

In vitro antibacterial activity of locally sourced natural honey against wound-derived staphylococcus aureus, Escherichia coli and klebsiella pneumoniae at Kiruddu national referral hospital, Uganda

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Abstract

Background:

Antimicrobial resistance among common wound pathogens has increased interest in locally available complementary antibacterial agents. Natural honey is traditionally used in wound care, but evidence against contemporary Ugandan clinical isolates remains limited.

Objective:

To determine the in vitro antibacterial activity of a locally sourced natural honey sample at varying concentrations against wound-derived *Staphylococcus aureus*, *Escherichia coli*, and *Klebsiella pneumoniae* isolates from Kiruddu National Referral Hospital.

Methods:

We conducted a laboratory experimental study using bacterial isolates recovered cross-sectionally from wound specimens. We processed 43 wound specimens and recovered 14 target isolates. Honey sourced from Matugga, Wakiso District, was tested at 25%, 50%, 75%, and 100% v/v using agar well diffusion. Each isolate was tested in triplicate. Meropenem was used as the positive control for *E. coli* and *K. pneumoniae* and gentamicin for *S. aureus*; sterile distilled water was the negative control.

Results:

Fourteen of 43 specimens (32.6%) yielded target organisms: *E. coli* 6/14 (42.9%), *S. aureus* 4/14 (28.6%), and *K. pneumoniae* 4/14 (28.6%). Mean inhibition zones increased from 10.22 to 17.32 mm for *E. coli*, 10.75 to 19.10 mm for *K. pneumoniae*, and 15.58 to 21.68 mm for *S. aureus* between 25% and 100% honey. Concentration significantly affected inhibition within each species ($p < 0.05$). Between-species differences were significant at 25%, 50%, and 75% but not at 100%. *S. aureus* was significantly more susceptible than *E. coli* at 25–75%.

Conclusion:

The locally sourced honey demonstrated concentration-dependent in vitro antibacterial activity against all three wound-derived pathogens. These findings justify larger laboratory and clinical studies but do not establish clinical efficacy.

Keywords: natural honey; antibacterial activity; wound infection; agar well diffusion; *Staphylococcus aureus*; *Escherichia coli*; *Klebsiella pneumoniae*; Uganda.

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1. Introduction

Wound infections remain an important cause of delayed healing, prolonged hospitalization, and healthcare expenditure, particularly where antimicrobial resistance complicates treatment [1–4]. *Staphylococcus aureus*, *Escherichia coli*, and *Klebsiella pneumoniae* are among the clinically important organisms recovered from infected wounds, and resistant phenotypes further restrict therapeutic options [3–7]. In Uganda, wound-related bacterial infections and antimicrobial resistance remain important concerns in tertiary-care settings [5,8]. Recent Ugandan evidence from Mbale Regional Referral Hospital further demonstrates the clinical relevance of these organisms: Mabonga et al. reported *Staphylococcus* spp., *E. coli*, and *Klebsiella* spp.

among important bacteria recovered from infected wounds and documented substantial antimicrobial resistance, including methicillin-resistant *S. aureus* [15].

Natural honey has attracted renewed scientific interest as a complementary antimicrobial material. Its antibacterial effects have been attributed to high osmolarity, acidic pH, hydrogen peroxide generation, and phytochemical constituents, including phenolic compounds [9,10]. Experimental studies have demonstrated activity against Gram-positive and Gram-negative pathogens, including resistant clinical isolates, although antibacterial potency varies according to floral source, geographical origin, processing, and test conditions [9–13].

Because antibacterial activity cannot be assumed to be identical across honey sources, locally generated evidence is important. The dissertation underlying this manuscript identified a gap in data evaluating Ugandan honey against freshly isolated wound pathogens from Kiruddu National Referral Hospital. The present study therefore evaluated a single locally sourced natural honey at graded concentrations against wound-derived *S. aureus*, *E. coli* and *K. pneumoniae* and compared the magnitude of inhibition among the three species.

