Original Research Article

COMPARATIVE ANTIBACTERIAL ACTIVITY OF HONEY AND GENTAMICIN AGAINST *KLEBSIELLA* SPECIES

Abstract

Klebsiella species are one of the major causes of systematic infections, theses gram negative organisms are also capable of housing various resistant genes to resist the potency of conventional 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 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 at the 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 infections, etc.

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

Introduction

"Klebsiella species are Gram-negative, non-motile bacilli, lactose-fermenting, facultative anaerobic rod-shaped encapsulated bacteria which can appear in mucoid lactose fermenter on MacConkey agar. Though, they are found in the normal flora of the mouth, skin, and intestines, they can cause critical changes to human and animal lungs if aspirated, precisely to the alveoli resulting in bloody sputum". [1]. 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 a mixture with vancomycin or penicillin" [2]. "At a concentration of 4.0µg/ml, it has great activity against gram-negative bacteria" [3]. "Likewise, honey has been related to antibacterial and antifungal activity" [4]. "Specifically, *Klebsiella* species were among the isolates that had their growth inhibited by honey".[5] Dekker and Frank, 2015 [6],[7],[8] reported "the 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".

The antibacterial activity of honey is influenced by various factors and working conditions. However, activities of honey is associated to the composition of hydrogen peroxide , phenol , pH, and osmotic pressure. Hydrogen peroxide is one of the most prominent backbone contributing to its activity , the antimicrobial activities of honey greatly depends on it concentration . 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 [9],[10].

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

A otal number of fifty isolates of *Klebsiella* species from various bacteriological sources (*Table 1*) were collected on 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 primarily identified on MaCconkey agar. The isolates were confirmed by different biochemical tests 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 [11] was employed to obtain the susceptibility pattern of the bacterial isolates against each undiluted honey and its fresh aq. Dilutions and 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 [12].

Table 1: Pathological sources of *Klebsiella* species

Clinical samples	Number of Isolates
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 2*). 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 3*). However, 39% of the clinical isolates showed resistance to gentamicin (4µg/ml).

Table 2: Results of Sensitivity Test on Honey, Gentamicin against *Klebsiella* species

NUMBER OF CLINICAL SAMPLES	BACTERIAL ISOLATES	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**
001 Urine	*Klebsiella. pneumoniae*	24.5	-	-	-	-	15.6	-	-	-	-	16.4
002 Urine	*K pneumoniae* sub *rhinoscleromatis*	12.4	-	-	-	-	10.8	-	-	-	-	15.5
003 Urine	*Klebsiella pneumoniae* sub *pneumoniae*	14.5	-	-	-	-	24.5	20.5	18.9	12.5	10.5	R
004 Urine	*Klebsiella oxycota*	20.5	-	-	-	-	10.5	-	-	-	-	17.5
005 Urine	*Klebsiella pneumoniae* sub *pneumoniae*	15.6	-	-	-	-	10.9	-	-	-	-	20.5
006 Urine	*Klebsiella platicola*	20.5	-	-	-	-	16.7	-	-	-	-	15.5
007 Urine	*Klebsiella pneumoniae* sub *pneumoniae*	27.5	20.5	14.5	12.2	-	24.9	18.9	12.5	10.5	8.5	R
008 Urine	*Klebsiella pneumoniae* sub *pneumoniae*	14.5	-	-	-	-	10.5	-	-	-	-	15.5
009 Urine	*Klebsiella pneumoniae* sub *pneumoniae*	26.7	-	-	-	-	20.9	-	-	-	-	16.6
010 Urine	*Klebsiella pneumoniae* sub *pneumoniae*	17.5	15.4	12.5		-	20.9	23.5	15.9	-	-	16.5
011 Urine	*Klebsiella pneumoniae*	16.8	-	-	-	-	-	-	-	-	-	R
012 Urine	*Klebsiella pneumoniae*	17.6	10.6	-	-	-	18.5	12.2	-	-	-	18.4
013 Urine	*Klebsiella pneumoniae* sub *ozaenae*	18.5	-	-	-	-	25.9	15.5	-	-	-	R
014 Urine	*Klebsiella pneumoniae* sub *pneumoniae*	-	-	-	-	-	-	-	-	-	-	R
015 Urine	*Klebsiella pneumoniae* sub *pneumoniae*	22.5	16.5	11.6	-	-	13.5	-	-	-	-	R
016 Urine	*Klebsiella pneumoniae*	-	-	-	-	-	-	-	-	-	-	R
017 Urine	*Klebsiella pneumoniae* sub *pneumoniae*	-	-	-	-	-	12.5	-	-	-	-	R

Table 2 Continued: Results of Sensitivity Test on Honey, Gentamicin against *Klebsiella* species

