XV. International Scientific Congress of Pure, Applied and Technological Sciences

MINAR Congress

المؤتمر العلمي الدولي الخامس عشر للعلوم الصرفة والتطبيقية والتكنولوجية

إســـــــطنبول

23 - 24 - 25 تموز

2025

XV. Uluslararası Fen, Uygulamalı ve Teknolojik Araştırmalar Kongresi

Rimar Academy
Publishing House

المؤتمر العلمي الدولي الخامس عشر للعلوم الصرفة والتطبيقية والتكنولوجية

XV. International Scientific Congress of Pure, Applied and Technological Sciences

FULL TEXT BOOK

2025

ISBN 978-625937770-4

Publishing House:	دار النشر:	Rimar Academy
Editor:	المحرر:	Prof. Dr. Ghuson H. MOHAMMED
Publication Coordinator:	تنسيق النشر:	AMIR MUAENI
ISBN:	نظام الترميز الدولى لترقيم الكتاب:	**978-625-93777-0-4**
DOI :	رقم معرف الكائن الرقمي:	http://dx.doi.org/**10.47832**/Minar Congress**15**
Printing:	تاريخ الطباعة:	**22/ 08 / 2025**
Date of The Congress:	تاريخ المؤتمر:	**23 - 24 - 25 / 07 / 2025**
Pages:	عدد الصفحات:	**90**
URL:	رابط النشر:	www.rimaracademy.com
Certificate Printing Press Number	رقم شهادة المطبعة:	**47843**

PREFACE

XV. International Scientific Congress of Pure, Applied and Technological Sciences was organized by Igdir University in collaboration with Rimar Academy. The primary objective of this event was to compile and disseminate valuable scientific knowledge and make a meaningful contribution to the future.

A substantial number of researchers from both local and international backgrounds demonstrated their interest in this conference. The scientific committee meticulously reviewed the submissions and ultimately accepted a select group of applicants—50 in total—of whom 45 were approved by the scientific committee.

The core of this conference was the presentation of 40 full research papers, while the remaining articles and research findings are set to be featured in forthcoming issues of the MINAR Journal.

I would like to extend my sincere appreciation to all the contributors and scholars who played an essential role in making this conference a resounding success. Your dedication and valuable contributions are deeply respected and acknowledged.

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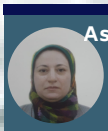

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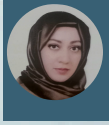

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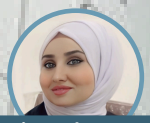

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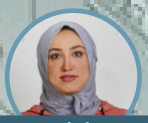

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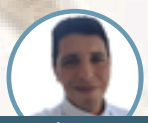

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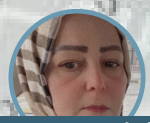

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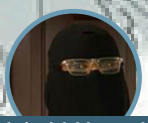

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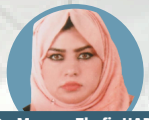

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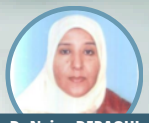

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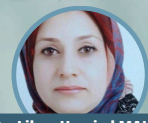

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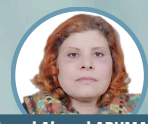

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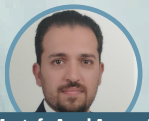

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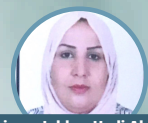

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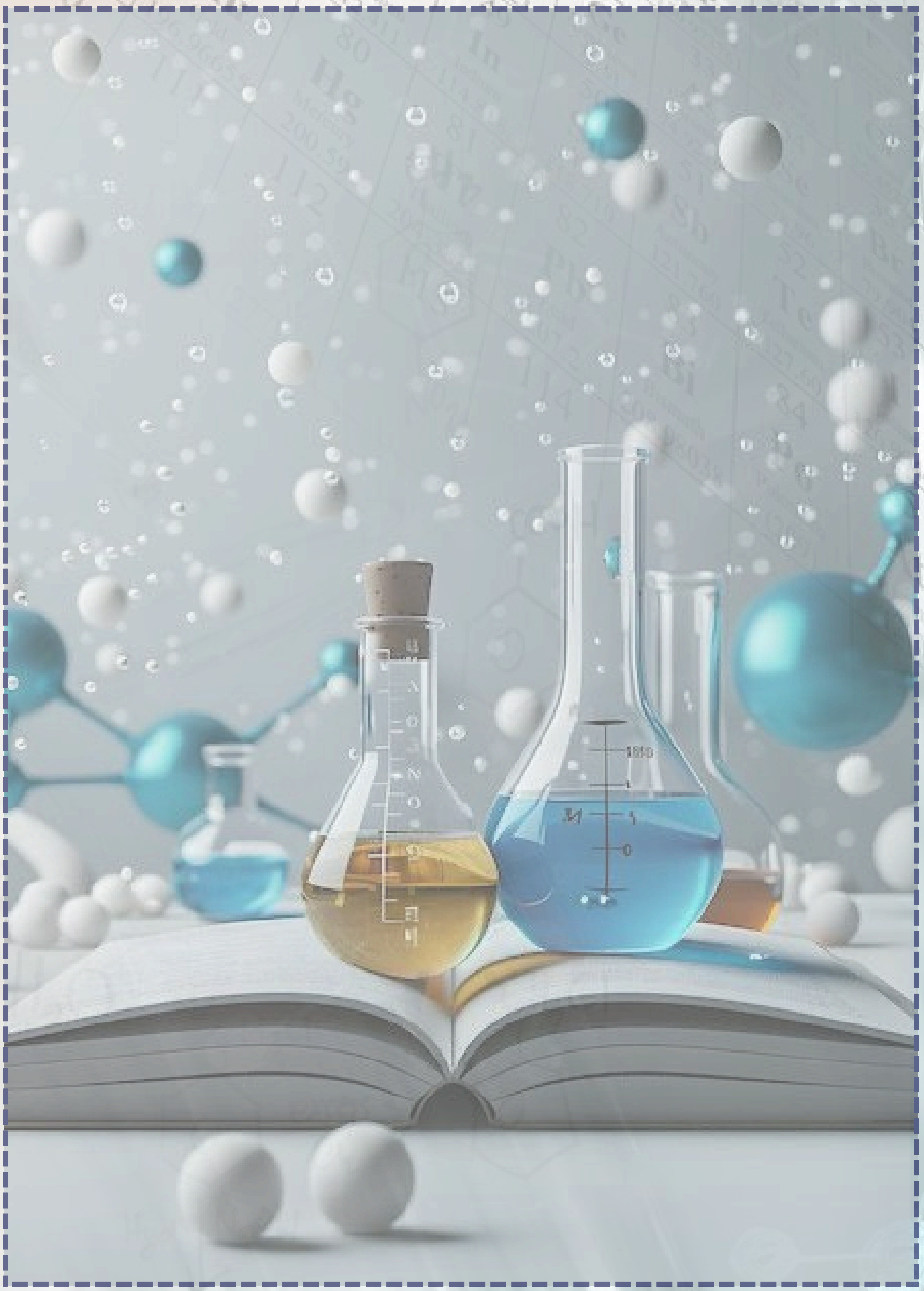

Production of Slime Layer and Some Antibiotic Resistance Enzymes by Escherichia Coli and Klebsiella Pneumoniae Isolates

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Maryam Kamel Mohammed [2]
Dimah Nazar Faraj [3]

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Abstract

One of the crucial public health problems worldwide is the urinary tract infections (UTIs) that are derived from uropathogenic bacteria (UPBs). Slime layer is known to have the ability to permit bacteria to achieve smooth surfaces attachment like catheters and prosthetic implants which in turn, facilitate biofilm formation and may cause lethal infections. On the other hand, Extended-spectrum beta-lactamase (ESBL) production is considered a growing concern among UPBs due to the limiting of the treatment options and contributes to resistance toward antibiotics. The principal study's point is the finding out the slime layer and ESBL production in *Escherichia coli* and *Klebsiella pneumoniae* of uropathogenic origin. Ten ready isolates (five isolates for each bacterial type) are gained from Department of Biology, College of Science, University of Baghdad. Disk diffusion method is employed to detect their antibiotic susceptibility towards Ciprofloxacin (CRO 10µg), Ceftazidime (CAZ 30µg) , Imipenem (IPM 10µg), Meropenem (MEM 10µg), Amoxicillin/ clavulanate (AMC 20/10 µg) and Aztreonam (ATM 30µg). Congo Red method is used to detect the production of slime layer for both types of bacteria. Black colonies mean slime layer production and pink colored colonies are not productive. Results show that all the tested bacterial isolates had ability to form slime layer even in different degrees. By using disk replacement method, ESBL production is detected. Results showed that two isolates (40%) of both *Escherichia coli* and *Klebsiella pneumoniae* are ESBL producers, while (60%) of both types are unable to produce these enzymes.

