Original Article

Assessing the Effect of Pomegranate Peel Extract against *Escherichia coli* and *Staphylococcus aureus* Isolated from Pregnant Women Suffering from Urinary Tract Infections in Al-Ramadi

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Abstract

Background: Bacteriuria, an excess of bacteria in the urine, may induce symptoms or be inconsequential. Urinary tract infections (UTIs) in pregnant women are frequently ascribed to *Staphylococcus aureus* and *Escherichia coli*, which are two well-known causative agents. The ethanolic extract of pomegranate peel may possess antibacterial properties that could protect against pathogenic microorganisms. **Objectives:** The objective of this research endeavor is to determine the effect of pomegranate peel ethanolic extract on *S. aureus* and *E. coli*. **Materials and Methods:** We utilized 100 samples from pregnant women diagnosed with chronic UTIs for the culture of the samples on selective and differential media. Subsequently, we employed biochemical assays to identify each isolate. The antibiotic susceptibility of the isolates was tested using the Vitek 2 system. We tested the antibacterial activity of pomegranate peel ethanolic extract at concentrations of 50, 25, 75, and 100 mg/mL using the agar well diffusion method. **Results:** Out of a total of 100 specimens, 65% were found to be infected with *E. coli* and 35% were infected with *S. aureus*. Antimicrobial sensitivity testing for *E. coli* revealed high resistance rates to ampicillin (75%), amoxicillin/clavulanic acid (82%), ceftriaxone (82%), and cefepime (85%), with lower resistance observed for gentamicin (49%). *S. aureus* exhibited high resistance to ciprofloxacin (85%) and amoxicillin/clavulanic acid (89%) while showing lower resistance to meropenem (50%). The antibacterial assay of the ethanolic extract of pomegranate peel indicated that, for *E. coli*, the lowest concentration (25 mg/mL) showed a lower percentage of inhibition zone at 10%, while the highest concentration (100 mg/mL) showed the highest percentage of inhibition zone at 48%. For *S. aureus*, the lowest concentration (25 mg/mL) displayed a lower percentage of inhibition zone at 12%, whereas the highest concentration (100 mg/mL) exhibited a higher percentage of inhibition zone at 38%. **Conclusion:** UTIs were most frequently caused by *E. coli*, followed by *S. aureus*; these specific isolates demonstrate significant resistance to the majority of antibiotics. Pomegranate peel ethanolic extract possesses antibacterial properties and may be considered for administration to humans.

Keywords: *Escherichia coli*, ethanolic extract, pomegranate peel, *Staphylococcus aureus*, UTI

Introduction

Whether bacteria are present in the urine and cause symptoms or not, this condition is referred to as bacteriuria.[1] Urinary tract infections (UTIs) are more common in females than males due to structural differences.[2] *Escherichia coli*, *Staphylococcus aureus*, and *Klebsiella* spp. are the bacteria most commonly associated with UTIs.[3]

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When *E. coli* is predominant, it can irritate the reproductive system and intestines. Some women may experience endometriosis, a condition where the inner lining develops outside of the uterus.[4] UTIs, the most common type of bacterial infection in pregnant women, are particularly prevalent during the third trimester.[5] The significance of *Escherichia*-associated tract infections in pregnant women remains uncertain, even though these specific strains of *E. coli* are equally dangerous as those that can cause severe complications for the expectant mother and her fetus.[6]

S. aureus is a common causative agent of UTIs; the bacterium is frequently detected in the blood, a phenomenon referred to as staphylococcal bacteremia, and can manifest as endocarditis.[7] *S. aureus* bacteriuria is associated with a significant financial and technological burden, as well as a high mortality rate.[8] Physicians face a significant therapeutic challenge due to the high incidence of UTIs among pregnant women. During pregnancy, changes in the morphology, physiology, and function of the urinary system correlate with an increased likelihood of ascending urethral infections.[9] Resistance of pathogenic microorganisms to antimicrobial drugs is a global concern.[10] Prominent microorganisms accountable for the development of drug resistance include *S. aureus* and *E. coli*.[11] The incidence of hospital-acquired infections induced by these pathogenic isolates has increased over the past few decades.[12]

As the number of drug-resistant diseases rises, standardized analytical methods for rapidly identifying and isolating novel bioactive compounds from medicinal plants are required.[13] Chemicals derived from medicinal plants may provide novel and straightforward methods of combating pathogenic microorganisms.[14]

An approach to protect against detrimental bacteria and other organisms involves the utilization of antimicrobial compounds present in fruit skins.[15] According to research,[16] pomegranate extracts may affect a diversity of microorganisms and viruses, among other things.

