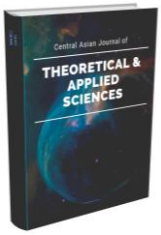

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Article

Evaluation of the Antibacterial Efficacy of Vinegar as a Feminine Wash Against Klebsiella pneumonia

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Abstract: Urinary tract infections (UTIs) are among the most common health problems affecting women worldwide. Klebsiella pneumoniae plays a significant role in the occurrence of UTIs, particularly in chronic cases, owing to its high capacity to acquire antibiotic resistance. This study aimed to evaluate the antibacterial efficacy of vinegar as a feminine wash against K. pneumoniae isolates obtained from women suffering from chronic urinary tract infections, and to compare this efficacy against commonly used antibiotics. The study included the collection of 50 clinical specimens from women with chronic UTIs. Bacterial identification was performed using culture methods, Gram staining, biochemical tests, and confirmed by the VITEK automated system. Antibiotic susceptibility testing was carried out using the Disk Diffusion Method. The antibacterial effect of vinegar at concentrations of 25%, 50%, and 75% was assessed by measuring inhibition zones. Results showed that K. pneumoniae was the most prevalent isolate (40%), with varying rates of antibiotic resistance. Meropenem achieved the highest sensitivity rate at 100%. Vinegar demonstrated concentration-dependent antibacterial activity: weak at 25%, moderate at 50%, and strong at 75% with large inhibition zones against K. pneumoniae isolates. The study concludes that vinegar possesses clear antibacterial efficacy, suggesting its potential use as an adjunctive or supportive agent in the prevention and management of female urinary tract infections. Future research is recommended to assess cellular safety and clinical effects before any medical application.

Keywords: Klebsiella pneumonia, Urinary Tract Infections (UTIs), Antibiotic Resistance, Vinegar (Acetic Acid), Antibacterial Activity, Agar Well Diffusion Method

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1. Introduction

Urinary tract infections (UTIs) are among the most prevalent bacterial infections in women globally and represent a significant health burden due to their recurrence and association with growing clinical and economic complications. Anatomical and hormonal factors contribute to women's increased susceptibility to these infections, particularly chronic or recurrent cases that are difficult to control with conventional therapies [1].

In this context, opportunistic Gram-negative bacteria — most notably Klebsiella pneumoniae — emerge as one of the principal pathogens associated with urinary tract infections, owing to their high capacity for causing infection, forming biofilms, and their increasing resistance to many common antibiotics [2].

The rapid spread of antibiotic-resistant strains has narrowed the available therapeutic options, prompting the scientific community to search for safe, effective, and low-cost alternatives or complementary measures. Among these alternatives, natural

products have attracted growing interest due to their antimicrobial properties and minimal side effects. Vinegar, particularly acetic acid vinegar, is one such substance that multiple studies have shown to possess antibacterial activity through mechanisms including reduction of pH and direct effects on bacterial cell membrane integrity [3].

Despite the traditional use of vinegar as a feminine wash in some communities, scientific data regarding its efficacy against urinary pathogens — particularly K. pneumoniae isolates from chronic UTI cases — remain limited and insufficient. Accordingly, this study aims to evaluate the antibacterial efficacy of vinegar as a feminine wash against K. pneumoniae isolates obtained from women with chronic urinary tract infections, in order to determine the feasibility of adopting it as a supportive or alternative option in infection prevention and control strategies, amid the increasing challenges of antibiotic resistance [4].

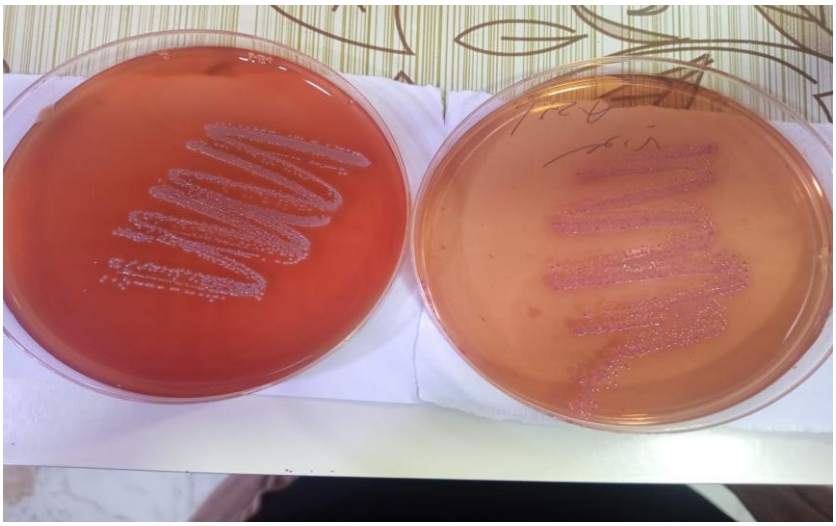

Figure1: Gram staining of bacteria showing purple Gram-positive cells and pink Gram-negative cells. *Klebsiella pneumoniae* appears as Gram-negative rod-shaped bacteria.

