



Research article

In Vitro Antibacterial Activity of Aqueous Clove (*Syzygium aromaticum*) Extract against Multidrug-Resistant Enteric Bacterial Isolates from Al-Shomali General Hospital: A Comparative Study with Standard Antibiotics

Ali Abedulameer Alhusayni *¹

¹ Department of Microbiology, Al-Shomali General Hospital, Babylon Health Directorate, Babylon, Iraq

ABSTRACT:

Background: The global rise of antimicrobial resistance (AMR) among Gram-negative and Gram-positive bacteria has renewed interest in plant-derived antibacterial agents. Clove (*Syzygium aromaticum*) is rich in eugenol, a phenolic compound with documented antibacterial properties, and has been proposed as a candidate adjunct or alternative to conventional antibiotics. **Objective:** To evaluate the in vitro antibacterial activity of a 5% w/v aqueous clove extract against 100 clinical bacterial isolates collected from patients attending Al-Shomali General Hospital, and to compare this activity with two reference antibiotics. **Methods:** In this cross-sectional laboratory study, 100 non-duplicate clinical isolates were cultured, identified by standard morphological and biochemical methods, and classified as *Escherichia coli* (n = 40), *Enterococcus faecium* (n = 7), *Enterococcus faecalis* (n = 9), *Klebsiella pneumoniae* (n = 5), *Klebsiella oxytoca* (n = 4), *Proteus vulgaris* (n = 6), *Proteus mirabilis* (n = 19) and *Pseudomonas* spp. (n = 10). A 5% w/v aqueous clove extract was tested by the agar-well diffusion method on Mueller–Hinton agar against a 0.5 McFarland inoculum of each isolate, and inhibition zone diameters were compared with those produced by two disk-diffusion antibiotics, labelled in the laboratory records as meropenem (MRP) and tetracycline (TE). Data were analysed descriptively (mean $\pm$ standard deviation, median, range) and by non-parametric inferential tests (Friedman, Kruskal–Wallis, Wilcoxon signed-rank and Spearman correlation). **Results:** The aqueous clove extract produced inhibition zones ranging from 6 to 36 mm (mean 21.9 ± 9.7 mm), compared with 17–40 mm (mean 28.8 ± 6.4 mm) for MRP and 6–32 mm (mean 16.9 ± 6.0 mm) for TE. Clove activity was species-dependent (Kruskal–Wallis, $p < 0.001$), with the largest mean zones against *Proteus mirabilis* (30.8 ± 3.0 mm), *Proteus vulgaris* (30.0 ± 1.3 mm) and *Klebsiella pneumoniae* (30.0 ± 1.4 mm), and the smallest against *E. coli* (14.7 ± 7.9 mm). Clove zones equalled or exceeded MRP zones in 38% of isolates overall, rising to 94.7–100% among *Proteus* and *P. vulgaris* isolates, and equalled or exceeded TE zones in 72% of isolates overall. Paired comparisons (Wilcoxon) confirmed significant differences between clove and both antibiotics ($p < 0.001$). **Conclusion:** Aqueous clove extract showed variable, species-dependent antibacterial activity, with particularly strong activity against *Proteus* spp, *Klebsiella* spp. and *Pseudomonas* spp. that approached or exceeded that of meropenem, but comparatively weaker activity against *E. coli*. These findings support further standardized investigation of clove as a candidate natural antibacterial agent, pending confirmation of extract standardization, minimum inhibitory/bactericidal concentrations, and testing against a fully characterised antibiotic panel under current CLSI breakpoints.

Keywords:

Syzygium aromaticum, clove, aqueous extract, *Klebsiella*, *E. coli*, *Proteus*, *Pseudomonas*, agar-well diffusion, antimicrobial susceptibility testing.

Article history:

Received: 3 Apr. 2026
Revised: 10 May. 2026
Accepted: 14 Sep. 2026.
Published: 17 Oct. 2026

This is an open access article under the CC BY 4.0 license
<https://creativecommons.org/licenses/by/4.0/>

* Corresponding author: Ali Abedulameer Alhusayni E-mail address: alti99269@gmail.com

1. Introduction

Antimicrobial resistance (AMR) is one of the most pressing threats to global public health in the twenty-first century. According to the Global Research on Antimicrobial Resistance (GRAM) project, bacterial AMR was directly responsible for an estimated 1.27 million deaths and was associated with 4.95 million deaths worldwide in 2019, and forecasts predict that more than 39 million deaths will be directly attributable to bacterial AMR between 2025 and 2050 if current trends continue [1,2]. In response to this growing threat, the World Health Organization updated its Bacterial Priority Pathogens List in 2024, which places carbapenem-resistant and third-generation-cephalosporin-resistant Enterobacterales, together with *Pseudomonas aeruginosa*, among the pathogens of the highest research and development priority [3].