Keywords: *Disk Replacement Method, Escherichia Coli, Esbls, Klebsiella Pneumoniae, Slime Layer.*

http://dx.doi.org/10.47832/MinarCongress15-1

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Introduction

Escherichia coli is a member of Enterobacteriaceae family that lives in human gastrointestine and warm-blooded animals as a gut microbiota (1). E. coli is a Gram-negative, non-sporulating, rod-shaped, facultative anaerobic (2). On the other hand, Klebsiella pneumonia is a commensal in human intestines, an opportunistic pathogen that is accountable for causing meningitis, lobar pneumonia, septicemia, urinary tract infections besides community and health care settings pyogenic infections (3,4).

Urinary tract infections (UTIs) are among the highly familiar infections worldwide. Uropathogenic Escherichia coli (UPECs) are the chief causal factor of UTIs. K. pneumoniae is the 2nd most common uropathogen after E. coli (5). UPECs adhere to epithelium of the bladder, followed by their urothelial epithelial cells invasion wherein their replication can occur to form biofilm of aggregated bacteria. This invasion can easily lead UPECs to establish and persist within urinary tract (6). Some bacteria can made enzymes known as Extended-spectrum beta-lactamases (ESBLs). They resulted in ineffectiveness of some antibiotics, that cause treatment of bacterial infection seriously harder. Treatment with full recommended course with correct dose is a cardinal issue. Even though, multiple bacteria construct ESBLs, they are particularly created by E. coli. Klebsiella, in addition can produce them (7). Members of this family that are ESBL-producing have a principal concern as they arise acquired infections in hospitals and community (8). Plasmid genes are accountable for ESBLs encoding, besides carrying genes of resistance to other antimicrobial agents (9). Layers of slime are amorphous and inaccordant in thickness. Relying on environment and cell type, they can be produced in diverse quantities. Protection of bacterial cells can be applied by these layers from dangers of the environment like desiccation and antibiotics. Moreover, they assist bacterial adhering to smooth surfaces (10).

Materials and Methods:

Bacterial isolates

Five isolates of E. coli and five K. pneumoniae are obtained from Biology Department, College of Science, University of Baghdad. Isolates are activated using nutrient broth and cultured on MacConkey agar and Nutrient agar (for both bacteria) in addition to use EMB for E. coli isolates.

McFarland is ready gained. It includes barium chloride (1%) and sulfuric acid (1%), which is approximately equals to 1.5 x10^{8} CFU/ml. It is used to prepare bacterial inoculum.

Detection of slime layer production

Production of slime layer is done by using Congo Red Agar which is prepared from the following components (11): Mueller-Hinton broth (21g/L), Agar-agar (15g/L), Sucrose (38g/L). whole ingredients are solvated in 900 ml of D.W. and autoclaved sucrose is sterilized by filtration by using 0.22µm filter unit then added to ingredients. However, Congo red stain (0.8 g) is dissolved in 100 ml of D.W. and autoclaved at 121°C/15 pound/inch to about 15 minutes, separately, then it is added to other ingredients after agar cooling to 55°C and poured in sterile Petri dishes.

Detection of some antibiotic resistance enzymes

Disk replacement method is performed to find out the Extended- spectrum Beta-lactamase (ESBL) production. It is conducted according to that described by Al-Jasser (2006) (12). Amoxicillin/ clavulanate disks (two instead of three) put in a plate of Mueller-Hinton that is previously inoculated with the test organism. Removal of these discs after putting for one hour at room temperature is conducted and replacement on the same spot by disks containing Ceftazidime and Aztreonam is done. Control these antibiotics discs (Ceftazidime and Aztreonam) are concomitantly placed at least 30 mm from these locations.

Increasing of a zone ≥5 mm for the discs that replaced Amoxicillin/clavulanate disks indicates a positive result for ESBL production when compared to the control disks that are placed alone immediately on inoculated plates of Mueller Hinton. By the assistance of a metric ruler, zones of inhibition are measured and recorded.

Results and Discussion

Results demonstrated that resistance percentage of E. coli isolates are 40%, 100% towards Ciprofloxacin and Ceftazidime respectively, whereas, full susceptibility (100%) to Imipenem and Meropenem is observed. Besides, K. pneumoniae isolates showed 100% resistance towards Ceftazidime and 80% of resistance towards Ciprofloxacin, with full susceptibility (100%) to Imipenem and Meropenem. Results of sensitivity test for each type of tested bacteria are compared with Standard inhibition zones according to CLSI (2023) (13) is shown in figure 1 and figure 2..

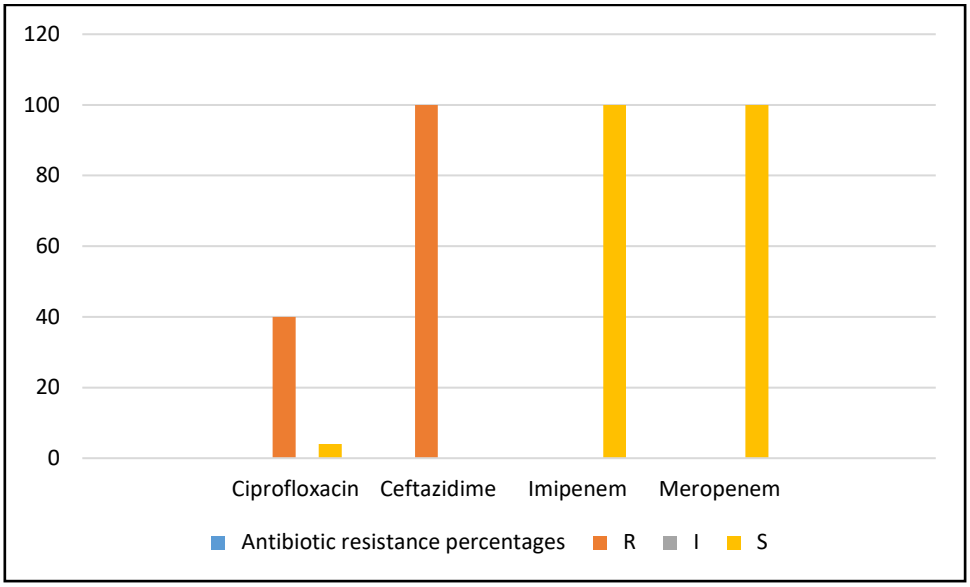

Figure 1: Sensitivity test for tested E. coli isolates towards Ciprofloxacin, Ceftazidime, Imipenem and Meropenem.

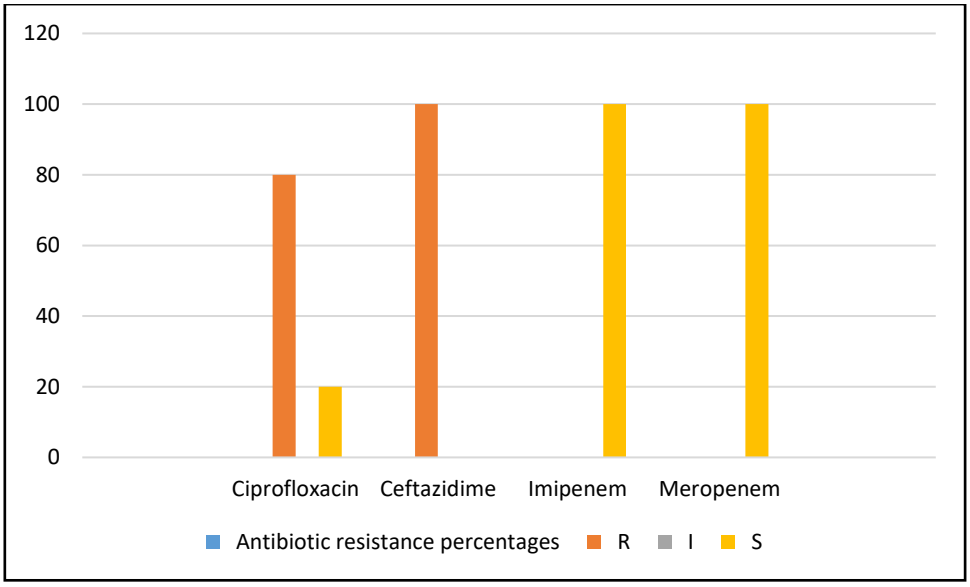

Figure 2: Sensitivity test for tested K. pneumoniae isolates towards Ciprofloxacin, Ceftazidime, Imipenem and Meropenem.

Every single isolates of E. coli is susceptible to ATM while all of the isolates (100%) are found to be resistant to CAZ. Also all isolates of K. pneumonia showed susceptibility to ATM, while (80%) are found to be resistant to CAZ. Among 10 isolates two E. coli isolates (E2 and E4) (40%) are found as ESBL producers and two K. pneumoniae (K4 and K5) (40%) are found as ESBL producers. As appeared in figures 3,4,5,6.

