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Molecular Diagnosis of Pseudomonas Aeruginosa Isolated from Urinary Tract Infections and Evaluation of the Effect of Solanum Nigrum Extract on its Growth

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Annotation: Background & aim: Antimicrobial resistance, biofilm formation, and a number of other virulence features make Pseudomonas aeruginosa a well-known gram-negative pathogenic bacterium. So, this study was aimed to molecular diagnosis of P. aeruginosa isolated from urinary tract infections and evaluation of the effect of Solanum nigrum extract on its growth.

Materials & Methods: During November 2024 to February 2025, 140 clinical samples of who had been admitted to Kirkuk Hospital in Kirkuk City after consulting with a specialist physician and being sent to the laboratory were gathered. The samples were then brought directly to the laboratory for culture on culture media.

Results: The findings demonstrated that, when grown in the optimal culture medium, 34 (24.3%) of the total samples had favorable results for bacterial growth. Out of all the samples, 106 (75.7%) had negative bacterial growth results. P. aeruginosa was highly responsive to gentamicin (82.3%), imipenem (91.2%), amikacin (97.1%), and ampicillin (88.3%). However, P. aeruginosa was 100% responsive to tobromycin. The findings showed that the ability of *S. nigrum* for bacteria inhibition, where the 50ul concentration showed the least efficiency against P. aeruginosa, as the diameter of the inhibition zone reached

6.13±1.07mm, while the 75ul concentration showed the highest inhibition, reaching 16.1±1.8mm, and the highest inhibition zone was recorded at the 100ul concentration, reaching 29.12±3.7mm. In the current study from 34 isolate, 34(100.0%) Pseudomonas aeruginosa isolates possessed *16S RNA* gene.

Conclusion: P. aeruginosa isolates are resistant to most antibiotics, with amikacin and topromycin being the most effective. S. nigrum extract exhibits antibacterial activity, and the 16S rRNA gene is a reliable marker for isolate identification.

Keywords: P. aeruginosa, 16S RNA, S. nigrum, Antimicrobial resistance.

Introduction

Natural products have been crucial to health care and disease prevention for thousands of years [1]. The plant has a variety of antibacterial, bacteriostatic, and chemotherapeutic properties [2]. In general, plants generate a large number of secondary metabolites, which are a significant source of insecticides, microbicides, and numerous pharmacological medications. Numerous polyphenols, including flavonoids, hydrolyzable tannins, and phenolic acid, exhibit antibacterial properties [3, 4]. Solanum nigrum is a member of the Solanumaceae family and has been shown to have anti-inflammatory, antibacterial, and anti-cancer properties [5]. Long used to treat a variety of ailments, the whole plant and its roots are employed, but the black fruits of S. nigrum are hazardous (mostly due to their high solanine content) and should not be consumed [6,7]. Gram-negative, aerobic, motile, and somewhat curved, P. aeruginosa is a bacterium that can be found in a variety of environments, including soil, water, air, and plant and animal tissues [8]. P. aeruginosa is a well-known opportunistic human pathogen that can cause a variety of deadly acute and chronic infections, such as meningitis, and infections of the urinary tract, especially in individuals with cystic fibrosis. Every year, it kills over 90,000 people and infects over 2 million people, making it one of the top three causes of opportunistic infections in humans [8,9]. High rates of morbidity and mortality are linked to P. aeruginosa UTIs in older adults. Compared to E. coli isolates, P. aeruginosa isolates from UTIs usually exhibit higher levels of antibiotic resistance [10,11]. One of the most significant bacteria producing complex clinical issues is P. aeruginosa [12]. One major issue facing world health is antimicrobial resistance. Antimicrobial resistance has developed as a result of the increased use of antibiotics in recent years, making infection treatment challenging [13]. So, this study was aimed to molecular diagnosis of Pseudomonas aeruginosa isolated from urinary tract infections and evaluation of the effect of *Solanum nigrum* extract on its growth.

Materials & methods

Specimen Collection

140 clinical samples were taken from Kirkuk Hospital in Kirkuk City between November 2024 and February 2025 from women who had been admitted after consulting with a specialist physician and being sent to the laboratory. The following procedures were part of the sample collection process: Women with UTIs between the ages of 20 and 68 had their urine samples collected. The samples were then sent straight to the laboratory for culture on culture media.

Bacterial Identification

Bacteria were diagnosed based on the following aspects:

Morphological diagnosis

Based on their cultural properties, the P. aeruginosa colonies growing on cetrimide and blood agar were identified, and they were subsequently incubated for 24 hours at 37.0C.