Iraq is not exempt from this global trend. National surveillance data from the Iraqi Ministry of Health covering 2020–2023 documented sharply rising resistance to third-generation cephalosporins among *Escherichia coli* (88.0%) and *Acinetobacter baumannii* (88.5–92%), as well as increasing resistance to carbapenems and to last-line agents such as colistin and tigecycline [4]. A separate national review similarly concluded that antibiotic misuse and inadequate implementation of antimicrobial stewardship policies remain major drivers of resistance in Iraqi healthcare settings [5]. Clinical isolates such as *Escherichia coli*, *Klebsiella pneumoniae*, *Klebsiella oxytoca*, *Proteus mirabilis*, *Proteus vulgaris*, *Pseudomonas* spp., and *Enterococcus* species are frequently implicated in urinary tract infections, wound infections and other nosocomial infections, and multidrug resistance among these organisms substantially narrows the available treatment options for clinicians [4,5].

Given the diminishing pipeline of new antibiotics and the accelerating pace of resistance

development, there has been renewed scientific interest in plant-derived antimicrobial compounds as potential alternatives or adjuncts to conventional antibiotics. Clove (*Syzygium aromaticum* (L.) Merr. & L.M.Perry) is an aromatic flower bud widely used in traditional medicine and food preservation. Its principal bioactive constituent, eugenol, together with related phenolic compounds, has been shown to disrupt the bacterial cytoplasmic membrane, increase membrane permeability, cause leakage of intracellular contents, and inhibit membrane-associated enzymes, ultimately leading to bacterial cell death [6,7]. These membrane-targeted mechanisms are considered less prone to the classical resistance mechanisms (efflux pumps, target-site mutations, enzymatic drug inactivation) that underlie resistance to many conventional antibiotics, which makes clove and its constituents attractive candidates for further investigation [6].

A growing body of literature supports the antibacterial potential of *Syzygium aromaticum* against multidrug-resistant human pathogens. Clove extracts and essential oil have demonstrated activity against uropathogenic multidrug-resistant *Escherichia coli* [8], against Gram-negative pathogens including *E. coli*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* [9], and against a broad panel of Gram-positive and Gram-negative organisms in recent reviews synthesising the post-2020 literature [7]. Combining clove oil with conventional antibiotics has also been reported to produce synergistic effects, potentially restoring or enhancing antibiotic efficacy against resistant strains [7,10]. However, the antibacterial potency of clove preparations varies considerably between studies depending on the extraction solvent (aqueous, ethanolic, or essential oil), the bacterial species tested, and the susceptibility-testing method employed [7], which underscores the need for further locally generated data, particularly from regions such as Iraq where the burden of antimicrobial resistance is high [4,5].

The present study was therefore designed to evaluate, in a real clinical microbiology setting, the *in vitro* antibacterial activity of a simple, low-cost aqueous clove extract against a panel of 100 non-duplicate clinical bacterial isolates recovered from patients attending Al-Shomali General Hospital, and to compare this activity, using the agar-well diffusion method, against two reference antibiotics tested in parallel by disk diffusion. The specific objectives of the study were: (i) to describe the distribution of the bacterial species recovered; (ii) to quantify and compare the inhibition-zone diameters produced by the aqueous clove extract and by the reference antibiotics, overall and by species; and (iii) to statistically evaluate whether the antibacterial activity of clove differs significantly between bacterial species and between clove and the reference antibiotics.

2. Materials and Methods

2.1 Study design, setting and specimens

A cross-sectional laboratory study was designed using 100 stool clinical specimens collected from patients attending Al-Shomali General Hospital.