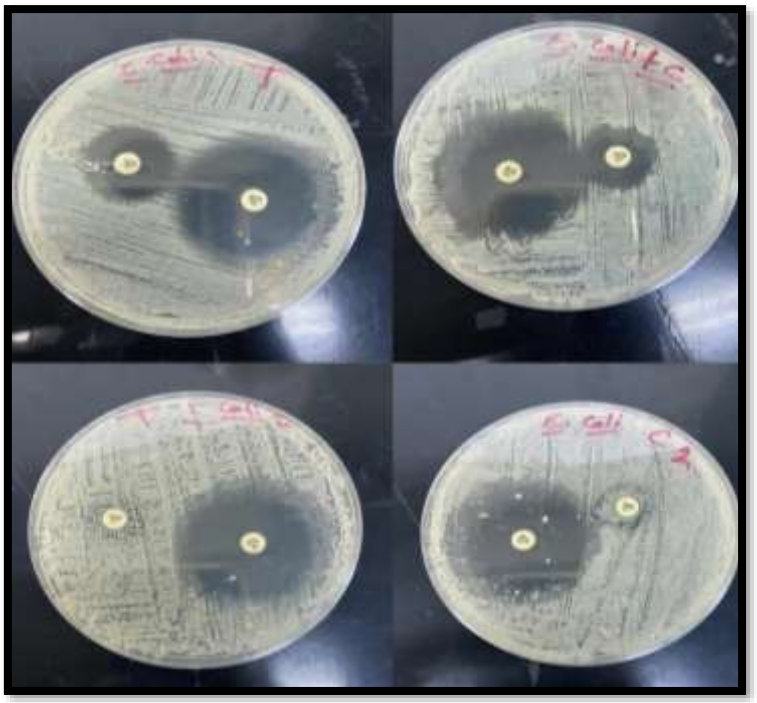

Figure 3: Production of ESBL by E. coli 1,2 isolates (on the left) compared with control same isolates (on the right).

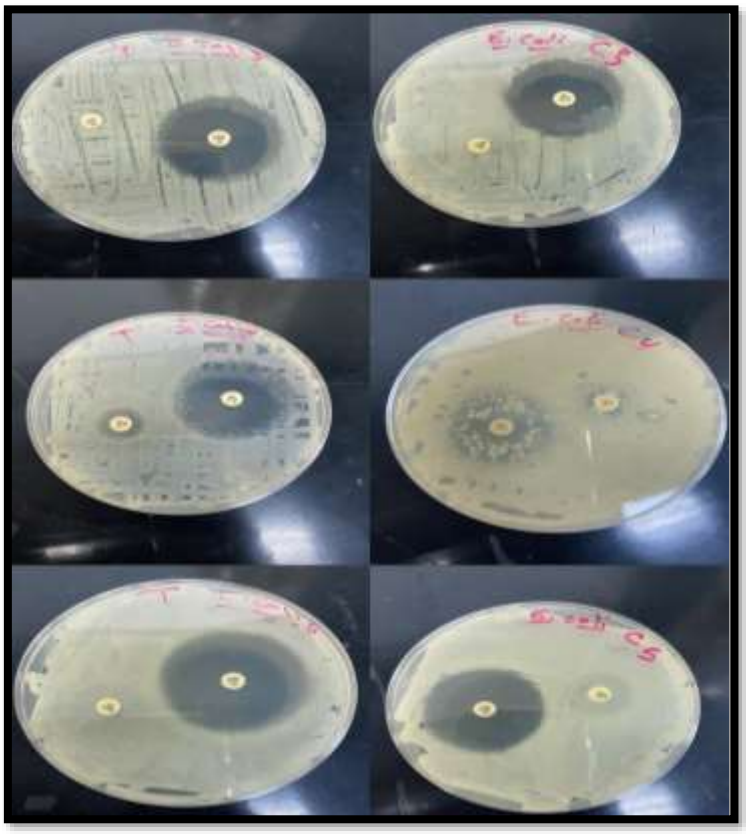

Figure 4: Production of ESBL by E. coli 3,4,5 isolates (on the left) compared with control same isolates (on the right).

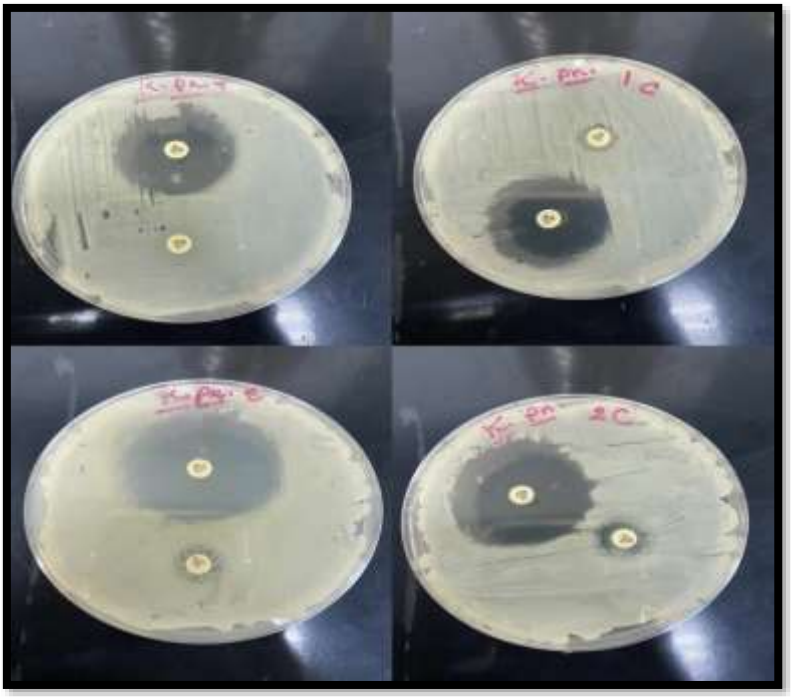

Figure 5: Production of ESBL by K. pneumoniae 1,2 isolates (on the left) compared with control same isolates (on the right).

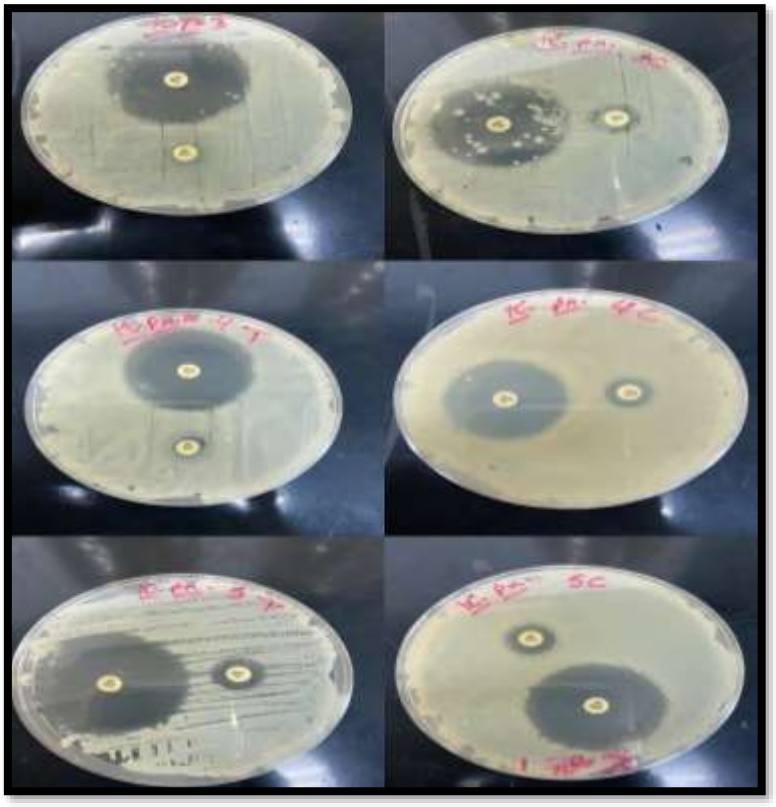

Figure 6: Production of ESBL by K. pneumoniae 3,4,5 isolates (on the left) compared with control same isolates (on the right).

For Gram-negative bacteria such as E. coli, both outer membrane that impede molecules permeability in addition to efflux pumps expression which reduce antibiotics intracellular concentration are the main contributors to the intrinsic resistance of bacteria (14). K. pneumoniae is observed to arise resistance towards antibiotics further easily than other bacteria via Extended Spectrum β-Lactamase (ESBLs) and Carbapenemase production. The prime predominant factor of antimicrobial resistance risk is the exposure of antibiotic. Antibiotics with intensive and prolonged usage are considered to be the principal contributors in the disclosure and highly resistant bacteria spreading (15). Gram-negative ESBLs producers represent significant challenge in infection management. The threat behind these organisms colonization or infection is ascribed to admission of intensive care unit, prolonged hospital stay, urinary and arterial catheterization, in addition to antibiotics exposure (16). Therapeutic options for the ESBL producers related infections have also become progressively limited. It is demonstrated an increase in ESBL producers occurrence among Enterobacteriaceae members isolated from clinical specimens (17).

