

## 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.<sup>13</sup> 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 penicillin<sup>7</sup>. At a concentration of 4.0µg/ml, it has great activity against gram-negative bacteria<sup>2</sup>. Likewise, honey has been related with antibacterial and antifungal activity<sup>17</sup>. Specifically, *Klebsiella* species were among the isolates that had their growth inhibited by honey.<sup>3</sup> Ibrahim<sup>6</sup> and Jeddar *et al.*<sup>8</sup> 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<sup>10, 16, 15, 4</sup>. 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 sterile nutrient agar (OXOID) slants from the Routine Section of the Medical Microbiology Laboratory of four

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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 purified on Macconkey agar. The isolates were confirmed by different biochemical test and then preserved on fresh nutrient agar slants in a refrigerator at 4 °C.

**Comment [U6]:** Whether ethical permission was obtained / statement from author about data regarding patients identity were not disclosed

**Comment [U7]:** MacConkey agar. Maintain uniformity

#### 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<sup>3, 14</sup> 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.<sup>14</sup>

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

| Pathological Source | Number of Isolate |
|---------------------|-------------------|
| 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                 |

**Comment [U8]:** Were all the isolates from OPD or IPD patients? Did author collect information about isolates which were part of hospital acquired infection? Coz these isolates are showing more resistance.

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#### 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

| ISOLATE NUMBER | 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 sub rhinoscleromatis</i>    | 12.4                   | -    | -    | -    | -   | 10.8                   | -    | -    | -    | -    | 15.5                   | 15.5 |
| 003 Urine      | <i>Klebsiella pneumoniae sub 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 sub 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 sub 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 sub pneumoniae</i> | 14.5                   | -    | -    | -    | -   | 10.5                   | -    | -    | -    | -    | 15.5                   | 15.5 |
| 009 Urine      | <i>Klebsiella pneumoniae sub pneumoniae</i> | 26.7                   | -    | -    | -    | -   | 20.9                   | -    | -    | -    | -    | 16.6                   | 16.5 |
| 010 Urine      | <i>Klebsiella pneumoniae sub 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 sub ozaenae</i>    | 18.5                   | -    | -    | -    | -   | 25.9                   | 15.5 | -    | -    | -    | R                      | R    |
| 014 Urine      | <i>Klebsiella pneumoniae sub pneumoniae</i> | -                      | -    | -    | -    | -   | -                      | -    | -    | -    | -    | R                      | R    |
| 015 Urine      | <i>Klebsiella pneumoniae sub 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 sub pneumoniae</i> | -                      | -    | -    | -    | -   | 12.5                   | -    | -    | -    | -    | R                      | R    |

**Comment [U10]:** Kindly specify in title of table. Mm represents Zone diameter? What was the breakpoint selected on the basis of standard guideline- mention in material and method

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**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><br>sub <i>pneumoniae</i>       | 11.5 | -    | -    | -    | -    | 21.5 | -    | -    | - | - | R    | R    |
| 041 Eye Swab | <i>Klebsiella pneumoniae</i><br>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><br>sub <i>pneumoniae</i>       | 14.6 | -    | -    | -    | -    | 15.5 | 12.5 | -    | - | - | 18.5 | 22.5 |
| 045 S.F.A    | <i>Klebsiella pneumoniae</i><br>sub <i>pneumoniae</i>       | -    | -    | -    | -    | -    | -    | -    | -    | - | - | 15.5 | 14.5 |
| 046 Urine    | <i>Klebsiella pneumoniae</i><br>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><br>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><br>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:** 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 settings<sup>11</sup>.

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<sup>11</sup>. Though honey is used widely in traditional medicine, its use in modern medicine is limited<sup>9</sup>. 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 quickly<sup>9</sup>.

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 to<sup>3</sup> that found undiluted honey to have more activity than Gentamicin when tested against some selected Gram negative bacteria. The variations recorded in the antibacterial activity of the types of honey tested were consistent with the reports<sup>1</sup> 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 activity<sup>1</sup>.

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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**Comment [U13]:** Whether any statistical test were applied for comparison?

**Comment [U14]:** ??? difficult to interpret

**Comment [U15]:** Part of mechanism of effect of honey on bacteria should be described in introduction section

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