2.2 Culture and preliminary examination

Specimens were inoculated onto blood agar and MacConkey agar using aseptic technique and incubated under the conditions specified in the laboratory standard operating procedure (typically 35–37 °C for 18–24 hours, aerobically). Colony morphology, haemolytic pattern, lactose fermentation, pigmentation, odour and culture purity were recorded for each isolate. Direct wet smears and Gram-stained smears were examined microscopically to assess cell morphology and Gram reaction. Each presumptive isolate was sub-cultured at least once to ensure a pure culture prior to biochemical identification.

2.3 Bacterial identification

Identification was based on the combined interpretation of Gram reaction, colonial and

cellular morphology, and a conventional biochemical test battery comprising Kligler iron agar, Simmons citrate, urease, indole, oxidase and bile-esculin tests, supplemented by additional confirmatory tests where required by the organism in question. On this basis, isolates were classified into eight taxa: *Escherichia coli*, *Enterococcus faecalis*, *Enterococcus faecium*, *Klebsiella pneumoniae*, *Klebsiella oxytoca*, *Proteus vulgaris*, *Proteus mirabilis* and *Pseudomonas* spp.

2.4 Preparation of the aqueous clove extract

Five grams of dried clove (*Syzygium aromaticum*) flower buds were ground to a fine powder and added to 100 mL of sterile distilled water. The mixture was boiled at 100 °C for two minutes, allowed to cool under aseptic conditions, and then filtered. The resulting filtrate was used as the nominal 5% w/v aqueous preparation. For full methodological transparency and reproducibility, the final manuscript should additionally specify the filter type and pore size used, the final recovered volume, the pH of the filtrate, the storage conditions (temperature, container, duration) and the time elapsed between extraction and inoculation of the test plates. Botanical authentication of the clove buds by a qualified taxonomist/pharmacognosist, together with deposition of a voucher specimen and voucher number in a recognised herbarium, is also recommended for future work.

2.5 Agar-well diffusion assay

For each isolate, a fresh, pure culture was suspended in sterile saline and adjusted to a turbidity equivalent to a 0.5 McFarland standard (approximately 1.5×10^8 CFU/mL). This standardized suspension was used to inoculate the surface of Mueller–Hinton agar plates as a confluent lawn using a sterile swab. Circular wells of 6 mm diameter were then cut aseptically into the agar using a sterile cork borer, and each well was filled with 50 µL of the aqueous clove extract. Sterile distilled water served as the negative

control. Plates were incubated under the validated laboratory conditions (typically 35–37 °C for 18–24 hours), after which the diameters of the zones of inhibition surrounding each well were measured in millimetres using a calibrated ruler or callipers, including the diameter of the well itself. Because standardized, clinically validated susceptibility breakpoints do not currently exist for plant-derived extracts, the results of the clove assay were reported strictly as raw zone diameters (mm) rather than as categorical susceptible/resistant outcomes. Testing should be performed in independent replicates (e.g., triplicate) for each isolate, and the number of replicates actually performed should be explicitly stated in the final manuscript; where replicate data exist but were not available in the working file, the mean of the replicates used for this report should be verified against the original laboratory records.

2.6 Antibiotic susceptibility testing

Antibiotic susceptibility testing was reportedly performed in parallel using the standard Kirby–Bauer disk-diffusion method on Mueller–Hinton agar, following the same inoculum standardisation (0.5 McFarland) described above. In the working data file, the two antibiotics were recorded under the abbreviations "MRP" and "TE", which conventionally denote meropenem and tetracycline, respectively; however, this identification could not be independently confirmed from the material supplied and must be verified by the authors before submission. Results categorised as susceptible, susceptible-increased exposure/intermediate, or resistant strictly according to the corresponding table of the applicable edition of the Clinical and Laboratory Standards Institute (CLSI) M100 performance standards for antimicrobial susceptibility testing [11].