Results of slime layer production

The results of the current study reveal that 100% of both bacterial isolates are slime layer producers, as they had the ability to form black colonies, but they ranged between strong, moderate and weak slime layer producers according to the density of black colonies formed on Congo red agar. Results are shown in figure 7 and 8.

Figure 7: Slime layer production by E. coli isolates. Black colonies indicates the ability of these isolates to form slime layer.

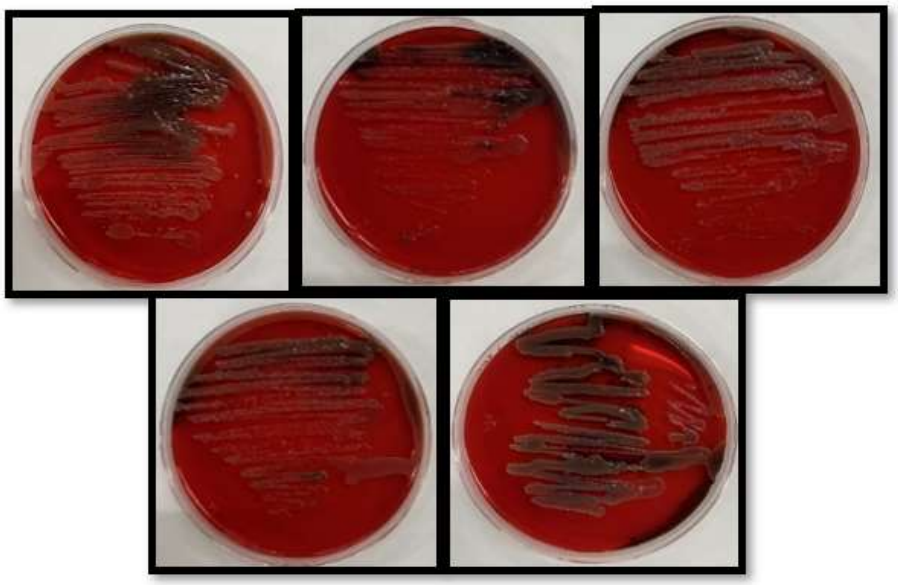

Figure 8: Slime layer production by K. pneumoniae isolates. Black colonies indicates the ability of these isolates to form slime layer.

From current results, it is clear that there isn't any isolate couldn't produce the slime layer even though they differed in the strength of its production. Colonies with black (or grey to black) color are considered as strong slime layer producers, red colonies are considered as non-slime layer producers (18). These results can indicate that both the bacterial types of uropathogenic origin can produce slime layer which, in turn, aid in the biofilm formation that protect bacteria from adverse conditions (19). Biofilm is a surface sticked bacterial cells embedded within extracellular polymeric substances matrix (20). It is previously observed that the most frequent pathogen is E. coli, follows by K. pneumoniae. They result in high UTIs percentage and can cause symptomatic UTIs (21). Bacteria can withstand adverse conditions by forming biofilm, even in the presence of antibacterial agents. These results are in accordance with previous results, which found that E. coli and Klebsiella are the predominant biofilm producers in the catheterized besides noncatheterized UTI patients (22,23,24). Previously, it is revealed that biofilm production and multiple antibiotics resistance have a vigorous correlation where MDR phenotypes are 100% biofilm producing strains (25,26,27). The cells proximity inside biofilm enable plasmids exchange, that may contribute to the spreading of antibiotic resistant strains (28).

Conclusion

E. coli and K. pneumoniae isolates showed excessive resistance to cephalosporins, and full sensitivity to carbapenem which can be a good choice to treat UTI infections by these bacteria. High correlation between slime layer production (biofilm formation) and antibiotic resistance (especially cephalosporins) is found and 40 % of both bacteria are ESBL producers that confer resistance to antibiotics. A crucial UTI virulent mechanisms is the thought to be the production of biofilm.

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Prevalence of Hepatitis B and Hepatitis C Infections in Children with Malignancy in Baghdad

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Abstract

Background: An estimated 10% of malignancies globally are thought to be caused by viral infections, with poor nations accounting for the great majority (>85%). The hepatitis viruses, such as hepatitis B virus and hepatitis C virus, which are also found in many malignancy cases, including leukemia, are the cause of the bulk of hepatitis.

Objectives: To evaluate hepatitis B and C virus infections prevalence in pediatric oncology patients and to assess which factors may raise the infection risk.

Methods: In the unit of pediatric oncology, a cross-sectional study is performed over a one-year period from February 2024 to February 2025. The study involved 84 patients diagnosed with malignancy for a minimum of six months duration. The patients are either undergoing active treatment or had completed treatment and are attending for follow-up. Individuals over 18 years of age and those diagnosed with cancer for a duration of less than six months are excluded from the study. Testing for HBV and HCV is conducted using samples of blood obtained from patients and subsequently analyzed at the hospital laboratory via PCR.

Results: The predominant proportion of patients is diagnosed with leukemia (46.4%). The incidence of hepatitis B is 9.5%, while that of hepatitis C is 6%. The quantity of blood transfusions, type of leukemia, and vaccination status shown a substantial correlation with the incidence of hepatitis virus infection.

Conclusion: Children with cancer have a significant incidence of hepatitis B and C infections. Compared to solid malignancies, hematological malignancies, more blood transfusions, and a lack of the hepatitis vaccine increase the risk of hepatitis B or C activation or infection.

Keywords: *HBV, HCV, Children, Malignancy, Prevalence, Viral, Iraq.*

http://dx.doi.org/10.47832/MinarCongress15-2

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Introduction

The global health issue that leads to hepatic dysfunction is hepatitis. The principal etiology of hepatitis is viral infections; however, non-viral factors such as poisons, pharmaceuticals, autoimmune disorders, bacterial infections, and parasitic infestations may also induce hepatitis (Usuda et al., 2024). Approximately 10% of global malignancies are linked to viral infections, with over 85% happening in developing nations (Schiller & Lowy, 2021). In various malignancy cases such as leukemias, HBV and HCV are common and constitute the majority of hepatitis patients (Shen et al., 2023). Infections like hepatitis B and C are recognized to alter immune system activity, potentially affecting cancer development and progression (Hao et al., 2025). Previous data for primary intraparenchymal brain showed that 19% of them are Glioblastoma multiforme (GBM) constitutes. The largest prevalence of primary brain tumors is attributed to glioblastoma multiforme, followed by meningioma (Ostrom et al., 2022). Chronic antigenic stimulation, especially from a virus such as HCV, substantially influences the development of non-Hodgkin lymphoma (NHL). A strong association between HCV infection and NHL has been conclusively established over the past twenty years, suggesting that around 8% of NHL cases globally may be associated with HCV (Zhang et al., 2023). Chronic HBV infection may contribute to the development of B-cell NHL (Zhao et al., 2025). The prevention and management of HCV and HBV infections are becoming more feasible and may reduce the incidence of NHL, particularly in regions with a high frequency of hepatitis virus infections (Lai et al., 2022). This study aimed to evaluate hepatitis B and C virus infections prevalence in pediatric oncology patients and to assess which factors may raise the infection risk.

Patients and Methods

A cross-sectional study is undertaken in the unit of pediatric oncology of Children Welfare Teaching Hospital / Medical City, Baghdad, Iraq, over a one-year period from February 2024 to February 2025.

This study comprised 84 patients diagnosed with cancer for a minimum of six months prior. They are either undergoing active treatment or had completed treatment and are attending for follow-up. Individuals over 18 years of age and those diagnosed with cancer for a duration of less than six months are excluded from the study.

Ethical considerations and official approvals: The conduction of this study is made in accordance with the ethical standards set by the Scientific Committee of the Surgery

Department at Ibn Sina University of Medical and Pharmaceutical Sciences regarding Human Experimentation, as well as the Helsinki Declaration of 1975, updates in 2013. Informed agreement is obtained from the patients' parents after a discussion about the project and its aims. The names are deleted and replaced with identification codes.

The researcher uses a questionnaire to collect the needed information. It includes age and gender, residence, type and duration of malignancy, number of platelets, packed RBC, and other blood products transfusion, liver function test (LFT), result of hepatitis screening test.

Sample collection and viral hepatitis test procedure: HBV and HCV testing is conducted using blood samples collect from patients and analyzed at the hospital laboratory via PCR. Two milliliters of blood are extracted from the volar aspect of the forearm of all trial participants to assess LFT, hepatitis B surface antigen (HBs Ag), and anti-hepatitis C antibody (anti-HCV).