Direct examination

Bacterial colonies were studied using a microscope to examine the morphological characteristics of the bacterial cells, focusing on their reaction with Gram stain, which allows differentiation of the type of reaction as well as the shape and arrangement of the cells.

Biochemical reaction and motility test

Multiple biochemical tests were performed to diagnose the bacteria, including H_2S, methyl red, citrate, urease, Voges-Proskauer, catalase, oxidase, and indole.

Identification by VITEK2

The VITEK 2 device, which represents advanced colorimetric technology and is one of the latest devices for microbial diagnosis, was used. All steps were carried out in accordance with the manufacturer's instructions, Biomerieux.

Antibiotic susceptibility test (AST)

Antibiotic susceptibility testing was performed on each isolate using the Kirby-Bauer method on Mueller-Hinton agar, in accordance with CLSI guidelines (2020).

Solanum nigrum leave extraction

In January 2025, the leaves were gathered from Kirkuk's local marketplaces. 150 mL of water was used to macerate the dried and powdered leaves (5 g) for 24 hours at room temperature. After three iterations of the extraction process, the solvent extracts were mixed and separated from the residue using a Buchner funnel and vacuum-assisted filtration using Whatman N. 1 filter paper. The extract of water was freeze-dried. [14,15].

The test of antimicrobial activity for extracts

The diffusion approach was used to assess the antibacterial activity on agar [16]. A bacterial strain culture was made with 1×106 cells per milliliter using the McFarland opacity standard. Using a technique known as the spreading method, 0.1 ml of each bacterial isolate (1×106 cells per ml) was added to the Mueller Hinton agar plates. Ten milliliters of extract were applied to each well as a control. Thirty minutes before the petri plates were put in the incubator, they were held. For a whole day, each petri dish was incubated at 37°C. Inhibitory zone measurements were performed.

Genetic Study

DNA extraction

The Wizard® Genomic DNA Purification Kit was used to isolate and separate DNA from Gram-negative bacteria. This kit enables the extraction of purified DNA from bacterial cells, facilitating its use in molecular analyses such as polymerase chain reaction (PCR) and gene sequencing, while maintaining the quality and density of the DNA to meet the requirements of rigorous laboratory experiments.

PCR procedure

Using 12.5 µl of the PCR Mastermix, 2 µl primers, 3 DNA templates, and 7.5 free nuclease water, the traditional PCR method was used to detect the 16S RNA sequences. Ten pmol/µl was the concentration utilized for all primers. Table 1 contains a list of the primers used in the research that was presented.

Table (1): 16S RNA gene PCR assay primer

Primer	Primer sequence	Length (bp)	Ref.
27F	`AGAGTTTGATCCTGGCTCAG-3`	1500	17
1492R	5`TACGGTTACCTTGTTACGACTT-3		

Agarose Gel Electrophoresis of DNA

Electrophoresis has been utilized to determine the outcome of the PCR interaction in the presence of standard DNA or to detect DNA fragments following the extraction process in order to differentiate the bundle size of the PCR interaction on the agarose gel.

Ethical approval

These investigations were accepted, according to the native ethics group, and all participating patients provided informed consent and knowledge about the purpose of the study.

Results and discussion

Samples distribution

The current investigation includes 140 urine samples from UTI patients (table 1). The data indicated that when cultivated in the optimal culture medium, 34 (24.3%) of the total samples exhibited favorable results for bacterial growth. Out of all the samples, 106 (75.7%) had negative results for bacterial growth.

Table (1): Distributed of study samples according to UTI

	No. (%) +ve culture	No. (%) -ve culture	Total No.(%)	P value
UTI Patients	34(24.3%)	106(75.7%)	140(100.0%)	0.001

Some studies revealed rates that were significantly lower than the current study; in Italy, the rate was 3.5% [18], whereas in Egypt and Saudi Arabia, the rates were 10% and 6.7%, respectively [19]. Concerning earlier research from Iraq and other nations, they documented varying patient P. aeruginosa infection rates. Baghdad, 36% [20], Kirkuk, 21.6% and Nasiriyah, 19.5% [21], 15.5% [22] Babylon, 25% [23], and Basra, 50.63% [24]. rates of 32.8% and 30% in Saudi Arabia and Egypt, respectively [25]. Variations in P. aeruginosa prevalence between studies may be due to different sanitary methods, geographical regions, examined populations, hospital types, and clinical specimen types.

Antibiotic susceptibility test

P. aeruginosa showed high resistant toward Ampicillin (88.3%), and a high sensitive toward Gentamicin (82.3%), Imipenem (91.2%) and Amikacin (97.1%). On the other hand, *P. aeruginosa* was completely sensitive (100%) toward Tobromycin respectively (table 2).