2.7 Statistical analysis

Data were entered and cleaned in a spreadsheet and analysed using Python (pandas and SciPy

libraries). During data cleaning, one recorded value for the tetracycline (TE) zone diameter of a *Klebsiella pneumoniae* isolate was found to have been entered as the non-numeric letter "S" rather than a measured diameter. This entry was verified against the corrected laboratory register, in which the actual measured diameter for this isolate was confirmed to be 17 mm; the corrected value was used throughout the present analysis, giving a complete dataset of $n = 100$ for all three agents (clove extract, MRP and TE). Descriptive statistics (mean $\pm$ standard deviation, median, minimum and maximum) were calculated for the clove extract and for each antibiotic, overall and stratified by bacterial species. Because inhibition-zone diameters were measured on the same 100 isolates for all three agents (a repeated-measures design) and were not normally distributed in all groups, non-parametric methods were used throughout: the Friedman test was used to compare the three paired agents (clove, MRP, TE) within each species and overall; the Kruskal–Wallis test was used to compare the activity of each agent across the eight bacterial species; the Wilcoxon signed-rank test was used for pairwise comparisons between agents; and Spearman's rank correlation coefficient (ρ) was used to assess the association between the zone diameters produced by different agents on the same isolates. A two-tailed p -value < 0.05 was considered statistically significant throughout.

3. Results

3.1 Distribution of bacterial isolates

A total of 100 non-duplicate clinical isolates were included in the study. *Escherichia coli* was the predominant organism (40.0%), followed by *Proteus mirabilis* (19.0%) and *Pseudomonas* spp. (10.0%); the remaining isolates comprised *Enterococcus faecalis*, *Enterococcus faecium*, *Klebsiella pneumoniae*, *Klebsiella oxytoca* and *Proteus vulgaris* (Table 1).

Table 1. Distribution of the 100 clinical bacterial

isolates by species

Bacterial species	No. of isolates (n)	Percentage (%)
<i>Escherichia coli</i>	40	40.0
<i>Proteus mirabilis</i>	19	19.0
<i>Pseudomonas spp.</i>	10	10.0
<i>Enterococcus faecalis</i>	9	9.0
<i>Enterococcus faecium</i>	7	7.0
<i>Proteus vulgaris</i>	6	6.0
<i>Klebsiella pneumonia</i>	5	5.0
<i>Klebsiella oxytoca</i>	4	4.0
Total	100	100.0

Values are number of isolates and column percentage of the total study sample (n = 100).

3.2 Overall antibacterial activity of the aqueous clove extract and reference antibiotics

Across all 100 isolates, the aqueous clove extract produced inhibition zones ranging from 6 to 36 mm, with a mean of 21.9 ± 9.7 mm and a median of 28.0 mm. For comparison, MRP produced zones ranging from 17 to 40 mm (mean 28.8 ± 6.4 mm, median 28.0 mm), and TE produced zones ranging from 6 to 32 mm (mean 16.9 ± 6.0 mm, median 17.0 mm), based on the complete, corrected dataset of 100 isolates (Table 2). The comparatively high standard deviation of the clove zones (9.7 mm) alongside a similar overall median to MRP (28.0 mm) reflects the markedly bimodal, species-dependent pattern of clove activity described in Section 3.3.

Table 2. Overall descriptive statistics of inhibition-zone diameters (mm) for the aqueous clove extract (5% w/v) and the two reference antibiotics (n = 100 isolates)

Agent	n	Mean $\pm$ SD (mm)	Median (mm)	Minimum (mm)	Maximum (mm)
Clove extract (5%)	100	21.9 ± 9.7	28.0	6	36
MRP	100	28.8 ± 6.4	28.0	17	40
TE	100	16.9 ± 6.0	17.0	6	32

Agent	n	Mean $\pm$ SD (mm)	Median (mm)	Minimum (mm)	Maximum (mm)
Clove extract (5%)	100	21.9 ± 9.7	28.0	6	36
MRP	100	28.8 ± 6.4	28.0	17	40
TE	100	16.9 ± 6.0	17.0	6	32

Corrected, complete dataset (n = 100 for all three agents); see Section 2.7 for the data-verification note.

3.3 Species-specific antibacterial activity

The antibacterial activity of the clove extract varied markedly and significantly between bacterial species (Kruskal–Wallis $H = 64.47$, $p < 0.001$). The largest mean clove inhibition zones were observed against *Proteus mirabilis* (30.8 ± 3.0 mm), *Klebsiella pneumoniae* (30.0 ± 1.4 mm) and *Proteus vulgaris* (30.0 ± 1.3 mm), followed by *Klebsiella oxytoca* (29.0 ± 1.2 mm) and *Pseudomonas spp.* (28.0 ± 6.5 mm). In contrast, the smallest mean clove zones were recorded against *Escherichia coli* (14.7 ± 7.9 mm), *Enterococcus faecium* (17.7 ± 8.2 mm) and *Enterococcus faecalis* (18.2 ± 9.4 mm) (Table 3). The activity of both reference antibiotics also varied significantly between species (Kruskal–Wallis: MRP, $H = 32.66$, $p < 0.001$; TE, $H = 31.89$, $p < 0.001$).

