIONS

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ABSTRACT

Objective: The present study compares the effect of Manuka and Jambhul honey on ten clinical isolates of *Klebsiella pneumoniae* producing Extended Spectrum Beta Lactamases (ESBLs) from burn wound infections.

Methods: The isolated organisms were identified and screened for ESBL production, which was further confirmed by Confirmatory Phenotypic Disc diffusion test and E-test. The antibacterial activity of Manuka and Jambhul honey was analysed using Agar cup method for varying concentrations of 25%, 50%, 75% and 100%. The Minimum inhibitory concentration (MIC) of Manuka and Jambhul honey against the isolates was determined using Agar dilution method.

Results: MIC was found to be in the range of 30%-40% (v/v) for both honey. The average MIC of Manuka for all the 10 isolates was estimated to be 34% whereas that of Jambhul, was 36.5% and this difference was found to be statistically insignificant (P>0.05 t test). Total phenolic content (TPC) in Manuka and Jambhul honey was estimated using Folin's Ciocalteau method and was found to be 1.22 mg TA/gm in Manuka honey and 1.18 mg TA/gm in Jambhul honey.

Conclusion: Thus, the study showed that honey may be an effective antimicrobial agent against ESBL producing *Klebsiella pneumoniae* isolated from burn wound infections. It also proved that antimicrobial activity of Indian Jambhul honey is comparable to New Zealand's Manuka honey.

Keywords: ESBL, Manuka honey, Jambhul honey, E-test, MIC, TPC.

INTRODUCTION

The emergence of drug-resistant organisms in both hospital and community is a major concern worldwide. The most widely used antibiotics for treatment of infections are Beta lactams due to their broad spectrum antimicrobial activity and proven clinical efficacy. There is a rise in resistance to these antibiotics because of production of Extended Spectrum Beta Lactamases (ESBLs) by the organisms [1]. ESBL producing bacteria are resistant to penicillins, third generation cephalosporins and monobactams which are generally used to treat Gram negative bacterial infections. They are commonly inhibited by Beta lactamase inhibitors such as Clavulanic acid, Sulbactam, and Tazobactam. [2] Inappropriate treatment of severe infections caused by ESBL producers have been associated with increased mortality. Hence it is very essential to know patterns of bacterial resistance while using antimicrobial agents for therapeutic purpose [3.]

Burn wound infections are one of the most important and potentially serious complications that occur in the acute period following an injury which is also a major cause of morbidity and mortality in hospitalised burn patients. Burn wound infections are largely hospital-acquired due to the transfer of microorganisms to a patient's skin surface on contact with contaminated external environmental surfaces, invasive diagnostic and therapeutic procedure and immune compromised status of the patients [3]. Burn wounds are subsequently colonized with microorganisms, including Gram-positive bacteria, Gram-negative bacteria and yeasts, which may be derived from the host's normal flora. Necrotic tissue & protein-rich exudates provide a rich growth medium for microorganisms and there is the release of toxic substances by the microbes, which if unchecked may lead to septic shock [4].

The common burn wound pathogens are *Pseudomonas aeruginosa, Klebsiella spp., E. coli* and *Staphylococcus aureus* [3, 4] *Klebsiella* is an opportunistic organism and extensive use of broad spectrum antibiotics in hospitalized patients has led to increase in development of multidrug resistant strains [5] Several studies from India and abroad have reported that hospital outbreaks of multidrug resistant *Klebsiella pneumoniae* are often caused by Extended Spectrum Beta Lactamase (ESBL) producers [6-8] Infections caused by ESBL-producing *K pneumoniae* are associated with higher mortality rate as compared to other ESBL producing organisms [9]. *Klebsiella pneumoniae* frequently contains plasmid encoded Extended spectrum beta lactamase (ESBL) producing genes, which are associated with beta lactam resistance and are easily transferred to other non-resistant strains [9]. With enhanced ability of transferable resistance for antibiotics, *Klebsiella* is responsible for majority burn wound associated infections [10]. With the increase in emergence of antibiotic resistant strains of *Klebsiella pneumoniae* in burn wound infections, evaluation of alternative potential therapeutic agents to control and eradicate them has become essential [6-10].

