

Original Research Article

COMPARATIVE ANTIBACTERIAL ACTIVITY OF HONEY AND GENTAMICIN AGAINST ~~CLINICAL ISOLATES~~ KLEBSIELLA SPECIES

Abstract

Klebsiella species are one of the major cause of systematic infections, these gram negative organisms are also capable of housing various resistant genes to resist the potency of convectional antibiotics. These observations account for the need to assess the antibacterial activity of Gentamicin and natural products against *Klebsiella*. A total of fifty (50) clinical isolates of *Klebsiella* species of different pathological sources were collected from four different Teaching Hospitals in Southwest, Nigeria. The identification of all the isolates was done using conventional biochemical tests. Antibiogram was carried out on all the 50 *Klebsiella* clinical isolates using multiple antibiotic discs and the sensitivity of honey was done using the agar diffusion method. In the antimicrobial susceptibility test on honey, two undiluted different samples of honey (Honey A Refined and Honey B Natural) showed high activity and 1:2 to 1:6 aq. dilutions showed less activity against the *Klebsiella* isolates. Gentamicin used in concentration of 4.0µg/ml has great activity against the isolates but was basically lower than the antibacterial activity of each undiluted honey. In the occasion of therapeutic disaster with gentamicin or any other associated antibiotics, honey offers an appropriate and improved alternative in dealing with infected burn wounds and other infections like urinary tract infections, nosocomial infection etc.

Keywords: *Klebsiella* species, Antibiotic resistance, Honey, Natural product.

Introduction

Klebsiella species are Gram-negative, non-motile bacilli, lactose-fermenting, facultative anaerobic rod-shaped encapsulated bacteria which can appear mucoid lactose fermenter on MacConkey agar. Though, they are found in the normal flora of the mouth, skin, and intestines, it can cause critical changes to human and animal lungs if aspirated, precisely to the alveoli resulting in bloody sputum.¹³ It was known over 100 years ago as a cause of community-acquired pneumonia. *Klebsiella pneumoniae* is clinically the most essential member of the *Klebsiella* Genus *Enterobacteriaceae*; it is narrowly connected to *Klebsiella oxytoca* from which it is differentiated by being indole-negative.

Gentamicin remains a standard antibiotic noted for its action against Gram-negative bacteria, particularly in mixture with vancomycin or a penicillin⁷. At a concentration of 4.0µg/ml, it has great activity against gram-negative bacteria². Likewise, honey has been related with antibacterial and antifungal activity¹⁷. Specifically, *Klebsiella* species were among the isolates that had their growth inhibited by honey.³ Ibrahim⁶ and Jeddar *et al.*^{6,8,8} reported bactericidal activity of honey on *Salmonella* spp. and *Shigella* spp. as also enteropathogens such as *E. coli*, *Vibrio cholerae* and other Gram-negative and Gram-positive bacteria.

A comparative study has however recognized honey as a more effective medicine than some antimicrobial compounds. This was the situation found between honey and certain antibiotics^{10, 16,15,4}. This study reports the antibacterial activity of honey from two different sources and of gentamicin on isolates of *Klebsiella* species from different pathological sources.

Materials and Methods

Bacteriology

Total number of fifty isolates of *Klebsiella* species from various bacteriological sources (Table I) were collected on

Formatted: Superscript

sterile nutrient agar (OXOID) slants from the Routine Section of the Medical Microbiology Laboratory of four different Teaching Hospitals across Southwest, Nigeria which are University College Hospital, Ibadan, Nigeria, Lagos University Teaching Hospital, Lagos, Nigeria, Ladoke Akintola Teaching Hospital and Federal Teaching Hospital, Ido-Ekiti, Ekiti State. The *Klebsiella* isolates were primary purified-identified on Macconkey-MaCconkey agar. The isolates were confirmed by different biochemical test and then preserved on fresh nutrient agar slants in a refrigerator at 4 °C.

Honey

Honey was gotten from two pure natural honey collection centers (A and B) in Ibadan, South West Nigeria. Every stock was used undiluted and also as fresh aq. dilutions of 1:2, 1:4, 1:6 and 1:8 against the respective bacterial isolates tested.

Gentamicin

Gentamicin sulphate (BP), a product of Greenlife Pharm co, India, was obtained in ampoule vials (2 ml) from a local pharmacy store. The antibiotic was used in 4µg/ml (aq.) dilutions alongside honey against every bacterial isolate.