2. Materials and Methods

2.1 Study design and setting

A laboratory experimental study was conducted using confirmed bacterial isolates cultured from wound specimens received cross-sectionally at the Microbiology Laboratory of Kiruddu National Referral Hospital, Kampala, Uganda. The experimental unit was the bacterial isolate rather than the patient.

2.2 Specimens, sampling and bacterial identification

All eligible wound swab or aspirate specimens received during the study period were enrolled consecutively. Specimens were cultured on blood agar, MacConkey agar and chocolate agar and incubated aerobically at 35–37°C for 24–48 hours. *S. aureus* was identified using Gram stain, catalase and coagulase tests; *E. coli* by Gram stain, lactose fermentation on MacConkey agar, indole and motility; and *K. pneumoniae* by colonial morphology and biochemical tests including catalase, oxidase, indole, citrate and urease.

2.3 Honey source and preparation

Natural unprocessed forest honey was obtained from a beekeeper in Matugga, Wakiso District. It was golden-yellow, viscous, characteristically aromatic, and had a measured pH of 4. No bacterial growth was detected when the honey was inoculated onto blood agar and MacConkey agar. Fresh 25%, 50%, and 75% v/v preparations were made with sterile distilled water; 100% honey was used undiluted.

2.4 Agar well diffusion assay

Bacterial suspensions equivalent to 0.5 McFarland were lawn-inoculated on Mueller-Hinton agar. Four 9-mm wells were prepared, and 100 µL of each honey concentration was dispensed. Meropenem discs served as positive controls for *E. coli* and *K. pneumoniae*, and gentamicin for *S. aureus*. Sterile distilled water served as the negative control. Plates were incubated at 37°C for 24 hours. Inhibition zones were measured in millimetres, and each isolate-concentration combination was tested in triplicate.

2.5 Data analysis

Triplicate readings were averaged for each isolate. Descriptive statistics summarized isolate frequencies and inhibition zones. Within-species concentration effects were assessed using the Friedman test and repeated-measures ANOVA. Between-species differences at each concentration were assessed using Kruskal-Wallis tests with pairwise comparisons, while a two-factor species × concentration ANOVA evaluated main and interaction effects. Statistical significance was set at $p < 0.05$.

2.6 Ethical considerations

Ethical approval was obtained from the Mengo Hospital Research and Ethics Committee (MH/8003), and permission was obtained from Kiruddu National Referral Hospital. Coded residual diagnostic isolates were used, and the ethics committee granted a waiver of individual informed consent.

Ethics Approval

Ethical approval was obtained from the Mengo Hospital Research and Ethics Committee (Approval Reference MH/8003). Permission was obtained from Kiruddu National Referral Hospital, and a waiver of individual informed consent was granted for use of residual diagnostic isolates.

3. Results

Forty-three wound specimens were processed. Fourteen (32.6%) yielded one of the three target organisms, 17 (39.5%) yielded other bacterial isolates, and 12 (27.9%) showed no growth.

Table 1. Distribution of wound specimens according to culture result.

Culture result	Frequency	Percentage (%)
Target isolates	14	32.6
Other isolates	17	39.5
No growth	12	27.9
Total	43	100

Interpretation: Of the 43 wound specimens processed, 14 (32.6%) yielded one of the three target organisms, 17 (39.5%) yielded other bacterial isolates, and 12 (27.9%) showed no bacterial growth. Thus, organisms outside the three prespecified target species represented the largest single culture-result category.