Research Objectives

1. Isolation and identification of bacteria causing urinary tract infections.
2. Evaluation of isolate susceptibility to antibiotics.
3. Testing the antibacterial effect of vinegar at concentrations of 25%, 50%, and 75%.
4. Comparing the efficacy of vinegar against the tested antibiotics.

2. Materials and Methods

2.1 Study Design and Sample Collection

This experimental laboratory study was conducted on clinical samples collected from women suffering from chronic urinary tract infections. Fifty midstream urine samples were collected from patients attending selected healthcare centers, using sterile containers under appropriate aseptic conditions. Samples were transported directly to the microbiology laboratory for necessary examination [5].

2.2 Isolation and Identification of Bacterial Isolates

Urine samples were cultured on appropriate standard culture media and incubated at 37°C for 24 hours. After bacterial growth appeared, colony morphological characteristics were examined, followed by Gram staining to differentiate between Gram-positive and Gram-negative bacteria [6].

Preliminary identification was carried out using a panel of standard biochemical tests, including: Catalase, Oxidase, Indole, Citrate utilization, Methyl Red (MR), Voges–

Proskauer (VP), Urease, and Motility tests. For final confirmation of isolate identity, particularly Klebsiella pneumoniae, the VITEK automated diagnostic system was used according to the manufacturer's instructions [7].

2.3 Antibiotic Susceptibility Testing

The susceptibility of K. pneumoniae isolates to several antibiotics was evaluated using the Disk Diffusion Method. The tested antibiotics included: Meropenem, Azithromycin, Flagyl (Metronidazole), Amikacin, and Gentamicin. Results were interpreted based on inhibition zone diameters, and isolates were classified as sensitive or resistant according to standard laboratory criteria [8].

2.4 Preparation of Vinegar Concentrations

A commercial acetic acid-based vinegar was used in this study. Three different concentrations were prepared using sterile distilled water: 25%, 50%, and 75% (v/v). Concentrations were prepared immediately before use to ensure stability and accuracy [9].

2.5 Evaluation of Antibacterial Efficacy of Vinegar

The antibacterial effect of vinegar was tested using the Agar Well Diffusion Method. Mueller-Hinton agar plates were inoculated with pure cultures of K. pneumoniae, then sterile wells were punched into the culture medium, and each well was filled with a specified amount of one of the prepared vinegar concentrations [10].

Plates were incubated at 37°C for 24 hours, after which the diameters of inhibition zones formed around the wells were measured. The antibacterial effect was classified as weak, moderate, or strong based on the size of the inhibition zone [11].

2.6 Statistical Analysis

Results were recorded and analyzed using descriptive analysis. Bacterial isolation results were expressed as percentages, and the efficacy of different vinegar concentrations was compared based on the diameters of the inhibition zones formed.

3. Results

3.1 Bacterial Isolation Results

Bacterial Species	Count	Percentage (%)
Klebsiella pneumoniae	20	40%
Staphylococcus spp.	20	40%
E. coli	5	10%
Streptococcus spp.	5	10%

3.2 Biochemical Test Results

Test	E. coli	Klebsiella	Staphylococcus	Streptococcus
Gram Stain	Gram-negative rods	Gram-positive clusters	Gram-positive chains	Gram-negative rods
Catalase	+	+	-	+
Oxidase	-	-	-	-
Indole	+	-	-	-
Citrate	-	+	Variable	-
Methyl Red (MR)	+	-	Variable	-
Voges-Proskauer (VP)	-	+	+	-
Motility	+	-	-	-
Urease	-	+	+	-

3.3 Antibiotic Susceptibility Results

Antibiotic	Sensitivity Rate
Meropenem	100%
Azithromycin	80%
Flagyl (Metronidazole)	90%
Amikacin	50%
Gentamicin	30%

3.4 Vinegar Efficacy as an Antiseptic

Vinegar Concentration	Effect	Inhibition Zone
25%	Weak	Small
50%	Good	Moderate
75%	Strong	Large