Table (2): Antibiotic susceptibility test of *P. aeruginosa*

Antibiotics	Sensitive %	Intermediate %	Resistant %	P value
Ampicillin	8.8	2.9	88.3	0.001
Clindamycin	47.1	2.9	50.0	
Trimethoprim	58.8	0.0	41.2	
Cefotaxime	70.6	5.9	33.5	
Gentamicin	82.3	0.0	17.7	
Imipenem	91.2	2.9	5.9	
Ciprofloxacin	61.8	11.8	26.4	
Levofloxacin	52.9	14.7	32.4	
Azithromycin	73.5	2.9	23.6	

Amikacin	97.1	0.0	2.9	
Tobramycin	100.0	0.0	0.0	

The most common and significant mechanism of resistance in Gram-negative bacteria is the production of β-lactamase enzymes by P. aeruginosa. The outer membrane of Gram-negative bacteria has porin channels that β-lactamase either diffuses through or directly passes through. P. aeruginosa lowered permeability and either enhanced efflux or decreased the synthesis of porins [26]. The results of the study by [27] showed that the percentage of isolates that were resistant to meropenem (77.5%), tobramycin (75%), ceftriaxone (75%), ciprofloxacin (67.5%), gentamicin (65%), and ceftazidime (65%) decreased gradually as the percentage of isolates that were resistant to cefepime (62.5%), norfloxacin (60%), and imipenem (with percentages as 55%). 20 isolates, or 42.6%, however, demonstrated ciprofloxacin resistance. This study's 42.6% resistance result was higher than the 31% from several clinical sources [29] and the 9% from a prior local investigation in Baghdad [28]. Our findings, however, were more in line with those of non-local research. As compared to 64% of 100 isolates from various clinical specimens in Iran [31] and 100% (81 isolates) in Tunisia [32], 38 clinical isolates of P. aeruginosa obtained from various clinical sources in north Lebanon shown 57.89% resistance to ciprofloxacin [30]. Isolation from various departments, patient groups, and undoubtedly variations in the dosage of this antibiotic administered by various institutions could all be reasons for variations in resistance rates. However, because of the abuse by the community and hospitals, the resistance rate is rising annually [33].

Table (4) showed the ability of *S. nigrum* for bacteria inhibition according to concentration. 50, 75, 100ul of *S. nigrum* was caused in inhibitory effect on *P. aeruginosa* isolates. The 50ul concentration showed the least efficiency against P. aeruginosa, as the diameter of the inhibition zone reached 6.13±1.07mm, while the 75ul concentration showed the highest inhibition, reaching 16.1±1.8mm, and the highest inhibition zone was recorded at the 100ul concentration, reaching 29.12±3.7mm.

Table (5): show the effect of aqueous extract of *S. nigrum* at different concentrations

Treatment / **Bacteria type**	***S. nigrum***		
	50ul	**75ul**	**100ul**
P. aeruginosa	6.13± 1.07	16.1± 1.8	29.12±3.7

The antibacterial properties of this plant are probably why it has been used traditionally to treat bacterial illnesses. Many steroidal alkaloids from Solanum may exhibit unique pharmacological properties in their biological activity, as suggested by Stebbins and Poddock [34]. Phytochemical screening has identified flavonoids, phenols, alkaloids, and tannis, all of which have been shown to possess antibacterial properties [35]. The search for solasodine glycosides in various plants has been the focus of multiple articles due to the importance of solasodine to the pharmaceutical industry [36]. Therefore, these compounds—solasodine in particular—may be responsible for the antibacterial effect. One possible mode of action for this component is to impede the production of proteins, membrane phospholipids, and nucleic acids [37]. The results of the current study contradict the assessment by Patil et al. [38] of the antibacterial properties of a complete methanol extract of Solanum surattense using the disk diffusion method on agar. The previous study demonstrated antibacterial activity against Gram-positive bacteria with limited effect against Gram-negative bacteria. In contrast, the results of the current study showed that the Solanum surattense extract possesses strong activity against Gram-negative bacteria. Depending on the phytoconstituent, the plant may exhibit antimicrobial activity through a number of mechanisms, including disruption of the cell membrane, inhibition of cell wall formation, deactivation of microbial adhesins, inhibition of enzymes, or inhibition of nucleic acid synthesis [39]. Because they shield the plants from infection, the physiologically recognized substances are effective. Potentially helpful structures for the creation of novel chemotherapeutic drugs can be found in the significant sources of medicinal plants. The antioxidative, antidiabetic, anticarcinogenic, antibacterial, antiallergic, antimutagenic, and anti-inflammatory properties of phytochemical phenolic substances are well-established [40]. It is well known that tannins have broad antibacterial and antioxidant properties [41], this explains the results of the current study, where Solanum nigrum extract was able to inhibit the growth of Pseudomonas aeruginosa.