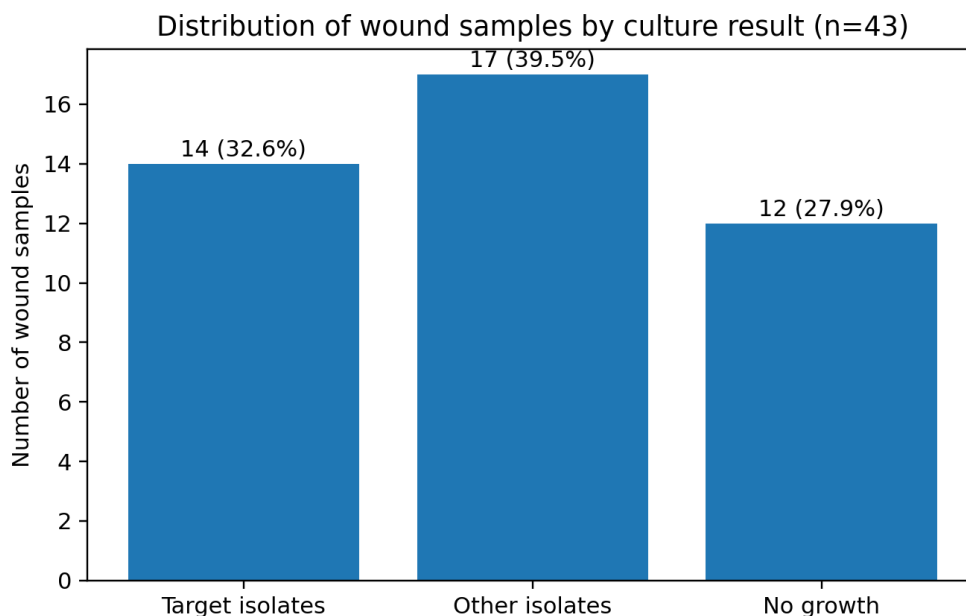


Figure 1. Distribution of wound specimens according to culture result (n=43).

Interpretation: The culture results show that 32.6% of specimens yielded one of the target organisms. Other bacterial isolates were recovered from 39.5% of specimens, while 27.9% had no growth, demonstrating that the three target organisms represented only part of the bacterial spectrum recovered from the wound specimens.

Table 2. Distribution of the 14 target bacterial isolates.

Species	Frequency	Percentage (%)
E. coli	6	42.9
S. aureus	4	28.6
K. pneumoniae	4	28.6
Total	14	100

Interpretation: Among the 14 target isolates, E. coli was the most frequently recovered species, accounting for 6 isolates (42.9%). S. aureus and K. pneumoniae each accounted for 4 isolates (28.6%). Because the number of

target isolates was small, these proportions should be interpreted as a description of this study sample rather than the wider hospital epidemiology.