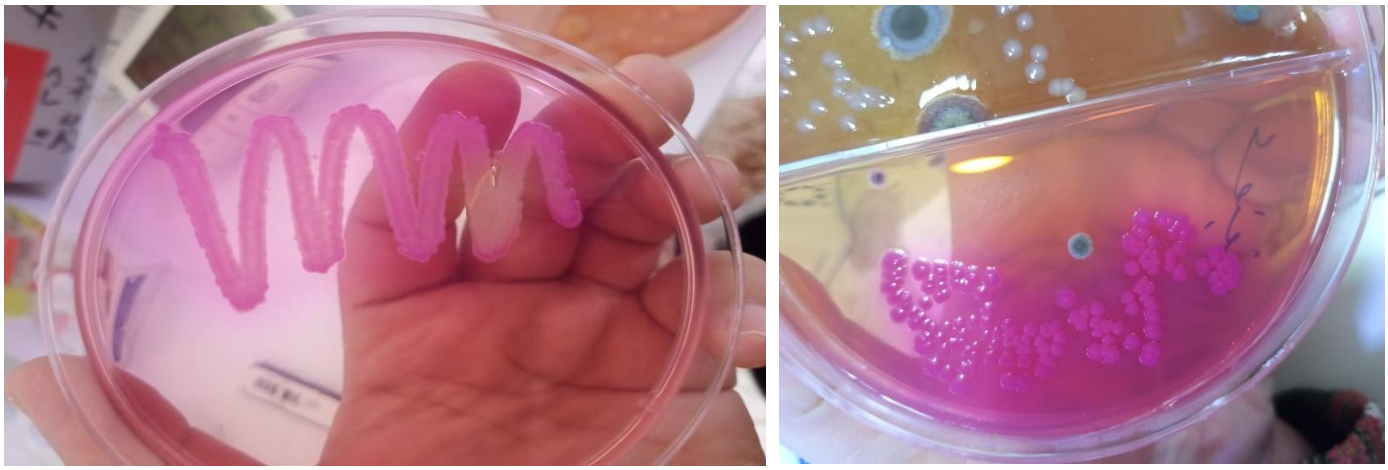

Figure 2. Gram staining of *Klebsiella pneumoniae* showing pink Gram-negative rod-shaped bacteria.

Figure 3. Gram staining of *Klebsiella pneumoniae* showing pink Gram-negative rod-shaped bacteria.

Figure 4. Gram staining of *Klebsiella pneumoniae* showing pink Gram-Positive rod-shaped bacteria.

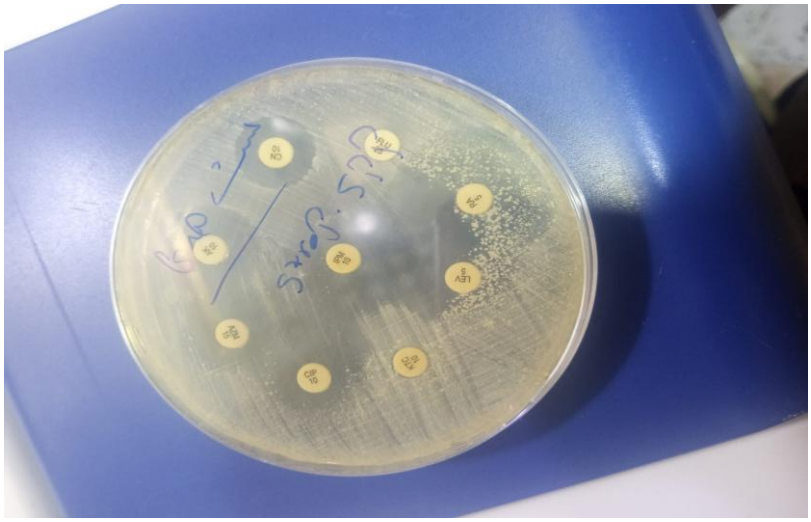

Figure 5. Antibiotic susceptibility test of *Klebsiella pneumoniae* using the disk diffusion method

Figure 6. Antibiotic susceptibility test of *Klebsiella pneumoniae* using the disk diffusion method