Sensitivity test

The agar-cup diffusion method^{3, 14} was employed to obtain the susceptibility pattern of the bacterial isolates against each undiluted honey and its fresh aq. Dilutions and of 4µg/ml of gentamicin. Considerations for the sensitivity and resistance of bacteria were based on the extent of the presence or absence of zones of growth inhibition.¹⁴

Table I: Pathological/Bacteriological sources of *Klebsiella* species

<u>Pathological Source/clinical samples</u>	<u>Number of Isolates</u>
Urine	22
Blood	6
Sputum	6
Wound	5
Semen	3
Eye Swab	2
Ear Swab	1
Stool	1
H.V.S	1
E.C.S	1
S.F.A	2

Result

Samples of honey from sources A and B, as also gentamicin in 4.0µg/ml dilutions, exhibited varying levels of antibacterial activity against the bacterial cultures tested as indicated by zones of growth inhibition (*Table II*). Undiluted honey from each source produced the strongest activity, followed by 1:2 and 1:4 dilutions in decreasing order. Undiluted honey from each source produced the strongest activity, followed by 1:2 and 1:4 dilutions in decreasing order; dilution 1:8 did not show any activity on majority of the isolates.

Relative percentage resistance of clinical isolates of *Klebsiella* species to honey. In honey A (Refined) 30% of the clinical isolates were resistance when not diluted, in honey B (Natural) only 22% showed resistance when not diluted (*Table III*). However, 39% of the clinical isolates showed resistance to gentamicin (4µg/ml).

Table II: Sample Results of Sensitivity Test on Honey, Gentamicin and Amoxicillin against *Klebsiella* species

NUMBER OF CLINICAL SAMPLES ISO LATE NUMBER	BACTERIAL ISOLATES CLINICAL SOURCES	HONEY (A) REFINED (mm)					HONEY (B) NATURAL (mm)					GENTAMICIN 4.0ug/ml **	
		0	1:2	1:4	1:6	1:8	0	1:2	1:4	1:6	1:8	A	B
001 Urine	<i>Klebsiella pneumoniae</i>	24.5	-	-	-	-	15.6	-	-	-	-	16.4	17.9
002 Urine	<i>K pneumoniae</i> sub <i>rhinoscleromatis</i>	12.4	-	-	-	-	10.8	-	-	-	-	15.5	15.5
003 Urine	<i>Klebsiella pneumoniae</i> sub <i>pneumoniae</i>	14.5	-	-	-	-	24.5	20.5	18.9	12.5	10.5	R	R
004 Urine	<i>Klebsiella oxycota</i>	20.5	-	-	-	-	10.5	-	-	-	-	17.5	16.5
005 Urine	<i>Klebsiella pneumoniae</i> sub <i>pneumoniae</i>	15.6	-	-	-	-	10.9	-	-	-	-	20.5	16.5
006 Urine	<i>Klebsiella platicola</i>	20.5	-	-	-	-	16.7	-	-	-	-	15.5	15.5
007 Urine	<i>Klebsiella pneumoniae</i> sub <i>pneumoniae</i>	27.5	20.5	14.5	12.2	-	24.9	18.9	12.5	10.5	8.5	R	R
008 Urine	<i>Klebsiella pneumoniae</i> sub <i>pneumoniae</i>	14.5	-	-	-	-	10.5	-	-	-	-	15.5	15.5
009 Urine	<i>Klebsiella pneumoniae</i> sub <i>pneumoniae</i>	26.7	-	-	-	-	20.9	-	-	-	-	16.6	16.5
010 Urine	<i>Klebsiella pneumoniae</i> sub <i>pneumoniae</i>	17.5	15.4	12.5	-	-	20.9	23.5	15.9	-	-	16.5	16.9
011 Urine	<i>Klebsiella pneumoniae</i>	16.8	-	-	-	-	-	-	-	-	-	R	R
012 Urine	<i>Klebsiella pneumoniae</i>	17.6	10.6	-	-	-	18.5	12.2	-	-	-	18.4	15.5
013 Urine	<i>Klebsiella pneumoniae</i> sub <i>ozaenae</i>	18.5	-	-	-	-	25.9	15.5	-	-	-	R	R
014 Urine	<i>Klebsiella pneumoniae</i> sub <i>pneumoniae</i>	-	-	-	-	-	-	-	-	-	-	R	R
015 Urine	<i>Klebsiella pneumoniae</i> sub <i>pneumoniae</i>	22.5	16.5	11.6	-	-	13.5	-	-	-	-	R	R
016 Urine	<i>Klebsiella pneumoniae</i>	-	-	-	-	-	-	-	-	-	-	R	R
017 Urine	<i>Klebsiella pneumoniae</i>	-	-	-	-	-	12.5	-	-	-	-	R	R