Pomegranates, belonging to the Punicaceae family, are formally referred to as *Punica granatum*. They provide an ample supply of phytochemicals, which are beneficial for health.[17] According to numerous studies, pomegranate peel, juice, seeds, and even the tree itself serve as sources of phytochemicals.[18] Pomegranates contain a variety of polyphenols, including anthocyanins, proanthocyanidins, flavonoids, ellagitannins, and gallotannins, as well as condensed tannins.[19]

Materials and Methods

Study design

The primary objective of this cross-sectional investigation was to examine pregnant women with persistent UTIs (bacterial infections). A total of 100 samples were randomly collected from pregnant women attending clinics and maternity facilities in Anbar Governorate between January and March of 2024. The samples were obtained from women of various ages.

Isolation of bacteria

A general urine examination was conducted for all samples, although data were not presented. Rapid culture on blood and MacConkey agar was initiated and continued aerobically at 37°C for a full day after sample collection. For *S. aureus*, samples were additionally purified on mannitol salt agar, and for *E. coli*, on eosin methylene blue. The positive results reported based on the inhibition zone around the well. Microscopic examination, biochemical tests, and, finally, the Vitek 2 system (Biomerieux, France) were all components of the standard bacteriological testing protocol used to identify the purified *S. aureus* and *E. coli* isolates.

Antimicrobial sensitivity test

Both Gram-negative bacteria, such as *E. coli*, and Gram-positive bacteria, such as *S. aureus* in this case, were subjected to antibiotic susceptibility testing using the Vitek 2 compact system with Vitek 2 antibiotic susceptibility testing (AST) cards by bioMérieux. For *E. coli*, the following drugs were examined: ampicillin, amoxicillin/clavulanic acid, piperacillin/tazobactam, cefoxitin, ceftriaxone, cefepime, imipenem, amikacin, gentamicin, ciprofloxacin, levofloxacin, and nitrofurantoin. The antibiotics found to be effective against *S. aureus* are as follows: ciprofloxacin, amikacin, norfloxacin, meropenem, tetracycline, cefotaxime, methicillin, ampicillin, nitrofurantoin, and amoxicillin/clavulanic acid.

Extraction of pomegranate peel plant

The pomegranate peel extracts in alcohol were prepared following the procedure described by Jaisinghani *et al.*[20] Pomegranate fruits were purchased from the local market. The skins of the pomegranates were carefully peeled off. The peel extract was prepared using the whole peel, including the albedo. The peels were washed thoroughly with sterile distilled water after rinsing with tap water. The samples were dehydrated at 45°C for 48 h and then ground into a fine powder using a sterile mixer grinder. Subsequently, they were stored in containers to prevent exposure to air.

The extraction process involved soaking 10 g of powdered peel in 90 mL of 96% ethanol for a full day. After this period, the peel particles were removed from the extract by filtering it using Whatman filter paper No. 1. Subsequently, the extract underwent vacuum concentration using a Heidolph VE-11, Paris rotating evaporator at temperatures below 40°C.

Antibacterial assay of pomegranate peel plant

An antibacterial experiment was conducted using the agar well diffusion technique.[21] Mueller–Hinton agar was sterilized and prepared using the resazurin microtiter plate assay, which was cooled to approximately 40°C, inoculated with various microbial cultures, and poured into plates. After solidification, 6 mm wells were created. Peel extract diluted in 10% DMSO was applied to the wells at several dilutions (25, 50, 75, and 100 mg/mL). The plates were left to incubate at 37°C overnight. After the incubation period, the zones of inhibition were measured and documented. Concurrent controls were conducted alongside the tests. The experiments were repeated three times.