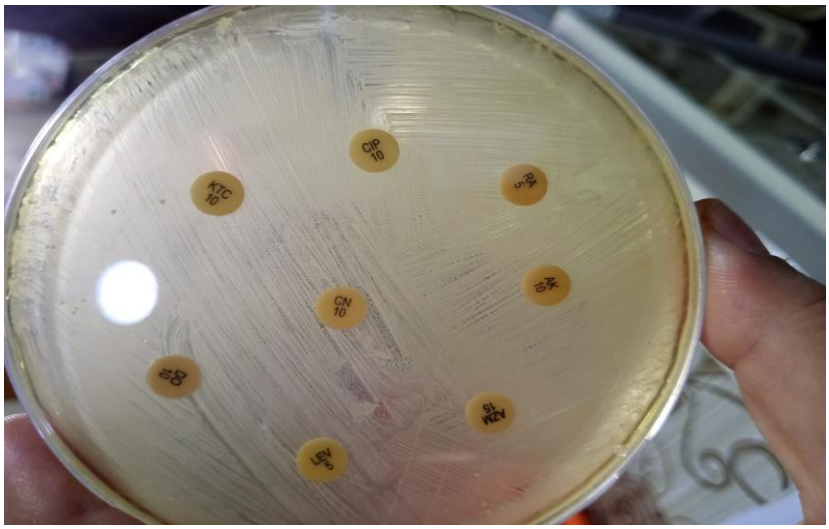

Figure 7. Antibiotic susceptibility test of *Klebsiella pneumoniae* using the disk diffusion method

Figure 8. Antibiotic susceptibility test of *Klebsiella pneumoniae* using the disk diffusion method

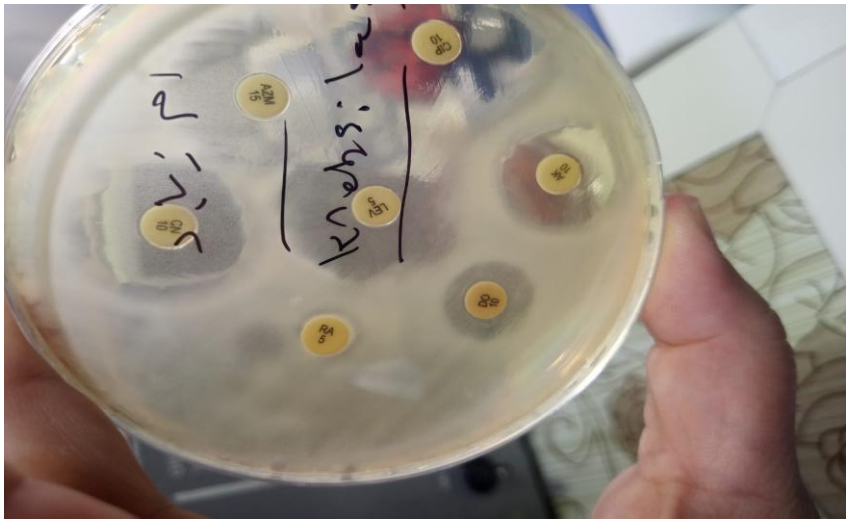

Figure 9. Antibiotic susceptibility test of *Klebsiella pneumoniae* using the disk diffusion method

Figure 10. Antibiotic susceptibility test of *Klebsiella pneumoniae* using the disk diffusion method

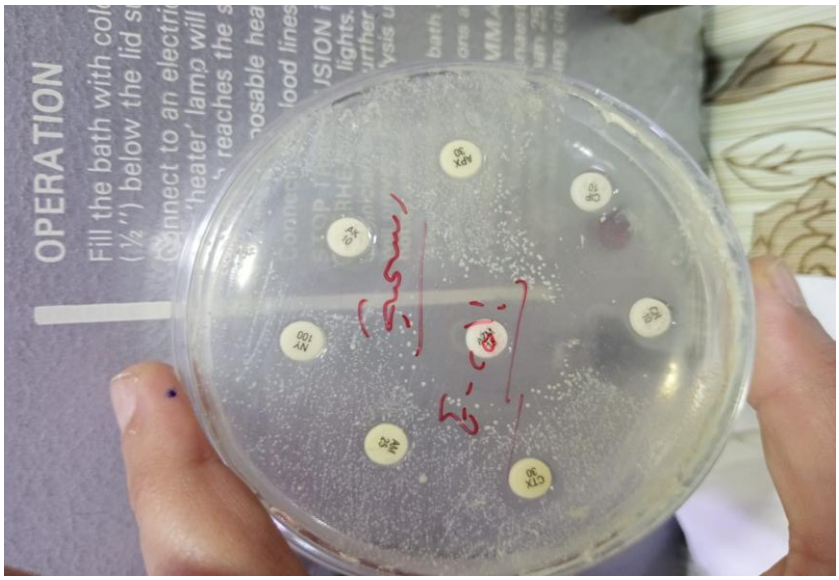

Figure 11. Antibiotic susceptibility test of *Klebsiella pneumoniae* using the disk diffusion method

4. Discussion

Chronic urinary tract infections (UTIs) in women represent a significant clinical challenge due to their high recurrence rate and the increasing involvement of multidrug-resistant bacteria. Among the most problematic uropathogens, *Klebsiella pneumoniae* has gained particular attention because of its strong virulence factors, ability to form biofilms, and marked capacity to acquire resistance to multiple classes of antibiotics. These characteristics complicate treatment outcomes and limit the effectiveness of conventional antimicrobial therapies [12].