Honey has been used as a traditional remedy for burn wound infections since ancient times because of its antimicrobial and tissue healing properties [11] It has been observed that Honey reduces inflammation and pain, increases the rate of healing by stimulation of angiogenesis and epithelisation [12]. On burn sites, its antibacterial and anti-inflammatory properties allows a moist healing environment to be maintained that protects the wound from deterioration and fibrosis [13]. The antimicrobial activity of honey is attributed largely to osmolarity, low pH, hydrogen peroxide production and the presence of other phytochemical components from its floral sources [12, 14]. The variation in its antibacterial activity depends on factors like geographical location, season, botanical source, harvesting and processing and storage conditions. Phytochemical components are derived from the floral source of the honey and can confer activity that is stable in the presence of enzyme, catalase, which destroys hydrogen peroxide [12, 14]. In burn wound infections where honey is used as a topical antimicrobial and wound dressing, non-peroxide activity may be advantageous as it is not destroyed by catalase present in body fluids [15]. This non-peroxide activity was first identified in unifloral Manuka honey which originates from the Manuka plant (*Leptospermum scoparium*) from New Zealand, where it is often marketed as the Unique Manuka Factor (UMF) and sold as a

therapeutic agent worldwide [16]. Another unifloral honey having medicinal properties used in this study is Jambhul honey obtained from plant *Syzygium cumini* found in western India [17].

There are many reports published about medicinal properties of Manuka Honey but very few reports about the medicinal value of Jambhul honey, [15-17] hence this research was carried out to compare the antibacterial activity of Manuka and Jambhul honey. The aim of this present study is to carry out *in vitro* evaluation of antibacterial activity of Manuka honey, a native from New Zealand and Jambhul honey, a native from India, against ESBL producing *Klebsiella pneumoniae* strains isolated from burn wound infections.

MATERIALS AND METHODS

Honey samples

Jambhul honey was procured from Phondaghat Pharmacy, Mumbai and Active 12+Manuka honey (Pure gold) from a departmental store. The sterility of both the honey was tested by inoculating a loopful of the undiluted honey on Blood agar, MacConkey's agar, and Sabouraud's agar. Blood agar and MacConkey's agar were incubated at 37 °c for 48 hours and Sabouraud's agar was incubated at room temperature for one week. The sterility of the honey used in the study was thus confirmed. Honey samples were stored in glass containers at room temperature in the dark and the same were used for the entire study [7, 18].

Bacterial strains

Strains of *Klebsiella pneumoniae* were isolated and identified by using standard Microbiological methods from burn wound infections of patients from a tertiary care hospital in Mumbai [19].

Antimicrobial susceptibility test (AST)

The antimicrobial susceptibility was determined by Kirby-Bauer disk diffusion method in accordance with the Clinical and Laboratory Standards Institute (CLSI) guidelines using commercially available antimicrobial discs (Hi Media, Mumbai.). The following antibiotics were used-Ampicillin (10 µg), Amikacin (10 µg), Ceftriaxone (30 µg), Ciprofloxacin (5µg), Gentamicin (10 µg), Amoxyclav (30 µg), Ceftazidime (30 µg), Imipenem (10 µg), Meropenem (10 µg), Piperacillin (10 µg), Piperacillin-Tazobactam (100/10). The bacterial suspensions were prepared using sterile saline to obtain turbidity equivalent to the 0.5 McFarland standards [20].

Screening test for ESBL

Screening test for ESBL production was done by analysing the size of an inhibition zone for Ceftazidime. If the size of an inhibition zone for Ceftazidime (CAZ 30µg) was ≤ 22 mm, ESBL production was indicated, which was further confirmed by phenotypic confirmatory tests as per CLSI guidelines. [20].

Confirmatory phenotypic disc diffusion test

Confirmatory test was carried out by spreading culture using a sterile swab on Mueller and Hinton agar. The turbidity of culture was equivalent to 0.5 McFarland standards. Antibiotic discs of Cefotaxime (30µg) and Ceftazidime (30µg) along with Cefotaxime/Clavulanic acid (30/10) and Ceftazidime/Clavulanic acid (30/10) were placed on the seeded agar. The *Klebsiella* strains were phenotypically confirmed as ESBL producers if there was>5 mm increases in the inhibition zone diameter of either Cefotaxime or Ceftazidime in combination with Clavulanic acid versus Cefotaxime or Ceftazidime alone. [20].