Results

Identification of bacteria

E. coli exhibited lactose fermentation on MacConkey agar, β hemolysis on blood agar, and on eosin methylene blue (EMB) agar showed metallic green sheen colonies (as shown in Figure 1). Biochemical tests revealed negative oxidase, positive indole, and negative citrate utilization. *S. aureus* exhibited β hemolysis on blood agar and appeared as golden yellow colonies on mannitol salt agar. Biochemical tests revealed negative oxidase, negative catalase, and positive coagulase. Out of 100 urine specimens collected from female patients with suspected UTIs, 65% were found to be infected with *E. coli*, and 35% were infected with *S. aureus* [Figure 2].

Determine the resistance of pathogenic bacteria

E. coli and *S. aureus* exhibited high resistance percentages against most antibiotics as follows: for *E. coli*, ampicillin (75%), amoxicillin/clavulanic acid (82%), piperacillin/tazobactam (55%), cefoxitin (75%), ceftriaxone (82%), cefepime (85%), imipenem (70%), amikacin (56%), gentamicin (49%), ciprofloxacin (77%), levofloxacin (75%), and nitrofurantoin (60%; see Figure 3).

For *S. aureus*: gentamicin (71%), ciprofloxacin (85%), amikacin (78%), norfloxacin (65%), meropenem (50%), amoxicillin/clavulanic acid (89%), levofloxacin (66%), tetracycline (83%), cefotaxime (60%), methicillin (70%), nitrofurantoin (85%), and ampicillin (79%; see Figure 4).

Antibacterial activity of ethanolic pomegranate peel extract

The results presented in Figures 5 and 6 indicate that extracts from pomegranate peel exhibited potent antibacterial effects against both Gram-positive (*S. aureus*) and Gram-negative (*E. coli*) bacteria.

The peel extracts demonstrated significant activity against both bacterial strains. For *E. coli*, the lowest concentration tested (25 mg/mL) resulted in a lower percentage of inhibition zone at 10%, while the highest concentration (100 mg/mL) exhibited a higher percentage of inhibition zone at 48%. Similarly, for *S. aureus*, the lowest concentration (25 mg/mL) showed a lower percentage of inhibition zone at 12%, while the highest concentration (100 mg/mL) showed a higher percentage of inhibition zone at 38%.

Discussion

As depicted in Figure 1, *E. coli* accounted for 65% of the specimens infected, while *S. aureus* accounted for 35%. Additional research further validates these findings. For instance, Forson *et al.*[22] demonstrated that UTIs occurring during pregnancy are frequently attributed to extraintestinal pathogenic *E. coli* (ExPEC). ExPECs can colonize specialized biological environments such as the urogenital tract due to the diverse and unique virulence factors (VFs) they possess.[23] In community medicine, UTIs rank as the second most common infectious diagnosis.[24] Approximately 150 million individuals

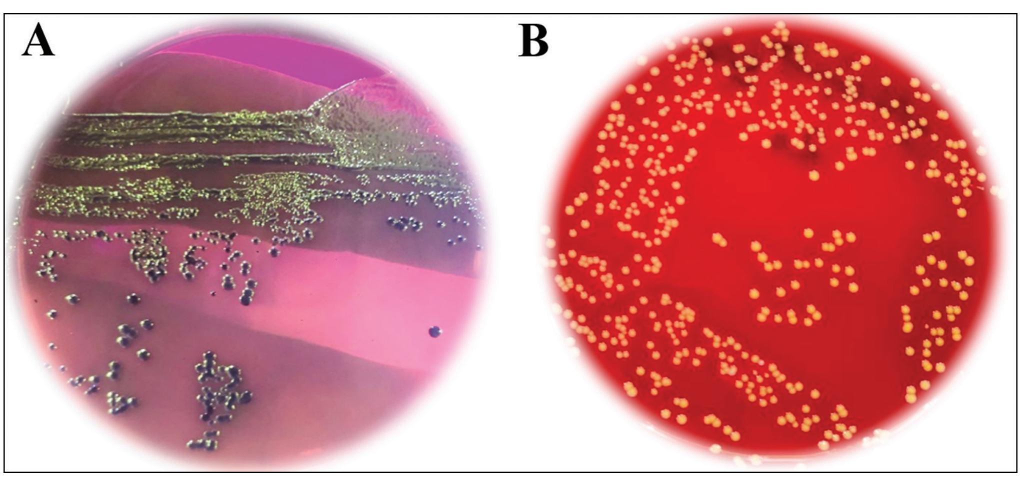

Figure 1: Morphological characterization of bacteria on culture media. (A) *E. coli* on EMB agar. (B) *Staphylococcus* on blood agar