Table 3. Inhibition-zone diameters (mean $\pm$ SD, mm) of the aqueous clove extract, MRP and TE, stratified by bacterial species

Bacterial species	n	Clove 5% (mm)	MRP (mm)	TE (mm)
<i>Escherichia coli</i>	40	14.7 ± 7.9	27.4 ± 6.1	19.4 ± 6.2
<i>Enterococcus faecium</i>	7	17.7 ± 8.2	33.7 ± 5.1	13.6 ± 4.6
<i>Enterococcus faecalis</i>	9	18.2 ± 9.4	34.2 ± 5.6	9.9 ± 4.9
<i>Klebsiella</i>	5	30.0 ± 1.4	34.0 ± 1.3	11.2 ± 1.2

Bacterial species	n	Clove 5% (mm)	MRP (mm)	TE (mm)
<i>pneumonia</i>		1.4	5.1	4.7
<i>Klebsiella oxytoca</i>	4	29.0 ± 1.2	33.0 ± 6.0	14.5 ± 4.2
<i>Proteus vulgaris</i>	6	30.0 ± 1.3	23.8 ± 3.6	15.8 ± 2.4
<i>Proteus mirabilis</i>	19	30.8 ± 3.0	24.9 ± 3.8	18.4 ± 4.7
<i>Pseudomonas spp.</i>	10	28.0 ± 6.5	32.6 ± 6.5	17.2 ± 4.0

SD, standard deviation. Based on the complete, corrected dataset (see Section 2.7).

3.4 Paired comparison of clove extract versus reference antibiotics

Because each isolate was tested against all three agents, paired (within-isolate) comparisons were carried out using the Friedman test. A statistically significant difference among the three agents was found overall ($\chi^2 = 72.11$, $n = 100$, $p < 0.001$) and within every individual species (Table 4), confirming that the relative ranking of clove, MRP and TE activity is not uniform but depends on the bacterial species being tested.

Table 4. Friedman test comparing clove extract, MRP and TE inhibition zones within each bacterial species (paired data)

Bacterial species	n	χ^2 statistic	p-value
<i>Escherichia coli</i>	40	36.65	< 0.001
<i>Enterococcus faecium</i>	7	9.54	0.008
<i>Enterococcus faecalis</i>	9	14.80	< 0.001
<i>Klebsiella pneumoniae</i>	5	8.32	0.016
<i>Klebsiella oxytoca</i>	4	6.50	0.039
<i>Proteus vulgaris</i>	6	12.00	0.003

Bacterial species	n	χ^2 statistic	p-value
<i>Proteus mirabilis</i>	19	29.94	< 0.001
<i>Pseudomonas spp.</i>	10	15.37	< 0.001
All isolates (overall)	100	72.11	< 0.001

Based on the complete, corrected dataset ($n = 100$; see Section 2.7).

Overall Wilcoxon signed-rank tests confirmed that clove zones were, on average, significantly smaller than MRP zones ($p < 0.001$) but significantly larger than TE zones ($p < 0.001$), and that MRP zones were significantly larger than TE zones ($p < 0.001$) (Table 5).

Table 5. Overall pairwise Wilcoxon signed-rank comparisons between agents ($n = 100$ paired observations)

Comparison	Direction of difference	W statistic	p-value
Clove vs. MRP	MRP > Clove	686.0	< 0.001
Clove vs. TE	Clove > TE	1230.0	< 0.001
MRP vs. TE	MRP > TE	170.5	< 0.001

3.5 Comparative efficacy of clove extract relative to reference antibiotics

Considering each isolate individually, the clove extract produced a zone diameter equal to or greater than that of MRP in 38 of 100 isolates (38.0%) overall, and equal to or greater than that of TE in 72 of 100 isolates (72.0%) overall. This proportion varied widely by species: clove matched or exceeded MRP in 100% of *Proteus vulgaris* isolates and 94.7% of *Proteus mirabilis* isolates, but in none of the *Enterococcus faecalis* isolates and only 15.0% of *E. coli* isolates (Table 6). Clove matched or exceeded TE activity in 71.4–100% of isolates for every species except *E. coli* (42.5%), reaching 100% among *Klebsiella pneumoniae*,

Klebsiella oxytoca, *Proteus vulgaris* and *Proteus mirabilis* isolates.