E-test

ESBL detection strips (Hi Media) are drug-impregnated strips in which upper half contains a concentration gradient of three antibiotics; Ceftazidime, Cefotaxime and Cefepime plus Clavulanic acid (MIX+Clav; MIC test range 0.032 to 4µg/ml) and lower half contains of Ceftazidime, Cefotaxime, and Cefepime (MIX; MIC test range 0.125 to 16µg/ml) in a concentration gradient in a reverse direction. If the ratio of the value obtained for MIX/MIX+Clav>8, then the strain was reported and confirmed as ESBL producer as per the application sheet supplied by the manufacturer.

A standard reference strain of *Escherichia coli* (ATCC 25922), susceptible to all antimicrobial drugs tested, and ESBL positive control strain *Klebsiella pneumoniae* ATCC 700603 were used as a quality control for Antimicrobial susceptibility test, Confirmatory phenotypic disc diffusion test and the E-test [20, 21].

Antibacterial activity of honey

The agar-well diffusion method prescribed by CLSI was employed in the antibacterial activity testing. Both Manuka and Jambhul honey were diluted using sterile distilled water to obtain varying concentrations of 25%, 50%, 75% and undiluted as 100%. Each Mueller Hinton (MH) agar plate was uniformly seeded with bacterial suspensions (0.5 McFarland) of the ten *Klebsiella* isolates. A sterile cork-borer was used to punch wells of 8 mm diameter in the MH agar. Approximately 100 µl of the diluted honey of varying concentrations i.e. 25%, 50%, 75% and 100% were added into each well and incubated at 37 °c for 24 hrs. The diameter of zones of inhibition was thereafter measured in mm for all the individual isolates, distilled water served as negative control. This experiment was performed in triplicate and means zone size inhibition was calculated to ensure the reproducibility of the results. [22].

Minimum inhibitory concentration (MIC)

The minimum inhibitory concentration (MIC) of Manuka and Jambhul honey was determined by Agar dilution method as per CLSI guidelines. Dilutions were prepared by mixing both honeys with sterile Mueller Hinton Agar to get final concentrations ranging between 5-50% (v/v). A plate of Mueller Hinton Agar without honey served as a control. These plates were seeded with bacterial suspensions using a loop and incubated at 37 °C for 24 hr. The MIC was recorded as the lowest concentration of honey at which visible bacterial growth was completely inhibited. [23, 24].

Total phenolic content

Total Phenolic content was estimated by using tannic acid (TA) as Standard solution for calibration curve preparation [25] Stock solutions 1 mg/ml of tannic acid was prepared in distilled water. From the above solution concentration of 100µg/ml was prepared and used in the experiment. Different concentration ranging from 4-20 µg/ml was prepared using distilled water. A volume of 0.5 ml FC reagent (1N) was added to each standard flask containing 0.5 ml of honey sample and then 2.5 ml of 20% sodium carbonate solution was added. The mixture was kept for 40 minutes and absorbance was recorded at 725 nm. The Total phenolic content (TPC) was expressed as milligram of Tannic acid equivalent (TA) in one gram of honey. [25, 26] The estimation of TPC for both honeys was carried out in triplicates.

RESULTS

Both Manuka and Jambhul honey were found to be sterile when inoculated on Blood agar, MacConkey's agar, and Sabouraud's agar. During the antibiotic sensitivity of ten strains of *Klebsiella pneumoniae,* it was found that 100% strains were resistant to Ampicillin, Amoxyclav, Ceftriaxone and Ceftazidime. 60% strains were resistant to Ciprofloxacin, 20% strains were resistant to Amikacin and Gentamicin, and in contrast all strains were sensitive to Imipenem, Meropenem and Piperacillin/Tazobactam. (table 1).

In vitro resistance of strains to Ceftazidime indicated the production of ESBL's. This was phenotypically confirmed by Phenotypic confirmatory disc test and E test. The ten strains showed an increase in inhibition zone diameter by>5 mm for both, Cefotaxime and Ceftazidime, when combined with Clavulanic acid as compared to the usage of Cefotaxime and Ceftazidime alone, thus confirming ESBL production. In the E-Test, the ratio obtained for MIX/MIX+Clav was>8, thus all the ten strains were phenotypically confirmed as ESBL producers.