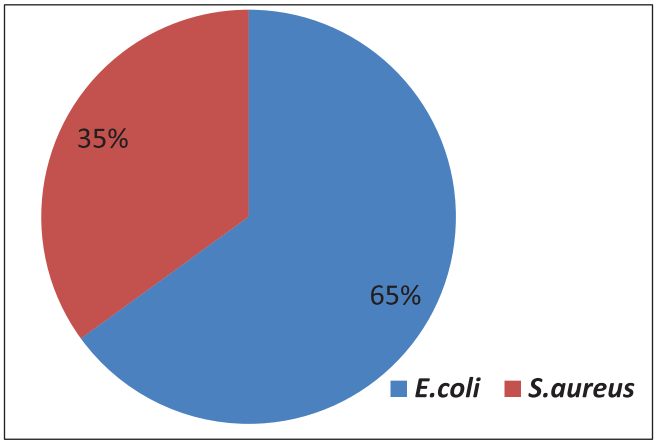

Figure 2: The percentage of *E. coli* and *S. aureus* bacteria from clinical samples

worldwide suffer from a UTI each year, with *E. coli* being associated with 82% of cases.[25]

Feminine contamination from the urethra, perineum, or other feminine areas is the most common route of transmission for UTIs caused by *E. coli* strains.[26] Around 50% of women will experience a UTI at some point in their lives, with 25% of this group developing recurrent infections. Sexual contact is also considered a plausible mode of transmission for uropathogenic *E. coli* isolates.[27]

According to Enupe *et al.*,[28] 40 (40%) of the 100 pregnant women tested positive for *S. aureus* infection, with the majority of them between the ages of 21 and 25. It is worth noting that most *S. aureus* bacteriuria infections do not present signs of a UTI.[29] The therapeutic significance of isolating *S. aureus* from urine in individuals with long-term