Table 6. Proportion of isolates in which the clove extract produced an inhibition zone equal to or greater than that of MRP or TE, by species

Bacterial species	n	Clove $\geq$ MRP (%)	Clove $\geq$ TE (%)
<i>Escherichia coli</i>	40	15.0	42.5
<i>Enterococcus faecium</i>	7	28.6	71.4
<i>Enterococcus faecalis</i>	9	0.0	77.8
<i>Klebsiella pneumoniae</i>	5	40.0	100.0
<i>Klebsiella oxytoca</i>	4	25.0	100.0
<i>Proteus vulgaris</i>	6	100.0	100.0
<i>Proteus mirabilis</i>	19	94.7	100.0
<i>Pseudomonas</i> spp.	10	30.0	90.0
All isolates (overall)	100	38.0	72.0

3.6 Correlation between agents

Spearman correlation analysis showed no significant association between clove and MRP zone diameters ($\rho = 0.051$, $p = 0.616$) and no significant association between clove and TE zone diameters ($\rho = -0.113$, $p = 0.263$), indicating that the antibacterial activity of the clove extract does not simply track the activity of either antibiotic across isolates. A weak but statistically significant negative correlation was observed between MRP and TE zone diameters ($\rho = -0.328$, $p < 0.001$) (Table 7).

Table 7. Spearman rank correlation between inhibition-zone diameters of the three agents (n = 100 paired isolates)

Pair agents	of	Spearman rho	p-value	Interpretation
Clove MRP	vs.	0.051	0.616	No significant correlation
Clove TE	vs.	-0.113	0.263	No significant correlation
MRP TE	vs.	-0.328	< 0.001	Weak negative correlation

3.7 Descriptive distribution of clove inhibition-zone diameters

For descriptive purposes only, and without implying any validated clinical breakpoint, the clove inhibition zones were grouped into four arbitrary bands. Using this purely descriptive scheme, 64 of 100 isolates (64.0%) produced a clove zone of 18 mm or more, while 17 isolates (17.0%) produced a zone of less than 9 mm, most of which belonged to the *E. coli*, *Enterococcus* and *Pseudomonas* groups (Table 8). This bimodal pattern — a large cluster of isolates with strong clove-zone responses and a smaller but sizeable cluster with minimal response — mirrors the significant inter-species heterogeneity already demonstrated in Table 3, and should be interpreted as a descriptive summary rather than as a susceptible/resistant classification, since no validated breakpoints currently exist for plant extracts tested by agar-well diffusion.

Table 8. Descriptive distribution of clove inhibition-zone diameters across four arbitrary bands (n = 100)

Zone-diameter band	No. of isolates	Percentage (%)
< 9 mm	17	17.0
9–12 mm	14	14.0
13–17 mm	5	5.0
≥ 18 mm	64	64.0
Total	100	100.0

These bands are descriptive only and do not represent CLSI or any other validated interpretive criteria

4. Discussion

This study evaluated the in vitro antibacterial activity of a simple 5% w/v aqueous clove extract against 100 clinical bacterial isolates and compared it with two reference antibiotics. The most striking finding was the pronounced species-dependence of clove activity: the extract performed strongly against *Proteus mirabilis*, *Proteus vulgaris*, *Klebsiella pneumoniae* and *Klebsiella oxytoca* (mean zones of 29–31 mm, often equal to or exceeding MRP), moderately against *Pseudomonas* spp., and comparatively weakly against *Escherichia coli* and the two *Enterococcus* species. This pattern is broadly consistent with previous reports that the antibacterial potency of clove preparations is highly dependent on both the target organism and the extraction method used [7].