Primary screening for *in vitro* antibacterial activity of honey samples was carried out by Agar well diffusion method. Unifloral, Manuka and Jambhul honey samples showed activity against all ten *Klebsiella* isolates. An increasing zone size was observed for 50%, 75% and 100% (50<75<100). The mean diameter size of inhibition zones for concentration of 50% was obtained in the range of 23-27 mm, for

75% concentration it was 33-36 mm and for 100% concentration it was 36-41 mm, for both Manuka and Jambhul honey. No zone of inhibition was seen for the concentration of 25% for both. (table 2).

Further, MIC was evaluated by Agar dilution method and the MIC for Manuka and Jambhul honey was found to be in range of 30-40%. (table 3).

Table 1: Pattern of antibiotic susceptibility of 10 *Klebsiella pneumoniae* isolates

Antibiotics	AK	AMP	AMC	CAZ	CIP	CTR	GEN	IMP	MRP	PIT
Resistant										
K. p isolates	2 (20)	10 (100)	10 (100)	10 (100)	6 (60)	10 (100)	2 (20)	0 (0)	0 (0)	0 (0)
Sensitive										
K. p isolates	8 (80)	0 (0)	0 (0)	0 (0)	4 (40)	0 (0)	8 (80)	10 (100)	10 (100)	10 (100)

Values in parenthesis indicates percentage, AK-Amikacin, AMP-Ampicillin, AMC-Amoxyclav, CAZ-Ceftazidime, CIP-Cipfrofloxacin, CTR-Ceftriaxone, GEN-Gentamicin, IMP-Imipenem, MRP-Meropenem, PIT-Piperacillin/Tazobactam.

Table 2: Mean diameter size of Inhibition Zone (mm)

Concn.	25%		50%		75%		100%	
	J	M	J	M	J	M	J	M
Isolate no.								
I-1	-	-	23.8 ± 0.3	24 ± 0	33.3 ± 0.5	33.7 ± 0.5	39 ± 0	39.3 ± 0.5
I-2	-	-	25.7 ± 0.6	25.7 ± 0.5	34.8 ± 0.2	35.3 ± 0.5	40.3 ± 0.5	39.8 ± 0.2
I-3	-	-	24.8 ± 0.8	24.5 ± 0.4	34 ± 0	34.2 ± 0.2	38.5 ± 0.4	39 ± 0
I-4	-	-	24.7 ± 0.6	25.5 ± 0.4	34.5 ± 0.4	33.7 ± 0.5	38.5 ± 0.4	40.5 ± 0.4
I-5	-	-	25.5 ± 0.5	26 ± 0	34.3 ± 0.5	35.7 ± 0.5	38 ± 0	41 ± 0
I-6	-	-	25.8 ± 0.3	24.3 ± 0.5	35.3 ± 0.5	35.5 ± 0.4	38.2 ± 0.2	39.7 ± 0.5
I-7	-	-	24.2 ± 0.3	26.5 ± 0.4	34.2 ± 0.2	34.3 ± 0.5	37.3 ± 0.5	39 ± 0.4
I-8	-	-	25.8 ± 0.3	27.3 ± 0.5	33.3 ± 0.5	34.8 ± 0.2	36.2 ± 0.2	39.7 ± 0.4
I-9	-	-	27 ± 0	26 ± 0	35.7 ± 0.5	36 ± 0	38.3 ± 0.5	39 ± 0
I-10	-	-	24.8 ± 0.3	25.8 ± 0.2	33.3 ± 0.5	35 ± 0	35.7 ± 0.5	37.2 ± 0.6

J-Jambhul honey, M-Manuka honey, '-'–no zone of inhibition

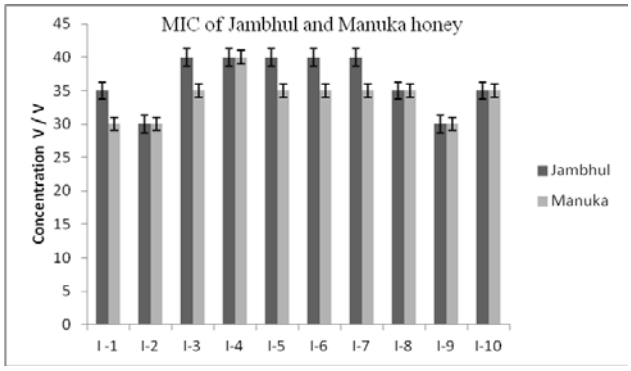

Fig. 1: Average MIC of Jambhul and Manuka honey against 10 clinical isolates of *Klebsiella pneumoniae*