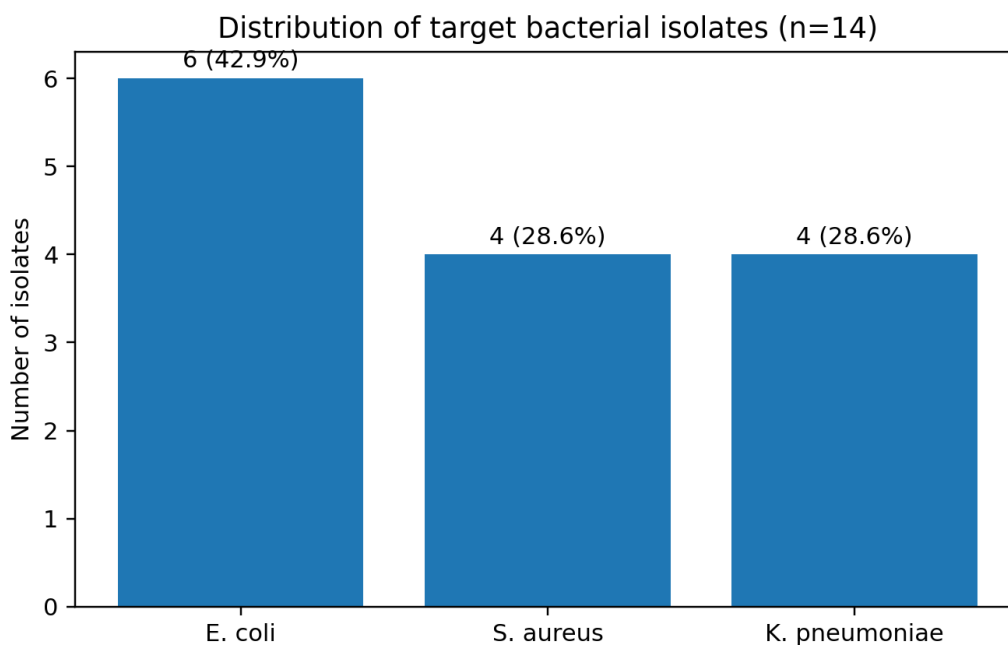


Figure 2. Distribution of the target bacterial isolates by species (n=14).

Interpretation: E. coli constituted the largest proportion of the target isolates (42.9%), whereas S. aureus and K. pneumoniae each contributed 28.6%. The figure summarizes species composition among the 14 target isolates rather than prevalence among all wound specimens.

The honey used for antibacterial testing was a forest honey sourced from Matugga, Wakiso District. It had a pH of 4 and showed no detectable bacterial growth under the culture conditions used.



Figure 3. Locally sourced natural honey used in the study.

Description: The photograph shows the locally sourced forest honey obtained from Matugga, Wakiso District, and used for antibacterial testing. The honey was golden-yellow and viscous, had a measured pH of 4, and showed no detectable bacterial growth under the culture conditions used.

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Figure 4. Prepared natural honey concentrations used for antibacterial testing.

Description: The photograph shows the prepared honey dilutions used in the agar well diffusion assays. Honey was tested at 25%, 50%, 75%, and 100% v/v, allowing assessment of whether antibacterial activity changed with increasing concentration.



Figure 5. Agar plates demonstrating zones of inhibition during honey susceptibility testing.

Interpretation: The agar plates demonstrate visible zones surrounding wells containing honey preparations. The clear areas represent inhibition of bacterial growth and formed the basis for measurement of zone diameters in millimetres. The pattern of larger zones at higher concentrations was subsequently quantified in Tables 3 and 4.

Table 3. Zones of inhibition (mm) produced by honey concentrations against individual isolates.

Sample ID	Positive control	Negative control	25%	50%	75%	100%
0001C	30	9	9	14	14.6	16
0002C	30	9	10	14.7	15.7	19
0003C	31	9	11.3	17.3	18	18
0004C	30	9	11	15.3	17	18.3
0005C	31	9	9.7	13.7	15.7	17.3
0006C	30	9	10.3	13.7	14.7	15.3
0001B	30	9	10	15.3	16	15.7
0002B	30	9	9	13.7	14	15
0003B	21	9	11.7	16.3	16.7	19.7
0004B	29	9	12.3	18.3	18	26
0001A	19	9	13	19	19.7	20.3
0002A	18	9	16.3	20.7	21.7	21.7
0003A	6	9	17.7	20.3	21	22
0004A	18	9	15.3	21.3	23.3	22.7

Interpretation: The negative control remained at the 9-mm well diameter for all isolates, indicating no measurable inhibition beyond the well. In contrast, inhibition generally increased as honey concentration increased. The strongest responses at 100% honey were observed among the *S. aureus* isolates, although isolate-level variability was evident, particularly for *K. pneumoniae*.

The negative control consistently measured 9 mm, corresponding to the physical well diameter and therefore

indicating no inhibition beyond the well. Inhibition generally increased with increasing honey concentration. At 25%, isolates 0001C and 0002B produced only the 9-mm baseline. Positive-control inhibition was high for *E. coli* and *K. pneumoniae* but variable for *S. aureus*; isolate 0003A had a 6-mm gentamicin zone while retaining a strong honey response.

Table 4. Mean $\pm$ SD zones of inhibition (mm) by species and honey concentration.