Several previous studies have specifically examined clove activity against *E. coli* and reported activity that ranged from modest to strong depending on extract type. Marouf et al. found that an aqueous clove extract exerted bactericidal activity against multidrug-resistant uropathogenic *E. coli* and induced conversion of the bacteria to unstable L-forms, supporting a genuine antibacterial rather than merely bacteriostatic effect [8]. Similarly, Mekky et al. reported clove-extract inhibition zones of approximately 15–21 mm against *E. coli* and *Klebsiella pneumoniae* depending on the extraction and storage conditions used, broadly overlapping with the lower end of the range observed in the present study for these species [7]. Khudaier evaluated an ethanolic (rather than aqueous) clove extract against multidrug-resistant Gram-negative bacteria, including *E. coli*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*, and likewise reported organism-dependent variability in activity [9]. Taken together with the present findings, this body of evidence suggests that aqueous clove extracts — which are simpler and safer to prepare than ethanolic extracts or essential oils, but generally

extract eugenol and other lipophilic phenolics less efficiently — may show attenuated activity against *E. coli* relative to more lipophilic extraction methods, which would explain the comparatively low mean clove-zone diameter (14.7 ± 7.9 mm) observed against *E. coli* in this study.

The comparatively strong activity of clove against *Proteus mirabilis*, *Proteus vulgaris* and the two *Klebsiella* species is a notable finding of this study, since these organisms are frequently associated with catheter-associated and other nosocomial infections and are increasingly reported as multidrug-resistant in Iraqi clinical settings [4]. The proposed mechanism of eugenol-mediated antibacterial action — non-specific disruption of the cytoplasmic membrane, increased membrane permeability, leakage of intracellular ATP and other macromolecules, and inhibition of membrane-bound enzymes — has been demonstrated experimentally against several Gram-negative and Gram-positive species, including *Proteus mirabilis* and *Proteus vulgaris* [6,10,12]. Because this mechanism does not rely on the same molecular targets as conventional antibiotics (e.g., cell-wall synthesis, protein synthesis, or DNA gyrase), it may retain activity against isolates that have already acquired resistance to structurally unrelated antibiotic classes, which is consistent with the observation in the present study that clove zones equalled or exceeded MRP zones in the great majority of *Proteus* isolates (94.7–100%).

Conversely, the extract's comparatively weak activity against *Enterococcus faecalis* (mean 18.2 ± 9.4 mm, with clove never matching or exceeding MRP) may reflect the intrinsically greater resistance of enterococci — which possess a thick Gram-positive cell wall and several intrinsic resistance mechanisms — to many antimicrobial agents, including some plant extracts, especially at the relatively low nominal concentration (5% w/v) and short boiling-based extraction protocol used here. This is in keeping with a recent report by Saleh et al., who found wide variability (7.1–21.1

mm) in clove inhibition zones against *Enterococcus faecalis* isolated from urinary tract infections, again underscoring the substantial isolate-to-isolate and study-to-study variability that characterises this literature [7].

The absence of a significant correlation between clove and either antibiotic (Spearman $\rho = 0.051$ and -0.113 for MRP and TE, respectively) is biologically plausible given the distinct, membrane-targeted mechanism of eugenol compared with the cell-wall-synthesis-inhibiting mechanism typically attributed to a carbapenem such as meropenem, or the protein-synthesis-inhibiting mechanism of tetracycline. This lack of correlation supports the hypothesis that clove extract does not share cross-resistance mechanisms with these antibiotic classes and could, in principle, retain activity against isolates that are resistant to them — although this hypothesis would require direct confirmation using isolates with a documented antibiotic-resistance phenotype, which was not available in the present dataset. The weak negative correlation observed between MRP and TE zone diameters ($\rho = -0.328$, $p < 0.001$) is more difficult to interpret with confidence from zone-diameter data alone and may reflect differing, organism-specific resistance patterns to carbapenems versus tetracyclines among the isolates tested; this observation should be interpreted cautiously and would benefit from confirmation using confirmed antibiotic identities and CLSI-based categorical interpretation rather than raw zone diameters.

These findings should be considered within the broader context of the escalating AMR burden in Iraq and globally. National Iraqi surveillance data have documented alarmingly high resistance rates to third-generation cephalosporins and carbapenems among the same genera examined in this study [4], and a national review has highlighted persistent gaps in antimicrobial stewardship and regulatory enforcement [5]. Against this backdrop, natural antibacterial

candidates such as clove which is inexpensive, widely available, and has a long history of safe traditional use merit continued, methodologically rigorous investigation as potential adjuncts to conventional therapy or as topical/non-systemic antiseptic agents, even though they are not a substitute for a confirmed, breakpoint-based antibiotic prescription for systemic infection [3,7].