NUMBER OF CLINICAL SAMPLES	BACTERIAL ISOLATES	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
018 Urine	*Klebsiella pneumoniae*	-	-	-	-	-	-	-	-	-	-	
019 Urine	*Klebsiella pneumoniae* sub *rhinoscleromatis*	26.5	22.5	16.5	11.6		22.5	16.5	10.5			17.5
020 Blood	*Klebsiella oxycota*	20.5	-	-	-	-	16.5	-	-	-	-	15.9
021 Blood	*Klebsiella pneumoniae* sub *pneumoniae*	-	-	-	-	-	12.5	-	-	-	-	R
022 Blood	*Klebsiella oxycota*	-	-	-	-	-	11.5	-	-	-	-	R
023 Blood	*Klebsiella aerogens*	17.5	-	-	-	-	22.9	-	-	-	-	16.5
024 Blood	*Klebsiella pneumoniae* sub *pneumoniae*	18.4	13.9	12.0	10.2	09.5	18.5	14.5	13.5	12.8	10.5	17.5
025 Blood	*Klebsiella pneumoniae* sub *pneumoniae*	15.5	12.0	11.5	11.5	10.5	15.5	13.5	12.5	10.8	10.5	R
026 Wound	*Klebsiella pneumoniae* sub *pneumoniae*	16.5	13.5	12.9	10.8	10.5	20.6	22.5	16.5	11.6	10.5	16.5
027 Wound	*Klebsiella oxytoca*	13.5	-	-	-	-	18.9	-	-	-	-	11.2
028 Wound	*Klebsiella pneumoniae* sub *pneumoniae*	-	-	-	-	-	22.6	20.5	16.5	-	-	R
029 Wound	*Klebsiella pneumoniae*	-	-	-	-	-	-	-	-	-	-	R
030 Sputum	*Klebsiella pneumoniae*	13.5	-	-	-	-	18.8	-	-	-	-	16.5
031 Sputum	*Klebsiella oxytoca*	20.5	-	-	-	-	26.5	22.5	20.6	20.2	18.4	17.2
032 Sputum	*Klebsiella pneumoniae*	14.5	12.5	10.8	-	-	28.5	16.5	13.0	10.5	-	17.5
033 Sputum	*Klebsiella pneumoniae*	-	-	-	-	-	-	-	-	-	-	11.5
034 Sputum	*Klebsiella pneumoniae* sub *rhinoscleromatis*	-	-	-	-	-	-	-	-	-	-	R

Table 2 Continued: Results of Sensitivity Test on Honey, Gentamicin against *Klebsiella* species

035 Sputum	*Klebsiella oxytoca*	-	-	-	-	-	22.5	18.5	-	-	-	R
036 Semen	*Klebsiella pneumoniae*	13.5	12.5	10.8	-	-	28.5	16.5	13.0	10.5	-	R
NUMBER OF CLINICAL SAMPLES	**BACTERIAL ISOLATES**	**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**
037 Semen	*Klebsiella pneumoniae* sub *pneumoniae*	-	-	-	-	-	-	-	-	-	-	R
038 Semen	*Klebsiella oxytoca*	-	-	-	-	-	-	-	-	-	-	
039 Ear Swab	*Klebsiella pneumoniae*	12.5	-	-	-	-	13.5	12.2	-	-	-	18.5
040 Ear Swab	*Klebsiella pneumoniae* sub *pneumoniae*	11.5	-	-	-	-	21.5	-	-	-	-	R
041 Eye Swab	*Klebsiella pneumoniae* sub *pneumoniae*	20.5	13.5	12.5	10.8	13.5	13.5	-	-	-	-	17.5
042 H.V.S	*Klebsiella pneumoniae*	10.9	-	-	-	-	12.5	-	-	-	-	14.6
043 E.C.S	*Klebsiella oxytoca*	20.5	-	-	-	-	20.5	-	-	-	-	R
044 S.F.A	*Klebsiella pneumoniae* sub *pneumoniae*	14.6	-	-	-	-	15.5	12.5	-	-	-	18.5
045 S.F.A	*Klebsiella pneumoniae* sub *pneumoniae*	-	-	-	-	-	-	-	-	-	-	15.5
046 Urine	*Klebsiella pneumoniae* sub *pneumoniae*	26.5	-	-	-	-	-	-	-	-	-	R
047 Stool	*Klebsiella oxycota*	-	-	-	-	-	26.5	20.5	-	-	-	17.5
048 Wound	*Klebsiella oxytoca*	26.5	20.5	15.5	10.6	-	22.5	16.5	10.5	-	-	19.5
049 Urine	*Klebsiella pneumoniae* sub *rhinoscleromatis*	22.5	19.5	14.5	12.6	-	23.5	18.5	15.5	-	-	17.5
050 Urine	*Klebsiella pneumoniae* sub *pneumoniae*	25.5	20.5	16.5	13.6	-	24.5	17.5	12.5	-	-	18.5

Key: R – Resistance, N.A – No Activity, 0 - Undiluted Honey,
** - Gentamicin 4.0µg/ml

Table 3: Resistance ratios of *Klebsiella* species against honey and 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 settings" [13].

"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 store [14]. Though honey is used widely in traditional medicine, its use in modern medicine is limited" [15]. "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, reducing pain and odour quickly" [16].

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 the 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 honey may have reduced their activity, since many studies have reported diluted honey to have high water activity. This is similar to that found in undiluted honey to have more activity than Gentamicin when tested against some selected Gram negative bacteria [5]. The variations recorded in the antibacterial activity of the types of honey tested were consistent with the reports [17] 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 to 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 concentration [17].

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

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