Species	25%	50%	75%	100%
<i>E. coli</i>	10.22 $\pm$ 0.85	14.78 $\pm$ 1.38	15.95 $\pm$ 1.33	17.32 $\pm$ 1.42
<i>K. pneumoniae</i>	10.75 $\pm$ 1.52	15.90 $\pm$ 1.93	16.18 $\pm$ 1.67	19.10 $\pm$ 5.04
<i>S. aureus</i>	15.57 $\pm$ 1.98	20.32 $\pm$ 0.97	21.43 $\pm$ 1.50	21.68 $\pm$ 1.01

Interpretation: Mean inhibition increased progressively with honey concentration for all three species. *S. aureus* showed the largest mean zones at every concentration, rising from 15.57 $\pm$ 1.98 mm at 25% honey to 21.68 $\pm$ 1.01 mm at 100%. *E. coli* had the smallest mean inhibition zones, while *K. pneumoniae* showed greater variability at 100% honey (19.10 $\pm$ 5.04 mm).

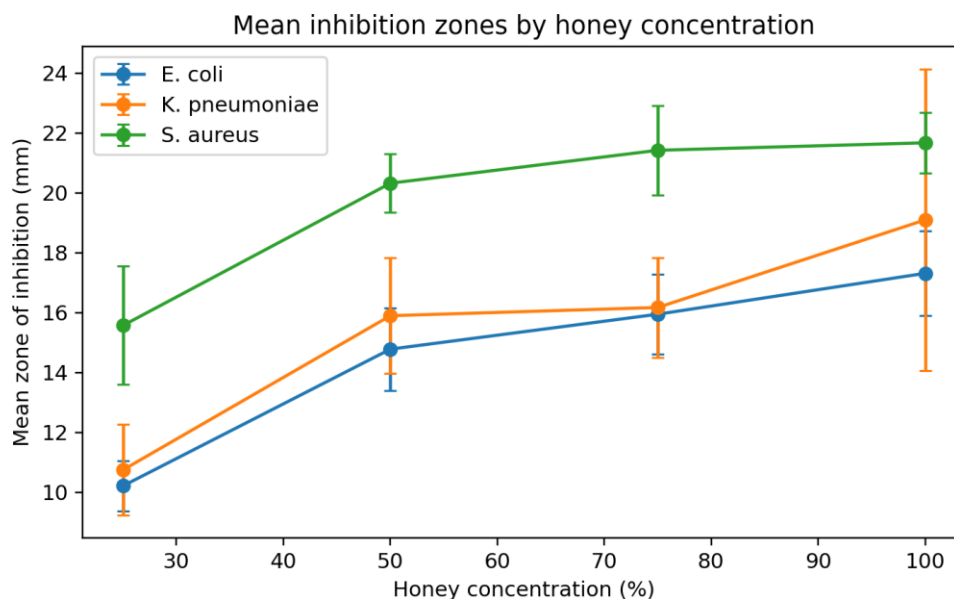


Figure 6. Mean zones of inhibition produced by increasing natural honey concentrations against *E. coli*, *K. pneumoniae* and *S. aureus*.

Interpretation: Mean inhibition increased with honey concentration in all three bacterial species. *S. aureus* consistently showed the greatest mean inhibition, whereas *E. coli* showed the lowest. The broadly parallel

concentration-response patterns are consistent with the two-factor analysis, which found significant main effects of species and concentration but no significant species-by-concentration interaction.

Table 5. Within-species comparison of inhibition zones across honey concentrations.

Species	Friedman χ^2	p-value	Repeated-measures ANOVA
<i>E. coli</i>	17.75	0.0005	F(3,15)=106.05, p<0.001
<i>K. pneumoniae</i>	9.90	0.019	F(3,9)=15.03, p=0.001
<i>S. aureus</i>	11.15	0.011	F(3,9)=39.31, p<0.001

Interpretation: Both the Friedman test and repeated-measures ANOVA demonstrated statistically significant concentration effects for *E. coli*, *K. pneumoniae*, and *S. aureus* (all p<0.05). These findings support a concentration-

dependent antibacterial response within each species under the experimental conditions used.