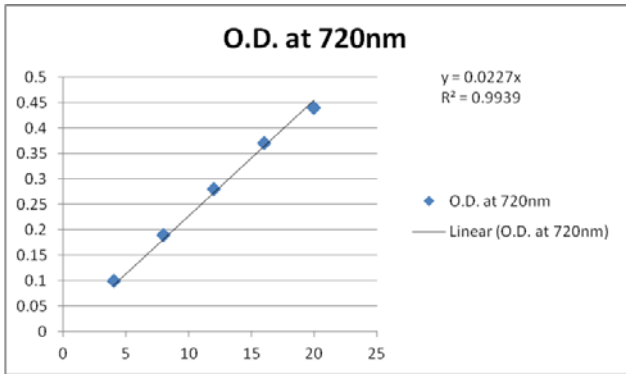

Fig. 2: Total phenolic content of Jambhul and Manuka honey

Statistical analysis

It was found by Statistical analysis that the difference in MIC of both the honeys for ten isolates of *Klebsiella pneumoniae* was insignificant (P^*>0.05 t test). The difference in TPC content of both was also found to be insignificant (P^*>0.05 t test).

DISCUSSION

In India, ESBL producing strains of Enterobacteriaceae have emerged as a challenge over last decade, majority of which are *Klebsiella* and *E. coli*. [7] The wide spread use of Cephalosporins and Aztreonam may be the probable reason for emergence of mutant strains which produce ESBL [8]. *Klebsiella pneumoniae* is a pathogen frequently found in burn wound infections and its management and treatment has become challenging due to lack of effective antibiotics [27].

Honey is generally recommended for healing of wound infections because it is non-irritant, non-toxic, easily available and inexpensive compared to antibiotics [28]. Though hydrogen peroxide is the major contributor to the antimicrobial activity of honey, however nonperoxide factors also play a critical role. Nonperoxide factors like phenolics, lysozyme, and flavonoids are related to the floral source and sometimes account for the major part of the antibacterial activity in Unifloral honey. Manuka and Jambhul honey are Unifloral honey, which indicates their medicinal benefits and thus warrants a need to study their role in curbing menace of ESBL producing pathogens. [29, 30].

This study was designed to study susceptibility pattern of ESBL producing strains of *Klebsiella pneumoniae* against antibiotics, Manuka and Jambhul honey under *in vitro* condition. All the strains were found to be resistant to cephalosporins and sensitive to carbapenems justifying the usage for carbapenem for treatment in infections caused by them [31]. Due to this reason, carbapenems have become the drug of choice to treat such infections. But excessive use of carbapenems has caused dissemination of

resistance determinant genes by the organism, which has led to the emergence of carbapenem resistant strains. Outbreaks due to these strains have been associated with higher morbidity and mortality [31]. Thus an alternative method of treatment should be considered to control this spreading resistance. Honey can be one such alternative medicine which has been used since ancient days. Besides its antimicrobial properties, honey can clear infection in a number of ways, including boosting the immune system, having anti-inflammatory and antioxidant activities, and via stimulation of cell growth.

Table 3: MIC of Jambhul and Manuka honey against 10 *Klebsiella pneumoniae* isolates by Agar dilution method

	Concentration % (v/v)									
Isolate no.	I-1	I-2	I-3	I-4	I-5	I-6	I-7	I-8	I-9	I-10
Honey										
Jambhul	35	30	40	40	40	40	40	35	30	35
Manuka	30	30	35	40	35	35	35	35	30	35

The average MIC of all the ten isolates for Manuka was found to be 34% (v/v) and for Jambhul it was 36.5% (v/v). (fig. 1). The total phenolic content estimated by Folin's Ciocalteu method for Jambhul honey was found to be 1.22 mg of TA/gm of honey whereas that of Manuka was found to be 1.18 mg of TA/gm of honey (fig. **2**). The standard curve equation is $y = 0.022x$, $R^2 = 0.993$.