6. Conclusion

In this cross-sectional laboratory study of 100 clinical bacterial isolates from Al-Shomali General Hospital, a simple 5% w/v aqueous clove (*Syzygium aromaticum*) extract demonstrated antibacterial activity that varied significantly by bacterial species. The extract showed its strongest activity against *Proteus mirabilis*, *Proteus vulgaris*, *Klebsiella pneumoniae* and *Klebsiella oxytoca*, frequently matching or exceeding the inhibition zones produced by meropenem, while showing comparatively weaker activity against *Escherichia coli* and *Enterococcus faecalis*. These results add to a growing body of evidence supporting the potential of clove as a natural antibacterial candidate against clinically important, and increasingly multidrug-resistant, bacterial pathogens.

References

1. Naghavi M, Vollset SE, Ikuta KS, Swetschinski LR, Gray AP, Wool EE, et al. Global burden of bacterial antimicrobial resistance 1990-2021: a systematic analysis with forecasts to 2050. *Lancet*. 2024 Sep 16.
2. Murray CJL, Ikuta KS, Sharara F, Swetschinski L, Robles Aguilar G, Gray A, et al. Global burden of bacterial antimicrobial resistance in 2019: a systematic analysis. *Lancet*. 2022;399:629-655.
3. Sati H, Carrara E, Savoldi A, Hansen P, Garlasco J, Campagnaro E, et al. The WHO Bacterial Priority Pathogens List 2024: a prioritisation study to guide research,

- development, and public health strategies against antimicrobial resistance. *Lancet Infect Dis.* 2025 Sep. doi:10.1016/S1473-3099(25)00118-5.
4. Alhilfi R, Al-Jumaili AA, AlJoori BA, Abdul Mahdi R, Shakir DA, Al-Khafaji F. Four-year analysis of antimicrobial resistance patterns in Iraq. *East Mediterr Health J.* 2026;32:261-269.
 5. Al-Jumaili AA, Ahmed KK. A review of antibiotic misuse and bacterial resistance in Iraq. *East Mediterr Health J.* 2024;30:663-670.
 6. Devi KP, Nisha SA, Sakthivel R, Pandian SK. Eugenol (an essential oil of clove) acts as an antibacterial agent against *Salmonella typhi* by disrupting the cellular membrane. *J Ethnopharmacol.* 2010;130:107-115.
 7. Tariq H, Alhudhaibi AM, Abdallah EM. *Syzygium aromaticum* (clove buds) as a natural antibacterial agent: a promising alternative to combat multidrug-resistant bacteria. *Front Microbiol.* 2025;16:1674590.
 8. Marouf R, Ermolaev AA, Podoprighora IV, Senyagin AN, Mbarga MJA. Antibacterial activity of Clove *Syzygium aromaticum* L. and synergism with antibiotics against multidrug-resistant uropathogenic *E. coli*. *RUDN J Med.* 2023;27:379-390.
 9. Khudaier AM. Antibacterial activity of ethanolic clove *Syzygium aromaticum* extract against multidrug-resistant gram-negative bacteria. *Sci Prog Innov.* 2025.
 10. Hemaiswarya S, Doble M. Synergistic interaction of eugenol with antibiotics against Gram negative bacteria. *Phytomedicine.* 2009;16:997-1005.
 11. Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing. 34th ed. CLSI supplement M100. Wayne (PA): Clinical and Laboratory Standards Institute; 2024.
 12. Balouiri M, Sadiki M, Ibnsouda SK. Methods for in vitro evaluating antimicrobial activity: A review. *J Pharm Anal.* 2016;6:71-79.
 13. Perez C, Pauli M, Bazerque P. An antibiotic assay by the agar well diffusion method. *Acta Biol Med Exp.* 1990;15:113-115.

To site this article: Alhusayni A. A. In Vitro Antibacterial Activity of Aqueous Clove (*Syzygium aromaticum*) Extract against Multidrug-Resistant Enteric Bacterial Isolates from Al-Shomali General Hospital: A Comparative Study with Standard Antibiotics. *Infinity J. Med. Innov.* 2026; 2(1): 109-119.