Statistical analysis: The Statistical Package for Social Sciences (SPSS) version 26 is used for analysis. The Chi-square test is utilized to assess the relationship between hepatitis screening outcomes and particular data. A threshold < 0.05 for P-value is considered significant.

Results

Patients' age ranged from 10 months to 14 years with a mean of 7.11 ± 3.8 years; 59.5% are males; 60.7% are living in urban area; 67.9% are vaccinated with hepatitis B vaccine; 46.4% are diagnosed with leukemia; 46.4% received platelet; 71.4% received blood; and LFT is high in 16.7% as shown in table (1).

Table 1: Distribution of study patients by general characteristics

Variable	No. (n= 84)	Percentage (%)
Age (Year)		
< 5	36	57.1
≥ 5	48	42.9
Gender		
Male	50	59.5
Female	34	40.5

Residence		
Urban	51	60.7
Rural	33	39.3
Hepatitis vaccination status		
Yes	57	67.9
No	27	32.1
Type of cancer		
Leukemia	39	46.4
Lymphoma	13	15.5
Other	32	38.1
Platelets Transfusion		
No	45	53.6
1 - 3	22	26.2
> 3	17	20.2
Blood Transfusion		
No	24	28.6
1 - 3	52	61.9
> 3	8	9.5
Place of transfusion		
Same hospital	61	72.6
Another place	23	27.4
LFT		
High	14	16.7
Normal	70	83.3

As shown in figure 1, the prevalence of hepatitis B is 9.5% and the prevalence of hepatitis C is 6%

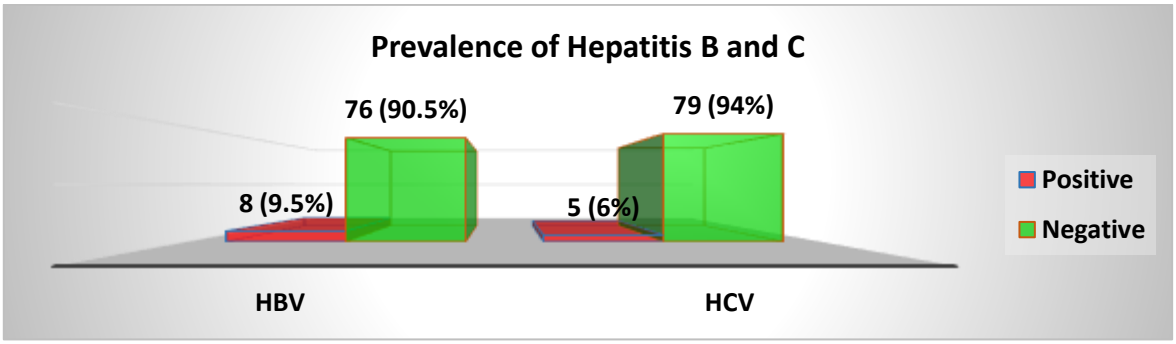

Figure 1: Prevalence of hepatitis B and C

It can be noticed that the highest prevalence of hepatitis infection is seen significantly among unvaccinated children (40.7%, P= 0.001), in patients diagnosed with leukemia (25.6%, P= 0.033), and in those with more than three times blood transfusion (50%, P= 0.015). No significant associations (P ≥ 0.05) between hepatitis infection and all other characteristics (Table 2).

Table 2: Association between hepatitis infection and patients' characteristics

Variables	Hepatitis B or C Infection		Total (%) n= 84	P - Value
	Positive (%) n= 13	Negative (%) n= 71		
Age (Year)				
< 5	5 (13.9)	31 (86.1)	36 (57.1)	0.727
≥ 5	8 (16.7)	40 (83.3)	48 (42.9)	
Gender				
Male	7 (14.0)	43 (86.0)	50 (59.5)	0.65
Female	6 (17.6)	28 (82.4)	34 (40.5)	
Residence				
Urban	5 (9.8)	46 (90.2)	51 (60.7)	0.073
Rural	8 (24.2)	25 (75.8)	33 (39.3)	
Hepatitis vaccination status				

Yes	2 (3.5)	55 (96.5)	57 (67.9)	< 0.001
No	11 (40.7)	16 (59.3)	27 (32.1)	
Type of cancer				
Leukemia	10 (25.6)	29 (74.4)	39 (46.4)	0.033
Lymphoma	2 (15.4)	11 (84.6)	13 (15.5)	
Other	1 (3.1)	31 (96.9)	32 (38.1)	
Platelets Transfusion				
No	5 (11.1)	40 (88.9)	45 (53.5)	0.444
1 - 3	4 (18.2)	18 (81.8)	22 (26.2)	
> 3	4 (23.5)	13 (76.5)	17 (20.3)	
Blood Transfusion				
No	2 (8.3)	22 (91.7)	24 (28.6)	0.015
1 - 3	7 (13.5)	45 (86.5)	52 (61.9)	
> 3	4 (50.0)	4 (50.0)	8 (9.5)	
Place of transfusion				
Same hospital	9 (14.8)	52 (85.2)	61 (72.6)	0.765
Another place	4 (17.4)	19 (82.6)	23 (27.4)	
LFT				
High	3 (21.4)	11 (78.6)	14 (16.7)	0.499
Normal	10 (14.3)	60 (85.7)	70 (83.3)	

Discussion

The survival rate of children with malignancy has significantly improved alongside advancements in therapeutic procedures, including intense chemotherapy. They necessitate numerous transfusions during rigorous treatment and have an elevated risk of infections that is blood-borne, including HBV, HCV, and HIV (Kebudi et al., 2019). The hazards associates

with these illnesses vary by country, contingent upon the prevalence of infections within the donor community and the blood screening technologies employed in each nation (Sebastião et al., 2025). Although the contact rate of HBV is comparable in children with benign and malignant hematological conditions, HBs Ag positive is significantly greater in children with malignancy. Survivors treated prior to the implementation of sufficient blood donor screening for HCV in the beginning of 1990s are at chronic liver damage risk (Usta et al., 2015).

In the current study, the prevalence of HBV is 9.5% and that of HCV is 6% which are lower than results found in studies conducted by Al-Jadiry MF et al study (Al-Jadiry et al., 2013), Salih AS et al (Salih & Abbas, 2018), Kareem HF et al (Kareem et al., 2022), and Raouf HE et al (Raouf et al., 2015). The study by Kebudi R et al reveals that none of the 100 patients recruited are seropositive for HBsAg, anti-HCV, or anti-HIV, both at diagnosis and during completion of treatment (Kebudi et al., 2019). The observed differences can be ascribed to the sample size of each study, alongside the considerable disparity in global HBV prevalence, varying from low in West of Europe, North of America, and Japan to high in Africa, Southeast of Asia, and China (Gnyawali et al., 2022). Another aspect is the efficacy of the active Immunization Expanded Programs in certain countries and the youthful demographic of the sample. The precision of hepatitis screening is contingent upon the methodology employed and the available kits quality; the presence or absence of PCR testing kits confirms the existence of hepatitis. Furthermore, the actions of the paramedical personnel facilitated the transmission of infection via the reuse of individual drug bottles or by the contamination of injectable medications or flush solutions (Xiao et al., 2020).

The current study clarifies that the number of transfused blood, type of cancer, and vaccine status have significant association on hepatitis virus infection prevalence. This is agreed with the results found by Al-Jadiry MF et al (Al-Jadiry et al., 2013), Salih AS et al (Salih & Abbas, 2018), and Kareem HF et al (Kareem et al., 2022) studies. Children with malignancy who need many transfusions during rigorous therapy face an elevated risk of bloodborne infections, including HBV and HCV. The risk of acquiring HCV infection from blood products has markedly diminished since the implementation of routine blood donor screening. In France, the estimated risk of transfusion-transmitted viral infection during the 2001-2003 period is 1 per 10 million for HCV and 1 per 640,000 for HBV (Hayes et al., 2024). Children residing in areas of intermediate and high endemicity for HBV are at infection risk, particularly those with malignancy undergoing rigorous cytostatic type of chemotherapy, which necessitates numerous transfusions of blood and induces

immunodeficiency. Vaccination against HBV for patients who are seronegative at the time of diagnosis may be advisable (Ocak et al., 2021).

In conclusion, the frequency of bloodborne hepatitis is analogous to findings in other published researches. Factors that may greatly influence the prevalence of hepatitis infections include the quantity of blood transfusions received, immunization status, and cancer type. It is proposed to establish transfusion protocols at cancer centers to minimize needless blood product transfusions, hence reducing the occurrence of bloodborne virus infections. Emphasis should be placed on the screening methods employed by blood banks to identify donors with hepatitis within the window period.