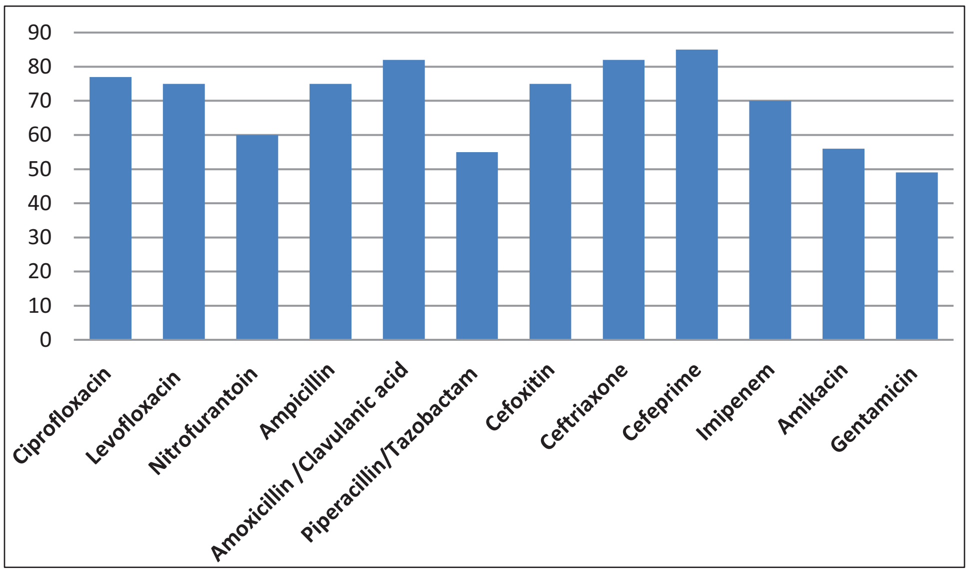

Figure 3: The percentage resistance of *E. coli* against antibiotics

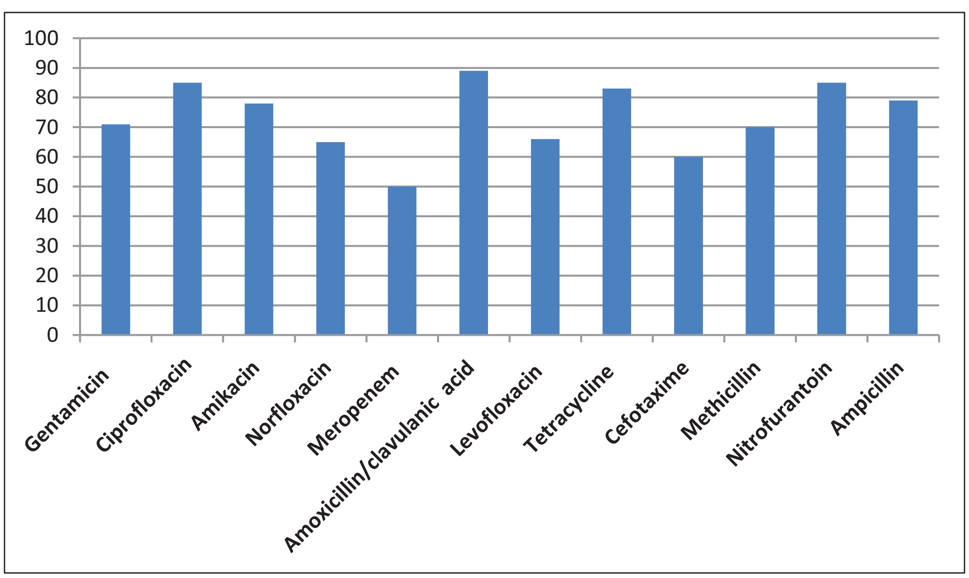

Figure 4: The percentage resistance of *S. aureus* against antibiotics

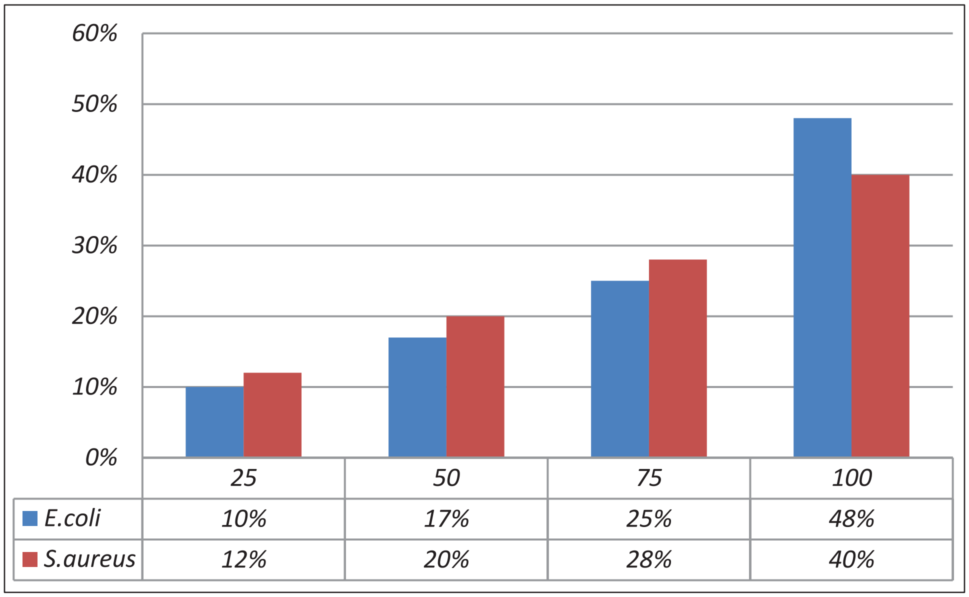

Figure 5: Antibacterial activity of ethanolic pomegranate peel against pathogenic bacteria