Thus honey has a multidirectional mode of healing infections. The agar cup assay showed that all the strains were inhibited by 50%, 75% and 100% of honey concentrations. Since all the ten strains of ESBL producing K. pneumoniae were inhibited by both the type of honey, it confirms their effectiveness in treating wound infections caused by them. The average MIC of all the ten isolates for Manuka was estimated to be 34% whereas that of Jambhul was 36.5 %. The difference in MIC of both the honey was found to be statistically insignificant (P>0.05 t test). The presence of phenolic compounds in both Unifloral honey, Manuka and Jambhul is indicative of their stable antibacterial activity and also emphasizes their promising medicinal benefits. The study thus highlighted that honey is capable of inhibiting ESBL producing *Klebsiella pneumoniae.* The anti-inflammatory activity of honey can slow down and reduce the release of exudates, thus preventing bacterial colonization. It is very unlikely that bacteria would pose resistance to honey because of its multidirectional mode of antibacterial action [32].

This study also proved that activity of Indian Jambhul honey is comparable with that of New Zealand's Manuka honey in inhibiting ESBL producing *Klebsiella pneumoniae* from burn wound infections. Jambhul being locally available in India, it will be easier to procure and shall also be more economical, if it is used for treatment of burn wound infections. Since the antibacterial activity of Jambhul was found to be on par with Manuka honey, there is a need to explore whether Jambhul honey contains some unique component like Manuka's UMF which will add more reliability and credibility to its antibacterial activity.

The emergence of ESBL production has posed a large threat to the use of many antibiotics especially the cephalosporins. With increase in resistance to the available antibiotics it has become essential to monitor the use of existing antibiotics and simultaneously search for new antimicrobials or else we will face a situation like pre-antibiotic era. It is also time to explore our traditional medicines and utilize them to treat infections. The therapeutic potential of honey is grossly underutilized, thus calling for clinical trials especially with native honeys like Jambhul and to ascertain their medicinal benefits *in vivo*.

CONCLUSION

In our study, under *in vitro* conditions both Manuka and Jambhul honey were effective in inhibiting ESBL producing strains of *Klebsiella pneumoniae*. Therefore both honeys have the potential to be used as a topical agent in burn wound infections caused by *Klebsiella pneumoniae* which are resistant to third generation Cephalosporins. Antibacterial activity of Jambhul honey was comparable to Manuka honey, indicating the potential of Jambhul honey to be used in prophylaxis of burn wound infections caused by *Klebsiella pneumoniae*.

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CONFLICT OF INTERESTS

The authors declare that they have no competing interest with whomsoever.

REFERENCES

1. Chaudhary U, Aggarwal R. Extended spectrum beta lactamases (ESBL)-an emerging threat to Clinical therapeutics. Indian J Med Microbiol 2004;22:75-80.
2. Paterson DL, Bonomo RA. Extended-spectrum Beta-lactamases A clinical update. Clin Microbiol Rev 2005;18:657–86.
3. Mehta M, Dutta P, Gupta V. Bacterial isolates from burn wound infections and their antibiograms: a eight-year study. Indian J Plast Surg 2007;40:25-8.
4. Sinai WM. Isolation and identification of aerobic pathogenic bacteria from burn wound infections. J Al-Nahrain University 2007;10:94-7.
5. Alaa H, Al-Charrakh, Samira YY, Al-Janabi SH. Occurrence and detection of Extended-spectrum beta lactamases in *Klebsiella* isolates in Hilla, Iraq. Afr J Biotechnol 2011;10:657-65.
6. Podschun R, Ullmann U. *Klebsiella* spp. as nosocomial pathogens: epidemiology, taxonomy, typing methods, and pathogenicity factors. Clin Microbiol Rev 1998;11:589–603.
7. Shukla I, Tiwari R, Agarwal M. Prevalence of extended-spectrum beta-lactamase producing *Klebsiella pneumoniae* in a tertiary care Hospital. Indian J Med Microbiol 2004;22:87-91.
8. Archana G, Krow A, David R, Lisa S. Extended spectrum beta lactamase-producing *Klebsiella pneumoniae* infections: a review of the literature. J Perinatol 2003;23:439–43.
9. Jason WB, Janelle LR, Duane RH, Steven EW, Kevin KC, Katrin M, *et al.* Impact of extended spectrum beta-lactamase producing klebsiella pneumoniae infections in severely burned patients. J Am Coll Surg 2010;211:391-9.
10. Rajeshwar SR, Jaya LL, S Pavani, Vijendra K, Jaya SP. Bacteriological profile, antibiogram of burn wound isolates and detection of MRSA and ESBL production at tertiary care hospital, Hyderabad. World J Pharm Pharm Sci 2014;3:1691-8.
11. Agbaje EO, Ogunsanya T, Aiwerioba OI. Conventional uses of Honey. Ann Afr Med 2006;5:78-81.
12. Molan P. The antibacterial activity of honey-2. Variation in the potency of the antibacterial activity. Bee World 1992;73:59-76.
13. Molan P. Potential of honey in the treatment of wounds and burns. Am J Clin Dermatol 2001;2:13-9.
14. Manisha DM, Shyamapada M. Honey: its medicinal property and antibacterial activity. Asian Pac J Trop Biomed 2011;4:154-60.
15. Molan PC. A brief review of the use of honey as a clinical dressing. Australian J Wound Management 1988;6:148-58.
16. Orla S, Anthony D, Rahma A, Alice P, Georgina G, Seamus C, *et al.* Comparison of the antimicrobial activity of Ulmo honey from Chile and Manuka honey against methicillin-resistant *Staphylococcus aureus, Escherichia coli* and *Pseudomonas aeruginosa*. BMC Complementary Altern Med 2010;10:47.
17. Sunita DD, Kirti SK. *In vitro* effect of some Indian honeys on Staphylococcus aureus from wounds. Indian J Exp Biol 2010;48:931-5.