Both non-parametric and parametric analyses showed significant concentration effects for all three species, confirming a dose-response relationship.

Table 6. Between-species comparison of inhibition zones at each honey concentration.

Honey concentration	Kruskal-Wallis H	p-value
25%	8.25	0.016
50%	8.58	0.014
75%	8.09	0.018
100%	5.40	0.067

Interpretation: Antibacterial activity differed significantly among the three species at 25%, 50%, and 75% honey concentrations, but the difference was not statistically

significant at 100% (p=0.067). Pairwise analysis showed greater inhibition of *S. aureus* than *E. coli* at 25- 75%, while

E. coli and *K. pneumoniae* did not differ significantly at any tested concentration.

Between-species differences were significant at 25%, 50%, and 75% but not at 100%. Bonferroni-corrected pairwise comparisons showed significantly greater inhibition of *S. aureus* than *E. coli* at 25–75% (adjusted $p=0.029-0.042$). *E. coli* and *K. pneumoniae* did not differ significantly at any concentration. Two-factor ANOVA showed significant main effects of species ($F=37.77$, $p<0.001$) and concentration ($F=37.18$, $p<0.001$), but no species $\times$ concentration interaction ($F=0.40$, $p=0.872$).

4. Discussion

The study has several strengths: freshly recovered clinical isolates were used; four graded honey concentrations were tested; isolate-concentration combinations were examined in triplicate; and both positive and negative controls were included. However, the findings are limited by the small number of target isolates, use of a single honey source, absence of formal botanical and physicochemical authentication, lack of MIC and MBC determination, and reliance on agar diffusion, which can be influenced by viscosity and diffusion characteristics. Most importantly, this was an in vitro study and did not assess wound healing, toxicity, dosing, or clinical outcomes. The findings therefore demonstrate laboratory antibacterial activity and justify further investigation, but they do not establish clinical efficacy.

An additional observation was the small gentamicin positive-control zone for one *S. aureus* isolate (0003A), despite a strong response to honey. This is potentially important in the context of antimicrobial resistance, which complicates management of wound infections in many settings [3-5,7,8]. However, the study did not perform a full standardized antimicrobial susceptibility profile for all isolates, and the observation involved only one isolate. It therefore cannot support a conclusion that honey is superior to conventional antibiotics or that it is clinically effective against resistant infections.

The between-species comparison was significant at 25%, 50%, and 75% honey but not at 100%. At the highest concentration, the mean inhibition zones of all three organisms increased, and the variability in *K. pneumoniae* widened, which may have reduced statistical separation among species. The two-factor analysis showed significant main effects of species and honey concentration but no significant species-by-concentration interaction. This suggests that species differed in overall susceptibility, while the general direction of increasing inhibition with increasing honey concentration was similar across the organisms.

S. aureus showed the greatest mean inhibition at every honey concentration and was significantly more susceptible than *E. coli* at 25–75%. One plausible explanation is the

structural difference between Gram-positive and Gram-negative bacteria. Gram-negative organisms possess an outer membrane that acts as an additional permeability barrier and can reduce intracellular access of antimicrobial compounds [14]. Nevertheless, *K. pneumoniae* showed intermediate responses and considerable variability at 100% honey, indicating that susceptibility cannot be explained by Gram classification alone. Strain-specific characteristics, capsule production, efflux, biofilm capacity, and differences in the composition of the tested honey may also influence inhibition [6,9,10].