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A Cross-Sectional Study of The Relationship between Dihydrofolate Reductase Enzyme and Some Biochemical Parameters in Iraqi Patients

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Abstract

Introduction: Dihydrofolate reductase (DHFR) is an enzyme that converted dihydrofolate to tetrahydrofolate. NADPH serves as an electron donor and can be transformed into the tetrahydrofolate cofactor utilized in 1-carbon transfer chemistry. DHFR transforms dihydrofolate to tetrahydrofolate, a compound that provides a methyl group necessary for the de novo synthesis of purines, thymidylate, and certain amino acids. The aim of this research is: The study of the relationship between DHFR enzyme and some Biochemical Parameters (FBG, B. Urea, ALT, AST, ALK, TBI, DBI, IBIL, Ca, Na, K, CL, CRE2, ALB) in Iraqi Patients.

Patients and Methods: The 48 serum samples are collected and divided into two groups: 20 constituted the control group, comprising patients, to ascertain the correlation between DHFR and other biochemical parameters in patients.

Results: A correlation is found between the DHFR enzyme and ALT1, sodium (Na) in the female patients' group, and between the DHFR enzyme and fasting blood sugar (FBS) in the male patients' group.

Keywords: *DHFR, FBG, B. Urea, ALT1, AST, ALK, TBI.*

http://dx.doi.org/10.47832/MinarCongress15-3

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1. Introduction

Dihydrofolate reductase, or DHFR, plays a key role, notably in folate metabolism. Its main job is converting dihydrofolate to tetrahydrofolate, necessary for DNA synthesis and repair. But DHFR is so important, especially in fast-growing cells, drugs, like antimetabolites, target it in cancer and autoimmune disease treatment [1]. But DHFR is more than just its basic function. Sometimes, the DHFR gene might change – these polymorphisms can alter how well the enzyme works, affecting how people respond to treatments and their risk of getting certain diseases [2]. DHFR's importance is clear in public health, especially for neglected tropical diseases like leishmaniasis. Here, drugs often don't work well because of how the enzyme functions. Studying the links between DHFR and other biochemical factors in patients may help us to find better ways to treat these illnesses. Dihydrofolate reductase (DHFR), a crucial enzyme, plays a vital role in numerous essential metabolic processes. Critically, it is essential for nucleic acid and amino acid production, both vital for cell growth and upkeep [3]. DHFR starts the conversion of folic acid to tetrahydrofolate, a vital step in folate metabolism that directly affects purine and thymidylate creation. Generally speaking, understanding DHFR activity isn't just important at the molecular level, but also for community health. Interestingly, variations in DHFR expression in Iraqi patients have been tied to several metabolic diseases, highlighting its clinical relevance. Moreover, DHFR is a key drug target; suppressing it is fundamental to medications that control abnormal cell growth, such as cancer treatments. Therefore, by looking at DHFR activity may give insights into its role in both normal physiology and disease, boosting our awareness of how metabolic pathways affect health outcomes [4,5].

2. Methods and Materials

2.1 Objectives of Research:

This study aims to explore how variations in the dihydrofolate reductase (DHFR) enzyme affects the chemical parameters in Iraqi patients. Through a detailed cross-sectional analysis, it examines the link between DHFR activity and factors like homocysteine and B9 level, which play key roles in metabolism and health. Beyond adding data on DHFR in this population, the research hopes to inform future treatments for related conditions, supporting the growing trend toward more personalized approaches in metabolic disease research [6,7].

2.2 Research Questions Addressed:

The current study explores intricate chemical marker correlations to probe fundamental research questions concerning dihydrofolate reductase's (DHFR) function in specific metabolic routes within Iraqi patients. The central role of this enzyme in folate metabolism and DNA production offers an important chance to elucidate its impacts on typical chemical parameters between control and patients [8]. The specialty of the investigation indicates to determine how DHFR activity relates to serum folate and its related metabolites, the key markers of metabolic well-being. Furthermore, this inquiry is generally aligned with wider discussions on health, especially emphasizing the need for innovative, which focused the treatments for parasitic ailments such as leishmaniasis, plus those connected to Apicomplexa, where, it must be said, treatment strategies remain quite limited [9]. Identifying DHFR connections are not only provided greater clinical understanding but, in most cases, also catalyzes future research improvements in therapeutic strategies [10].

2.3 Population and Sample Selection Criteria:

In any cross-sectional study, notably when we're looking closely at the Dihydrofolate Reductase enzyme alongside which related to the biochemical markers in Iraqi patients, paying close attention to both the population and how the researchers select our sample is vital [11]. Essentially, a solid framework that's based on clear selection criteria underpins both representation and how valid our results turn out to be; this is essential when drawing meaningful conclusions. Ideally, the population will need to reflect the wider Iraqi patient demographic; this means looking at a spread of things like age, gender, and even any pre-existing health conditions, as this helps improve data reliability. What's also key is that the sampling strategy is crystal clear, using precise inclusion and exclusion criteria; this helps researchers properly isolate the specific variables that matter. For example, Leishmaniasis impacts millions worldwide, showing how vital good population studies are, particularly when they address the therapeutic needs of those affected [12]. This really highlights why we need to have well-defined populations, especially if we're aiming to advance treatments for often-overlooked diseases [13]. Similarly, discussions at scientific conferences are constantly emphasizing the importance of having rigorous sampling methods if we want to push analytical chemistry forward, and that's especially relevant when we're studying biochemical parameters. In this study, the researchers are collected 48 serum samples; these are divided into two groups. The control group consisted of 10 males and 10 females, while the patient group is comprised 14 males and 14 females, all between 18 and 40 years old. These are

gathered from the Teaching Laboratories of Medical City in Baghdad between March and June of 2023. All subjects gave informed consent. Authorization for the study came from the Ethical Committee of the Ibn Sina University of Medical and Pharmaceutical Sciences, located in Baghdad, Iraq.

2.4 Dihydrofolate reductase enzyme assay:

The DHFR enzyme activity is determined generally using the technique reported by Haurani et al. [14], with minor variations. The DHFR experiment is carried out at 37°C using a spectrophotometric technique, with the reduction in absorbance at 340 nm indicating the conversion of NADPH and dihydrofolate to NADP and tetrahydrofolate, respectively. Enzyme activity is measured in nanomoles of dihydrofolate reduced per milligram of protein. The reaction mixture consisted of 2 mL of NADPH, 0.6 mL of dihydrofolate, and 50 mL of enzyme suspension; the absorbance is monitored every minute for 3 minutes.

The Clinical Significance of Dihydrofolate Reductase (DHFR) Deficiency

- Megaloblastic anemia is caused by impaired DNA synthesis in rapidly dividing cells, particularly in bone marrow [15].

- Immunodeficiency is due to the enzyme's role in lymphocyte proliferation and function [16].

- Congenital defects, especially neural tube defects, are linked to folate metabolism disturbances during pregnancy [17].

- Increased cancer risk, such as colorectal and breast cancer, as abnormal DHFR activity may contribute to unregulated cell growth and genomic instability [18].

- Drug resistance or sensitivity, particularly to antifolate drugs like methotrexate, which target DHFR to inhibit DNA replication in cancer and autoimmune disease [19].

Given its central role in cellular metabolism and disease pathogenesis, evaluating DHFR levels or activity can provide important diagnostic and prognostic information and may guide therapeutic decisions in clinical practice [20].

3. Result

In our cross-sectional study, we found some interesting links between how active the dihydrofolate reductase (DHFR) enzyme is and certain biochemical markers in Iraqi patients

[21]. It seems changes in DHFR activity can mess with folate levels, and that might change how well treatments work for diseases that need folate metabolism, like leishmaniasis—a big health problem there. What's more, when we looked at their genes, we noticed some specific variations in the DHFR gene are important [22]. These variations seemed to affect how well the enzyme worked and, as a result, the patients' biochemical profiles. Other studies have pointed out that genetic factors play a role in who gets sick and how they respond to treatment, so this kind of fits with that. It looks like getting a grip on the relationship between DHFR activity and these biochemical parameters could help us come up with better treatments and improve health for these folks who are already at risk [23].

In this research, the clinical laboratory analysis is descriptive for all parameters shown in Table-2,3,4. Table 5 and Figure 1 demonstrate the link between the DHFR enzyme and other parameters in the female patient group. However, as Table 6 and Figure 1 demonstrate, there is also a link between the DHFR enzyme and other parameters in the male patient group, as seen in Figure 2. Finally, Table 7 shows the significant differences between the DHFR enzyme and other parameters in the control group and the patient group, as determined by the T-test.

A DHFR (Dihydrofolate Reductase) emerges as a promising candidate for comparative investigations concerning the interplay between structure and function [24]. Lately, attention has centered on DHFR as a plausible genetic marker [25]. In the current study, correlations are observed between the DHFR enzyme and ALT1 and Na in the female patient cohort (Table 5 and fig-1), while a correlation between the DHFR enzyme and FBS is noted in the male patient group (Table 6 and fig-2).