Table II Continued: Sample Results of Sensitivity Test on Honey, Gentamicin and Amoxicillin against *Klebsiella* species

038 Semen	<i>Klebsiella oxytoca</i>	-	-	-	-	-	-	-	-	-	-	R	R
039 Ear Swab	<i>Klebsiella pneumoniae</i>	12.5	-	-	-	-	13.5	12.2	-	-	-	18.5	17.6
040 Ear Swab	<i>Klebsiella pneumoniae</i> sub <i>pneumoniae</i>	11.5	-	-	-	-	21.5	-	-	-	-	R	R
041 Eye Swab	<i>Klebsiella pneumoniae</i> sub <i>pneumoniae</i>	20.5	13.5	12.5	10.8	13.5	13.5	-	-	-	-	17.5	17.5
042 H.V.S	<i>Klebsiella pneumoniae</i>	10.9	-	-	-	-	12.5	-	-	-	-	14.6	16.4
043 E.C.S	<i>Klebsiella oxytoca</i>	20.5	-	-	-	-	20.5	-	-	-	-	R	R
044 S.F.A	<i>Klebsiella pneumoniae</i> sub <i>pneumoniae</i>	14.6	-	-	-	-	15.5	12.5	-	-	-	18.5	22.5
045 S.F.A	<i>Klebsiella pneumoniae</i> sub <i>pneumoniae</i>	-	-	-	-	-	-	-	-	-	-	15.5	14.5
046 Urine	<i>Klebsiella pneumoniae</i> sub <i>pneumoniae</i>	26.5	-	-	-	-	-	-	-	-	-	R	R
047 Stool	<i>Klebsiella oxytoca</i>	-	-	-	-	-	26.5	20.5	-	-	-	17.5	17.5
048 Wound	<i>Klebsiella oxytoca</i>	26.5	20.5	15.5	10.6	-	22.5	16.5	10.5	-	-	19.5	16.5
049 Urine	<i>Klebsiella pneumoniae</i> sub <i>rhinoscleromatis</i>	22.5	19.5	14.5	12.6	-	23.5	18.5	15.5	-	-	17.5	17.5
050 Urine	<i>Klebsiella pneumoniae</i> sub <i>pneumoniae</i>	25.5	20.5	16.5	13.6	-	24.5	17.5	12.5	-	-	18.5	17.5

Key: R – Resistance, N.A – No Activity, 0 - Undiluted Honey,

** - Gentamicin 4.0µg/ml

Table III: Resistance ratios of *Klebsiella* species against honey and gentamicin
 Relative Percentage
 Resistance of Clinical Isolates of *Klebsiella* species to honey, Gentamicin.

HONEY A (REFINED)					HONEY B (NATURAL)					GENTAMICIN
0*	1.2	1.4	1.6	1.8	0*	1.2	1.4	1.6	1.8	4.0µg/ml
30%	72%	74%	82%	92%	22%	60%	72%	84%	88%	39%

Key

0* = undiluted honey

Discussion and Conclusion

Klebsiella spp are opportunistic pathogens mainly involved in infections of the urinary and respiratory tracts of patients with underlying conditions. The bacterium appears to rapidly develop resistance to many antimicrobials, and it is frequently involved in outbreaks in hospital settings¹¹.

Honey is the natural sweet substance obtained from the secretions of the living parts or excretions of plants which the honey bees (*Apis mellifera*) collect and store¹¹. Though honey is used widely in traditional medicine, its use in modern medicine is limited⁵. Honey is used for the treatment of many infections and also used effectively as wound dressing including surgical wounds, burns and skin ulcers, mainly because it speeds up the growth of new tissues and helps to heal the wound, reduces pain and odour quickly⁹.