A clear concentration-response relationship was observed. Mean inhibition of *E. coli* increased from 10.22 ± 0.85 mm at 25% honey to 17.32 ± 1.42 mm at 100%; *K. pneumoniae* increased from 10.75 ± 1.52 mm to 19.10 ± 5.04 mm; and *S. aureus* increased from 15.57 ± 1.98 mm to 21.68 ± 1.01 mm. Both non-parametric and repeated-measures analyses confirmed statistically significant concentration effects within every species. Similar dose-dependent effects have been described in studies where increasing honey concentration produced progressively greater inhibition of bacterial growth [9,12,13]. Dilution may reduce osmotic pressure and the effective concentration of antimicrobial constituents, although some peroxide-generating honeys can show complex dilution effects [9,10].

The locally sourced honey inhibited *E. coli*, *K. pneumoniae* and *S. aureus*, with measurable activity becoming more pronounced as concentration increased. This finding is consistent with previous experimental work demonstrating broad-spectrum antibacterial activity of honey against Gram-positive and Gram-negative organisms [9,11-13]. Honey's antimicrobial effects have been attributed to several interacting properties, including high osmolarity, acidic pH, hydrogen peroxide generation, and phytochemical constituents such as phenolic compounds [9,10]. In the present study, the honey had a pH of 4 and was culture-negative before testing, but hydrogen peroxide and phytochemical concentrations were not measured. Consequently, these mechanisms provide biologically plausible explanations rather than mechanisms directly demonstrated by this experiment.

Fourteen of the 43 wound specimens (32.6%) yielded one of the three target organisms, and *E. coli* accounted for the largest share of the target isolates. This distribution differs from many wound-infection studies in which *S. aureus* is frequently dominant [3,5,7]. The difference may reflect the small number of target isolates, the short sampling period, local case mix, previous antimicrobial exposure, and the fact that the study deliberately focused on only three species. The additional recovery of other bacterial isolates from 39.5% of specimens further indicates that wound microbiology at the study site was broader than the three target organisms. Therefore, the observed species distribution should not be interpreted as evidence of a

change in wound-infection epidemiology at Kiruddu National Referral Hospital. This contrasts with the Mbale Regional Referral Hospital study by Mabonga et al., in which *Staphylococcus* spp. were the most frequent isolates among culture-positive wound specimens, followed by *E. coli* and *Klebsiella* spp. [15]. The contrast emphasizes that organism distribution may vary by hospital, patient population, wound characteristics, prior antimicrobial exposure, and sampling period.

5. Conclusion

Locally sourced natural honey from Matugga, Wakiso District demonstrated significant concentration-dependent in vitro antibacterial activity against wound-derived *E. coli*, *K. pneumoniae*, and *S. aureus* isolates from Kiruddu National Referral Hospital. *S. aureus* showed the greatest overall susceptibility, while *E. coli* and *K. pneumoniae* showed comparatively smaller inhibition zones. The findings provide preliminary local laboratory evidence supporting further investigation of Ugandan honey as a potential complementary antimicrobial material. These results should not be interpreted as evidence that natural honey can replace standard clinical wound care or antibiotic therapy. Larger studies using multiple honey sources, more clinical isolates, MIC/MBC assays, physicochemical and phytochemical characterization, and controlled clinical evaluation are required before clinical recommendations can be made.

6. Recommendations

1. Undertake appropriately designed safety, in vivo, and clinical studies before considering locally sourced honey for routine wound-management recommendations.
2. Extend laboratory evaluation to minimum inhibitory concentration, minimum bactericidal concentration, physicochemical and phytochemical characterization, and standardized testing against antimicrobial-resistant isolates.
1. Conduct larger studies using honey from multiple Ugandan floral and geographical sources and a broader panel of well-characterized clinical wound isolates.

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Conflict of Interest

The authors declare no conflict of interest.

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Author Contributions

Najjemba Joyce: investigation, data collection, laboratory work, analysis, and original dissertation preparation. Kasozi James and Mabonga Habert: supervision, methodological guidance, and critical review. Final authorship contributions should be confirmed by all authors before journal submission.

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