18. Omoya FO. Sterility examination and comparative analyses of inhibitory effect of honey on some gram negative and gram positive food borne pathogens in south west nigeria. Int J Med Health Pharm Biomed Eng 2013;7:64-6.
19. Washington C, Allen SD, Janda WM, Koneman EW, Schreckenberger PC. Color atlas and textbook of diagnostic microbiology. 6th ed. UK: Lipincott Williams Wilkins Pubishers; 2005.
20. Clinical Laboratory Standards institute. Performance Standards for Antimicrobial Susceptibility Testing. 22nd Informational Supplement. M100-S22;32:3, Wayne, PA; 2012.
21. Wafaa J, Rotimi VO, Fatima K, Rolla S, Aleyamma P, Ghyada AH. Prevalence of Extended-spectrum beta-lactamases in enterobacteriaceae, Pseudomonas and stenotrophomonas as determined by the VITEK 2 and E test systems in a kuwait teaching hospital. Med Princ Pract 2005;14:325–31.
22. Adeleke OE, Olaitan JO, Okpekpe EI. Comparative antibacterial activity of honey and gentamicin against *Escherichia coli* and *Pseudomonas aeruginosa*. Ann Burns Fire Disasters 2006;19:201-4.
23. Clinical Laboratory Standards institute. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically; Approved Standard. 9th Ed. M 07-A9;32:2, Wayne, PA; 2012.
24. Mullai V, Menon T. Antibacterial activity of honey against Pseudomonas aeruginosa. Indian J Pharmacol 2005;37:403.
25. Makkar HP, Bluemmel M, Borowy NK, Becker K. Gravimetric determination of tannins and their correlations with chemical and protein precipitation methods. J Sci Food Agric 1993;61:161-5.
26. Suhad SH, Viorica I. *Lythrum salicaria* (purple loosestrife) medicinal use, extraction and identification of its total phenolics compounds. Farm 2009;57:192-200.
27. Christian GG, Dominique LM, Otto C, Yehuda C. Clinical and economic impact of common multidrug resistant gram-negative bacilli. Antimicrob Agents Chemother 2008;52:813-21.
28. Zumla A, Lulat A. Honey–a remedy rediscovered. J R Soc Med 1989;82:384-5.
29. Willix DJ, Molan PC, Harfoot CG. A comparison of the sensitivity of wound infecting species of bacteria to the antibacterial activity of Manuka honey and other honey. J Appl Bacteriol 1992;73:388-94.
30. Irish J, Blair S, Carter DA. The Antibacterial activity of honey derived from australian flora. PLoS ONE 2011;6:e18229.
31. Khajuria A, Praharaj AK, Kumar M, Grover N, Aggarwal A. Multidrug resistant NDM-1 metallo-beta-lactamase producing *Klebsiella pneumoniae* sepsis outbreak in a neonatal intensive care unit in a tertiary care center at central India. Indian J Pathol Microbiol 2014;57:65-8.
32. Bernard Dixon. Bacteria can't resist honey. Lancet Infect Dis 2003;3:116.