The growing global concern regarding antibiotic resistance has encouraged renewed interest in alternative and complementary antimicrobial agents, especially those derived from natural sources. Natural products are often considered advantageous because of their low cost, wide availability, and generally lower incidence of adverse effects when compared with synthetic antimicrobial drugs. In this context, vinegar—whose main active

component is acetic acid—has been historically used as a disinfectant and antiseptic in both medical and domestic settings [13].

The antibacterial activity of vinegar is primarily attributed to its acidic nature, which leads to a reduction in environmental pH, disruption of bacterial cell membrane integrity, denaturation of cellular proteins, and inhibition of essential metabolic enzymes. These mechanisms can impair bacterial growth and survival, particularly in Gram-negative bacteria such as *Klebsiella pneumoniae*, whose outer membrane may be destabilized under acidic conditions. Previous in vitro studies have demonstrated that acetic acid can exert bacteriostatic or bactericidal effects against a wide range of pathogens, depending on concentration and exposure time [14].

Despite its traditional use as a feminine wash in certain communities, the scientific evaluation of vinegar's antibacterial efficacy against uropathogens—especially *Klebsiella pneumoniae* isolated from chronic UTI cases—remains limited. Assessing its activity under controlled laboratory conditions is therefore essential to determine whether such traditional practices have a valid microbiological basis. Furthermore, evaluating different concentrations of vinegar allows for an understanding of whether its antimicrobial effect is concentration-dependent, which is a critical factor when considering any potential clinical or preventive application. The Agar Well Diffusion method employed in this study is a widely accepted technique for evaluating the antimicrobial activity of liquid substances. It provides a direct visual and measurable indication of bacterial growth inhibition and allows for comparison between different concentrations of the tested agent. In parallel, assessing antibiotic susceptibility patterns of the isolates provides important context regarding current resistance trends and highlights the necessity of exploring supportive antimicrobial options [15].

This study is based on the hypothesis that vinegar exhibits antibacterial activity against *Klebsiella pneumoniae* and that this activity increases with higher concentrations. If confirmed, such findings may support the potential role of vinegar as an adjunctive or preventive agent rather than a replacement for antibiotics, particularly in reducing bacterial load or preventing recurrent infections. However, any suggested application must be approached cautiously, as further investigations are required to evaluate cytotoxicity, mucosal safety, and clinical effectiveness before recommending routine medical use [16].

5. Conclusion

The results of this study revealed that chronic urinary tract infections in women were caused by multiple bacterial species. Four different bacterial groups were isolated from 50 urine samples. Klebsiella pneumoniae and Staphylococcus spp. recorded the highest isolation rates at 40% each, while Escherichia coli and Streptococcus spp. were less prevalent, each accounting for 10%.

Biochemical tests and Gram staining showed that K. pneumoniae isolates were Gram-negative rods, oxidase-negative, catalase-positive, capable of citrate utilization, and non-motile — findings that confirmed their identification in comparison with standard reference data and VITEK system results.

Regarding antibiotic susceptibility, K. pneumoniae isolates showed notable variation in sensitivity and resistance rates. Meropenem recorded the highest sensitivity at 100%, followed by Flagyl at 90% and Azithromycin at 80%, whereas isolates showed high resistance to Gentamicin (30% sensitivity) and Amikacin (50% sensitivity).

The antibacterial efficacy test results demonstrated that vinegar's effect was concentration-dependent. At 25%, the antibacterial effect was weak with small inhibition zones. At 50%, a moderate effect was observed. At 75%, a strong effect with wide inhibition zones against K. pneumoniae isolates was recorded — indicating that vinegar possesses clear antibacterial activity at higher concentrations.

In conclusion, vinegar shows considerable antibacterial efficacy, suggesting its potential use as a supportive or adjunctive agent in the prevention and management of female urinary tract infections. However, further research is recommended to evaluate cellular safety and clinical effects before its adoption in medical applications.

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