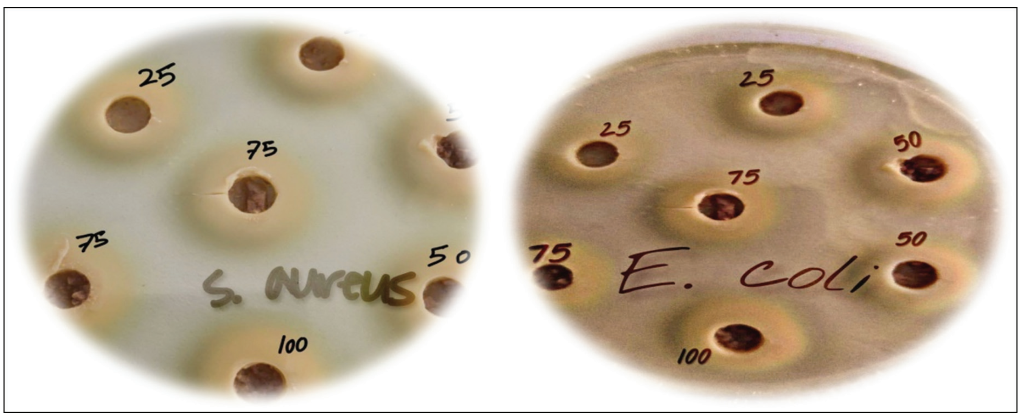

Figure 6: Inhibition diameter zone of *E. coli* and *S. aureus* bacteria

urinary catheterization is unclear, given that bacteriuria is almost always associated with this condition.[30] Although urinary *S. aureus* could potentially be the source of staphylococcal bacteremia,[31] the incidence of bacteremia in individuals with persistent *S. aureus* bacteriuria remains unknown.

It is estimated that between 5% and 10% of pregnant women may experience a UTI, with the exact percentage varying according to factors such as parity, race, socioeconomic status, and the anatomical and functional changes that occur during pregnancy.[32] In Mexico, premature delivery is responsible for 75% of neonatal fatalities and 50% of neurological sequelae.[33]

Changes in hormone levels during pregnancy are believed to sensitize the uterus to various assaults.[34] UTIs can trigger preterm labor by releasing cytokines such as interleukin (IL)-6, IL-8, and tumor necrosis factor-alpha, as well as activating the complement system.[32]

Although cervicovaginal infections and vaginosis are recognized as causes of preterm membrane rupture, there is currently no clear evidence indicating that UTIs act as a risk factor in this regard.[35]

However, as pregnancy progresses, the microflora of the vaginal tract becomes less harmful to the fetus.[36]

Being female poses a risk factor due to several factors, including the short urethra, close proximity to the vagina and anus, and the challenge women face in fully emptying their bladders.[22] Sexual activity and specific contraceptive methods are also believed to increase the risk.[27] The close anatomical proximity between the female urethra and vagina increases the risk of damage during sexual intercourse. Furthermore, this proximity may facilitate the transfer of bacteria from the vagina into the urethra and bladder during pregnancy or childbirth.[35]

Figures 2 and 3 demonstrate that most antibiotics are ineffective against *E. coli* and *S. aureus*, consistent with findings from several research studies.

E. coli isolates exhibited notable resistance to ampicillin and tetracycline, according to Shin *et al.*[37] The widespread and prolonged use of these medications is likely a contributing factor to the high prevalence of resistance.[38] Levofloxacin was found to have the highest percentage of resistance to the *E. coli* pathogen in a study by Ayub *et al.*[39] This contrasts with earlier findings indicating an 80% resistance rate to amoxicillin in cases of UTIs caused by *E. coli* in pregnant women.

The majority of UTIs in pregnant women were found to be caused by Gram-negative bacteria, as reported by Gessese *et al.*[40] and Hussein *et al.*[41] The following antibiotic susceptibility profiles were observed in Gram-negative (*E. coli*) bacteria: chloramphenicol 70%, tetracycline 30%, gentamycin 80%, ciprofloxacin 80%, ceftazidime amoxiclav 10%, ampicillin 50%, and cefotaxime 80%.