In Table -7, there are significant differences between the DHFR enzyme and FBG, B. urea, AlK, and Cre2 in the control group and the patient group, but there are no significant differences between the DHFR enzyme and other parameters in the same groups. This result agrees with Ahmed et.al, in 2013[14].

DHFR, or Dihydrofolate Reductase, is ubiquitously present in every dividing prokaryotic and eukaryotic cell. Although the sequences of mammalian enzymes are highly similar, every kind of bacterium is unique. The DHFR sequence in humans has 30% of its similarities with E. Coli and 70% of its similarities with other DHFRs in mammals [26]. Consequently, targeting DHFR has emerged as a prominent strategy in developing new antibacterial therapeutics. DHFR is a key target for treating a variety of microbial infections and holds potential as an antifungal, antimalarial, anti-tuberculosis, anti-Leishmaniasis, and

anti-Trypanosomiasis agent. Furthermore, understanding the mechanisms of resistance to antifolates and devising strategies to overcome them are crucial areas of research [27,28].

Interpretation of Statistical Results

The current study reveals that the several statistically significant differences and associations regarding DHFR enzyme levels and various biochemical parameters between patients and the control group. These results suggest a potential role of DHFR in metabolic and biochemical alterations.

1. Comparison Between Control and Patient Groups (T-test Results):

DHFR levels are significantly higher in patients (Mean = 30.32 U/ml/min) compared to the control group (Mean = 20.00 U/ml/min), with a p-value of 0.002, indicating a strong association between disease status and elevated DHFR activity.

Fasting Blood Glucose (FBG), Blood Urea, Alkaline Phosphatase (ALK), and Creatinine also showed statistically significant increases in the patient group, suggesting potential links between altered DHFR activity and impaired kidney or liver function and glucose metabolism.

Other parameters (e.g., ALT, AST, Albumin) did not show statistically significant differences, which may indicate that the relationship between DHFR and these markers is less direct or requires a larger sample to be detected.

2. Correlation in Female Patients:

A highly significant positive correlation is observed between DHFR and ALT ($r = 0.658$, $p = 0.005$), suggesting that liver enzyme activity may influence or reflect DHFR levels.

A significant positive correlation is also found between DHFR and sodium (Na) ($r = 0.607$, $p = 0.011$), which could indicate electrolyte imbalance linked to enzyme activity.

Other correlations (e.g., with FBG or Creatinine) approached significance, suggesting possible weak or moderate associations.

3. Correlation in Male Patients:

A significant positive correlation is found between DHFR and Fasting Blood Glucose ($r = 0.586$, $p = 0.028$), indicating that glucose levels may influence or be influenced by DHFR activity in males.

Most other parameters show weak or no significant correlations with DHFR, which might suggest gender-specific variations or different mechanisms of interaction.

The elevation of DHFR in patients may reflect a compensatory response to oxidative stress, metabolic disruption, or pathological overexpression.

These findings are preliminary and should be interpreted with caution. Future studies with larger sample sizes and more advanced analysis (e.g., multivariate regression) are needed to validate and clarify these relationships.

Interpretation of Graphical Results (Figures 1 & 2)

Figure 1: The Relationship between DHFR Enzyme and Other Parameters in Female Patients

This figure illustrates the correlation between DHFR enzyme activity and various biochemical markers in the female patient group. The most notable observations include:

A strong positive association between DHFR and ALT, suggesting a potential link between hepatic function and DHFR enzyme expression in females.

A moderate correlation trend with serum sodium (Na), which may indicate an indirect relationship between electrolyte balance and DHFR levels.

Other parameters, such as urea, bilirubin, or calcium, showed weaker or inconsistent patterns, possibly due to biological variability or small sample size.

These associations suggest that DHFR activity in female patients may be influenced by fluctuations in liver enzymes and electrolyte status, warranting further investigation.

Figure 2: The Association between Fasting Blood Sugar (FBS) and DHFR Enzyme in Male Patients

This figure highlights a clear positive correlation between fasting blood glucose levels and DHFR enzyme activity in male patients. The data indicates that:

Higher FBS values are associated with elevated DHFR activity, implying a possible metabolic link between glucose regulation and folate metabolism.

This could reflect an adaptive enzymatic response to oxidative stress or altered glucose metabolism in male patients.

Such a finding aligns with the hypothesis that DHFR activity may be upregulated in metabolic stress conditions, including hyperglycemia.

Together, the graphical data support the statistical findings and reinforce the potential gender-specific roles of DHFR in biochemical and metabolic markers. While the correlations are promising, larger studies are needed to determine causality and clinical significance.

4. Discussion

The study's results emphasize the complex connection between the dihydrofolate reductase (DHFR) enzyme and certain biochemical measures in Iraqi patients, perhaps hinting at some clinical applications. This connection may mirror the underlying metabolic routes affected by genetic [29], dietary, and environmental components unique to this group of people. We can learn more about conditions like cardiovascular diseases and some malignancies, which may be common in this group, by looking at the enzymatic activity of DHFR along with measures like homocysteine levels and folate concentrations. For example, the link between significant blood loss during surgery for pelvic tumors may indicate how significant such biochemical interactions are. Furthermore, understanding how metabolic pathways [30], like those seen in cancer research using drugs like Metformin, might change how vulnerable one is to disease or how one responds to treatment, underscores the importance of customized treatment strategies based on individual biochemical profiles [31].

This study's findings—examining the link between dihydrofolate reductase enzyme levels and certain biochemical parameters in Iraqi patients—are, in most cases, in line with previous research; research that highlights the essential role this enzyme plays in various metabolic pathways [32]. It's worth noting, however, that prior studies have sometimes produced contrasting results; these results illustrate the complexities inherent in dihydrofolate reductase's involvement across different populations, a complexity often attributed to genetic variability and other environmental factors. By way of example, while some reports emphasize the enzyme's significance in the pathogenesis of diseases like leishmaniasis, indicating a need for effective therapeutic approaches, others have focused on pathogenic interactions; interactions that complicate treatment in aquaculture, thereby demonstrating the enzyme's critical role in broader biological contexts [33]. This comparative analysis underscores the ongoing necessity for region-specific studies. These studies would help us

better understand the biochemical interactions that have a direct effect on health outcomes, reflecting the multifaceted implications of dihydrofolate reductase that extend well beyond simply traditional metabolic pathways [34].

"Although the current study did not stratify subjects by age groups, previous literature suggests that DHFR enzyme activity may be influenced by age-related factors. Enzyme activity may decline with aging due to reduced cellular proliferation and changes in folate metabolism. Future studies are recommended to investigate age-specific variations in DHFR activity [35,36].

Closing Remarks on Future Directions

Looking ahead, to really nail down how Dihydrofolate Reductase (DHFR) enzyme activity and biochemical stuff connect in Iraqi patients, there are a few big areas to explore. It's clear we need some genome-wide association studies to find more genetic quirks that mess with how the enzyme works and who's more likely to get sick, especially given the mixed genetic makeup of the population. Also, taking a page from the book of studying Trypanosomas' weird enzyme tricks using fancy computer tools and molecular simulations, could give us a better look at how pathways talk to each other and where we might aim treatments [37,38]. Plus, with drug resistance being a pain like in Leishmaniasis, future research should work on making drugs that not only shut down DHFR well but also aren't too toxic and are easy to get for treatment. All this together could help tailor medicine to individuals in the area [39].

Key Findings and Recommendations

Key Findings:

1- DHFR enzyme activity is significantly elevated in the patient group compared to the control group ($p = 0.002$), indicating its potential involvement in disease-related metabolic or cellular changes.

2- A strong positive correlation is observed between DHFR and ALT levels in female patients ($r = 0.658$, $p = 0.005$), suggesting a link between DHFR activity and liver function.

3 - In male patients, DHFR showed a significant correlation with Fasting Blood Sugar (FBS) ($r = 0.586$, $p = 0.028$), indicating a possible relationship between folate metabolism and glucose regulation.

4 - Urea, creatinine, and alkaline phosphatase levels are significantly elevated in the patient group, pointing to possible liver or kidney dysfunction associated with DHFR elevation.

5 - Despite variations in biochemical markers, some parameters, like bilirubin, albumin, and potassium, did not show significant differences between groups.

Recommendations:

1- Routine screening of DHFR enzyme activity is suggested, especially in patients with metabolic or liver/kidney-related symptoms, as early detection may support timely intervention [36].

2- Further research is needed to explore gender-specific differences in DHFR associations, especially regarding glucose and liver markers[37].

3- Monitoring DHFR levels in patients receiving antifolate therapy, such as methotrexate, is recommended to predict drug response or possible side effects [39].