Genetic study

In the current study from 34 isolate, 34(100.0%) Pseudomonas aeruginosa isolates possessed *16S RNA* gene as shown in figure (1).

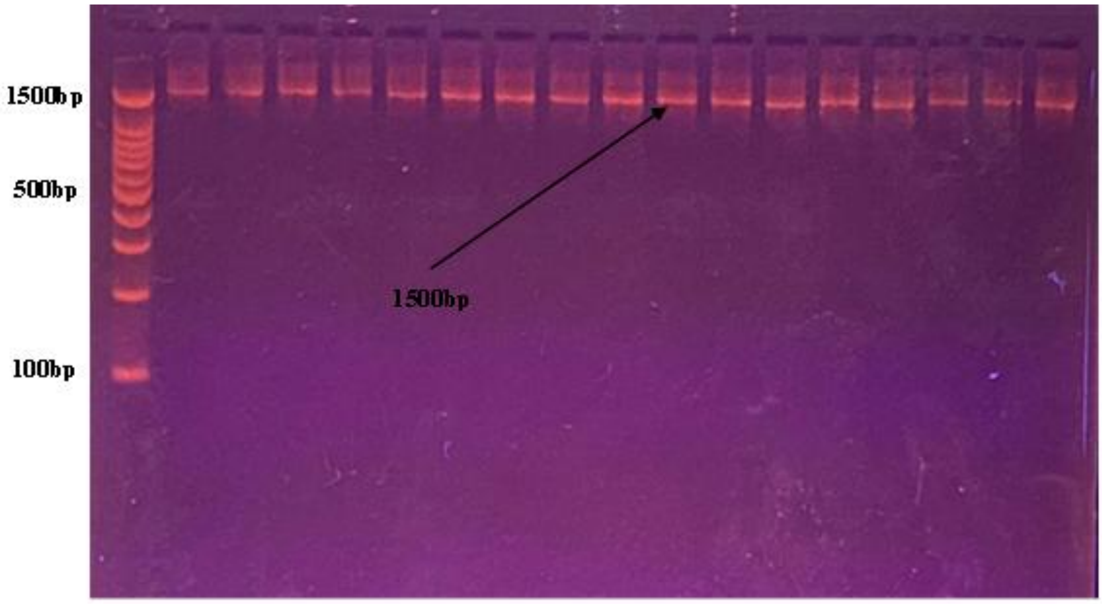

Figure (1): PCR amplification of 1500bp *16S RNA* gene by 1.4% agarose gel electrophoresis.

Ladder: M, Lane (1-17): PCR product of 17 *P. aeruginosa* isolates from urine samples

In the current study, all 34 (100%) Pseudomonas aeruginosa isolates were found to contain the 16S rRNA gene. This finding provides strong evidence that the 16S rRNA gene is a reliable marker for identifying this bacterium at the genome level, as it is a gene with a conservative sequence and can be used to identify microorganisms with high accuracy [42]. It also demonstrates that all analyzed isolates are indeed Pseudomonas aeruginosa, further enhancing the effectiveness of genetic identification techniques in verifying bacterial identity. It is noteworthy that the presence of the 16S rRNA gene in all isolates reflects the stability and widespread distribution of this gene among Pseudomonas aeruginosa species, making it a valuable tool for classifying this bacterium even in cases of variation in its morphological or biochemical characteristics [43]. Although this gene is widely used in identifying the genetic identity of bacteria, the presence of the 16S rRNA gene in 100% of the isolates may also indicate that these isolates have not undergone genetic processes that could cause mutation or loss of this gene [44]. The 16S rRNA gene can be considered an effective criterion for the diagnosis of P. aeruginosa in microbiological studies, but it remains important to explore other techniques such as microscopic examination or biochemical reactions to ensure the accuracy of the diagnosis in clinical settings [45].

Conclusions

Based on the results of the current study, P. aeruginosa isolates exhibit high resistance to many current antibiotics, and the best antibiotics are Amikacin and Tobromycin. S. nigrum extract also demonstrated high antibacterial activity against P. aeruginosa. The 16S RNA gene can also be considered a good indicator for identifying 16S RNA gene isolates.

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