Most *S. aureus* isolates were found to be resistant to ciprofloxacin (80%) and methicillin (70.5%), as reported by Ghaima *et al.*[42] Among the several Gram-positive bacteria that may cause UTIs in pregnant women, *S. aureus* is among the most common.[43] Concerningly, several *S. aureus* strains have demonstrated reduced sensitivity to vancomycin and resistance to methicillin.[44]

The capacity of *S. aureus* to adapt and acquire antibiotic resistance has enabled its widespread presence in human populations, rendering it a significant pathogen in various environments.[45] Among all *S. aureus* infections that may arise in the healthcare setting, methicillin-resistant *S. aureus* (MRSA) is the most common.[42] According to Hasan *et al.*,[44] plasmid-mediated synthesis of β-lactamases and other activities are linked to antibiotic resistance in *S. aureus.*

With its thicker and weakly cross-linked cell wall, vancomycin-intermediate *S. aureus* can sequester glycopeptides by forming targets at the cell's periphery.[46] Patients suspected of having a UTI exhibited a clear resistance pattern to medications typically used to treat staphylococcal infections, and Mitiku *et al.*[47] found that MRSA was prevalent in these isolates. Possible explanations for the discrepancies in the studies include variances in the pathogens, physiological changes in the hosts, and the prevalence of pathogenic islands containing VFs in *E. coli* and *S. aureus*.[39]

Antibiotic use invariably leads to either the rapid or gradual evolution of resistance in bacteria, stemming from their genetic adaption mechanisms.[48] Given the escalating prevalence of antibiotic-resistant bacteria, it is imperative to monitor the prevalence of various infections in the region and promptly report any changes.[42]

The global public health crisis of antimicrobial resistance (AMR) is increasing worldwide, with poor- and medium-income countries facing particularly acute challenges.[49] As part of its Global Action Plan to fight AMR, the World Health Organization has prioritized surveillance efforts.[41]

In addressing AMR, the Global Antimicrobial Resistance Surveillance System (GLASS) establishes global standards for data collection, analysis, and exchange. The surveillance data sent to GLASS originates from results obtained from AST on clinical samples.[50]

The effects of ethanol-extracted pomegranate peel on both Gram-positive (*S. aureus*) and Gram-negative (*E. coli*) bacteria were investigated in this study. A recent study by Kupnik *et al.*[51] confirmed that the peel extracts possessed antibacterial capabilities. The growth inhibition of *S. aureus* and *E. coli* was used to demonstrate the apparent antibacterial activity of pomegranate aril and peel extracts.

The inhibitory zone widths for *E. coli*, *S. aureus*, and *Bacillus subtilis* were reported as 12 mm, 22 mm, and 12 mm, respectively, in a study by Nozohour *et al.*,[52] which tested pomegranate fruit extracts in ethanol and methanol. According to Jaisinghani *et al.*,[20] pomegranate cultivar-specific ethanol peel extracts inhibit the growth of both Gram-negative and Gram-positive bacteria. The numerous health benefits of pomegranate are attributed to the bioactive chemicals present in the fruit's pulp and skin.[53] The antimicrobial activities of pomegranate peel extract are believed to be attributed to the polyphenols found in the peel, particularly tannins, which have demonstrated actions against viruses, fungi, and bacteria.[54]

Antibacterial agents in the form of polyphenols exert their antibacterial effects by forming complexes with proteins in bacterial cell walls, leading to cell lysis. These compounds are characterized by their aromatic rings containing hydroxyl groups.[55] Polyphenols inhibit sulfhydryl groups present in soluble and extracellular microbial proteins, while their hydroxyl groups function to delocalize electrons and act as proton exchangers, thus reducing the pH gradient adjacent to the cell membrane.[56] Consequently, pharmaceuticals and efforts to combat antibiotic resistance could potentially benefit from isolating bioactive constituents from the skins of *P. granatum*, especially those demonstrating antibacterial properties.[52]

Conclusions

E. coli was identified as the most common bacteria causing UTIs, followed by *S. aureus*, with both isolates displaying high resistance to a wide range of antibiotics. The ethanolic extract of pomegranate peel exhibited significant antibacterial activity, suggesting its potential application as a medicinal treatment for humans. This not only reduces the costs and risks associated with antibiotic usage but also highlights the added value of utilizing leftover peels for health benefits, as well as in food preservation and pharmaceutical applications. Regular monitoring is necessary to establish reliable data on uropathogenic resistance patterns, facilitating appropriate empirical treatment of patients with UTIs.

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Conflicts of interest

There are no conflicts of interest.

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