In this study, undiluted honey from each source produced the strongest activity, followed by 1.2 and 1.4 dilutions in decreasing order; dilution 1:8 does not show any activity on majority of the isolates. Also in this study all undiluted honey and some 1:2 aqueous dilution of honey had more activity than Gentamicin even at 4.0ug/ml and 8.0ug/ml. The result observed between diluted and undiluted honey may be due to the fact that the presence of water in the diluted does honey may have reduced their activity, since many studies have reported diluted honey to have high water activity. This is similar to² that found undiluted honey to have more activity than Gentamicin when tested against some selected Gram negative bacteria². The variations recorded in the antibacterial activity of the types of honey tested were consistent with the reports¹ and have been attributed to delayed levels of hydrogen peroxide/thermal stability of the glucose oxidase enzyme, non-peroxide factors, and the plant/floral source. In this study both refined and natural honey showed activity against clinical isolates *Klebsiella* species. Undiluted natural honey had the highest activity when compared gentamicin. The activity shared by both refined and natural honey at the undiluted phase recorded a fewer number of resistance compared to 4µg/ml of gentamicin.

Variation in the inhibitory activity of honey could be a reflection of differences in honey antibacterial activity¹.

Honey has shown to be remediation as antibacterial activity was found on resistant isolates. Undiluted honey natural honey is more active against *Klebsiella* infection.

Formatted: Superscript

REFERENCES

1. Adeleke O.E and Olaitan P.B (2006). The Antipseudomonal Property of Honey and Gentamicin Annals of Burns and Fire Disasters - vol. XIX - n. (4)140-143.
2. Adeleke O.E., Olaitan J.O., Okpekpe E.I (2006). Comparative Antibacterial Activity of Honey and Gentamicin against *Escherichia coli* and *Pseudomonas aeruginosa*. *Annals of Burns and Fire Disasters - vol. XIX - n. (4)*201-204.
3. Allen K.I., Radwan S., Reid G.M.: Antimicrobial potential of honey on some microbial isolates. *J. Medical and Pharmaceutical Sciences*, 2: 75-9, 2000.
4. Cowan S.T.: "Cowan and Steel's Manual for the Identification of Medical Bacteria" (2nd ed.), 1-30, Cambridge University Press, London, 1974.
5. Greenwood, J. (1993). Reflective practice: a critique of the work of Argyris and Schon. *Journal of Advanced Nursing*, 18(8), 1183-1187.
6. Ibrahim A.S.: Antibacterial action of honey. *Bull. Islam Med.*, 1: 363-5, 1985.
7. Kilka L.J., Goodman J.N.: Antibiotic Interactions. *J. American Medical Association*, 248: 1309, s.d.
8. Jeddar A., Kharsany A., Ramsaroop U.G., Bhamjee A., Hafejee I.E., Moosa A.: The antimicrobial action of honey. *South Afr. Med. J.*, 67: 257-8, 1985.
9. Lusby P.E, Coombes A, Wilkinson JM. (2002). Honey: a potent agent for wound healing. *J Wound Ostomy Continence Nurs.* (6):295-300.
10. Molan P.: "The Curative Property of Honey: The Nature of the Antibacterial Activity and the Bee World", 10-15, Waikato University Press, New Zealand, 2000.
11. Moore, C. ., Moore, S. ., Leecaster, M. ., & Weisberg, S. . (2001). A Comparison of Plastic and Plankton in the North Pacific Central Gyre. *Marine Pollution Bulletin*, 42(12), 1297-1300.
12. Podschun, R., P. Heineken, and H. G. Sonntag. 1996. Hemagglutinins and adherence properties to HeLa and Intestine 407 cells of *Klebsiella pneumoniae* and *Klebsiella oxytoca* isolates. *Zentbl. Bakteriologie, Mikrobiol. Hyg. Ser. A* 263:585-593.
13. Ryan K, Stanley F. 2004. *Sherris Medical Microbiology*, 4th ed. (GC Ray and KJ Ryan, Eds.). McGraw Hill.
14. Singleton P.: "Bacteria in Biology, Biotechnology and Medicine" (4th ed.), 333-8, John Wiley & Sons Ltd, New York, 1999.
15. Stokes E.S., Ridway G.I., Wren G.M.: "Clinical Microbiology" (7th ed.), 20-30, Arnold, London, 1993.
16. Subrahmanyam M., Shahapure A.G., Nagame N.S. et al.: Effects of topical application of honey on burn wound healing. *Annals of Burns and Fire Disasters*, 14: 3-5, 2001.
17. Tysett C., de Rautlin de la Roy Y.: Assays on the study of osmophilic yeasts, organisms causing fermentation of honey collected in France. *Faculty of Pharmacology, University of Nancy Bull.*, 134: 1-26, 1993.