4- Educational campaigns and nutritional counseling should be encouraged, particularly for individuals with folate metabolism disorders, to promote awareness and preventive care [40].

5- Larger-scale and longitudinal studies are essential to validate the current findings and to establish DHFR as a reliable biomarker in clinical diagnostics [41].

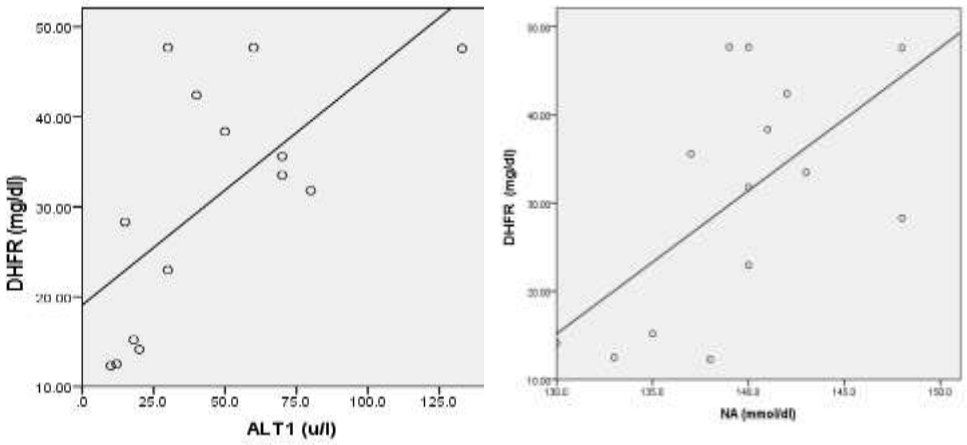

Figure 1: The relationship between the DHFR enzyme and other parameters in the female patients' group

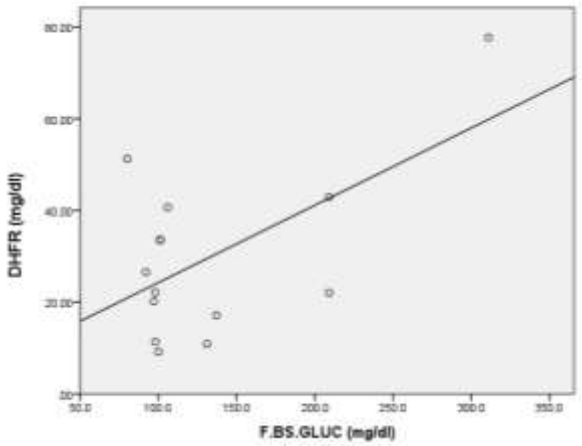

Figure 2: The association between FBS and the DHFR enzyme in the group of male patients

Table 1: The Biochemical Parameters in the present study

The Biochemical Parameters	Abbreviated symbols	Normal Values
Fasting Blood Sugar	FBG	65-110 mg/dL
Blood Urea	B. Urea	15-45 mg/dL
Alanine aminotransferase	ALT1	12-78 U/L
Asparlate aminotransferase	AST	15-37 U/L
Alkaline Phosphatase	ALK	46-116 U/L
Total Billirubin	TBI	0.2-1 mg/dL
Direct Billirubin	DBI	0.00-0.2 mg/dL

Indirect Billirubin	IBIL	0.00-0.8 mg/dL
Calcium Blood Test	Ca	8.5-10.1 mmoL/dL
Sodium Blood Test	Na	136-155 mmoL/dL
Potassium Blood Test	K	3.5-5.5 mmoL/dL
Chloride in the Blood	CL	98- 107 mmoL/dL
Creatinine	CRE2	0.55-1.3 mg/dL
Albumin	ALB	3.4-5 g/dL
Dihydrofolate-reductase enzyme	DHFR	Male: 15-20 U/ml/min Female:20-25 U/ml/min

Table 2: Description of all parameters in the control group

Descriptive Statistics					
CONTROL	**N**	**Min**	**Max**	**Mean**	**±SD**
CON_F.BS.GLUC	10	79.00	95.00	87.50	±5.19
CON_B.UREA	10	25.00	41.00	34.70	±5.85
CON_ALT1	10	25.00	60.00	39.20	±12.15
CON_AST	10	19.00	30.00	25.60	±3.86
CON_A1K	10	50.00	90.00	80.30	±12.89
CON_TBI	10	0.30	0.90	0.55	±0.21
CON_DBI	10	0.08	0.18	0.14	±0.03
CON_1B1L	10	0.20	0.50	0.40	±0.10
CON_CA	10	8.40	9.30	8.92	±0.33
CON_NA	10	133.00	147.00	138.80	±4.05
CON_K	10	4.00	4.90	4.43	±0.33
CON_CL	10	98.00	105.00	101.60	±2.27
CON_CRE2	10	.56	1.00	0.79	±0.17
CON_ALBUMIN	10	3.50	4.80	4.11	±0.38
CON_DHFR	10	17.00	23.00	20.00	±1.83

Table 3: The description for all parameters in the female patients' group

FEMALE PATIENTS	N	Min	Max	Mean	±SD
FE_PA_F.BS.GLUC	14	65.00	350.00	142.50	±93.37
FE_PA_B.UREA	14	13.00	171.00	46.21	±43.58
FE_PA_ALT1	14	10.00	133.00	45.57	±34.54
FE_PA_AST	14	10.00	200.00	36.57	±13.33
FE_PA_A1K	14	57.00	200.00	119.50	±44.27
FE_PA_TBI	14	0.30	2.00	0.70	±0.42
FE_PA_DBI	14	0.08	0.35	0.15	±0.07
FE_PA_1B1L	14	0.20	1.60	0.43	±0.35
FE_PA_CA	14	6.00	10.90	8.75	±1.19
FE_PA_NA	14	130.00	148.00	139.57	±5.02
FE_PA_K	14	3.00	7.00	4.23	±1.24
FE_PA_CL	14	60.00	110.00	100.29	±12.36
FE_PA_CRE2	14	0.40	15.00	2.45	±1.04
FE_PA_ALBUMIN	14	3.50	5.20	4.20	±0.49
FE_PA_DHFR	14	12.30	47.70	30.72	±13.42

Table 4: The description of all parameters in the male patients' group

MALE PATIENTS	N	Min	Max	Mean	±SD
MA_PA_F.BS.GLUC	14	80.00	311.00	133.57	±65.25
MA_PA_B.UREA	14	15.00	171.00	67.50	±65.57
MA_PA_ALT1	14	8.00	152.00	40.64	±12.02
MA_PA_AST	14	10.00	216.00	34.14	±14.19
MA_PA_A1K	14	54.00	288.00	120.43	±61.71
MA_PA_TBI	14	0.40	1.70	0.61	±0.34
MA_PA_DBI	14	0.09	0.39	0.15	±0.08

MA_PA_1B1L	14	0.21	1.61	0.46	±0.35
MA_PA_CA	14	5.00	10.50	8.69	±1.23
MA_PA_NA	14	134.00	146.00	141.07	±3.71
MA_PA_K	14	3.50	7.20	4.47	±1.15
MA_PA_CL	14	57.00	108.00	100.64	±12.83
MA_PA_CRE2	14	0.43	14.49	2.47	±1.01
MA_PA_ALBUMIN	14	3.50	5.00	4.14	±0.43
MA_PA_DHFR	14	9.15	77.70	29.92	±18.77

Table 5: The relationship in the group of female patients between the DHFR enzyme and other parameters

Correlations														
		F.BS.	B.UREA	ALT1	AST	A1K	TBI	DBI	1B1L	CA	Na	K	CL	CRE2	ALBN
DHFR	Pearson correlation	0.406	0.020	0.658**	-0.297	0.004	0.130	-0.414	0.029	-0.187	0.607*	-0.448	0.416	-0.457	0.188
	Sig.	0.075	0.473	0.005 HS	0.151	0.495	0.329	.070	0.461	0.261	0.011 S	0.054	0.069	0.050	0.259
	N	14	14	14	14	14	14	14	14	14	14	14	14	14	14
	Regression			0.432							0.368				
****. Correlation is high significant at the 0.01 level.**															
***. Correlation is significant at the 0.05 level.**															

Table 6: The relationship in the group of female patients between the DHFR enzyme and other parameters

Correlations														
		F.BS.GLUC	B.UREA	ALT1	AST	A1K	TBI	DBI	1B1L	CA	NA	K	CL	CRE2	ALBUMIN
DHFR	Pearson correlation	0.586*	-0.305	0.018	-0.147	0.179	-0.253	-0.190	-0.201	0.284	-0.080	-0.347	0.120	-0.328	0.192
	Sig.	0.028	0.290	0.953	0.615	0.540	0.382	0.515	0.491	0.324	0.786	0.224	0.683	0.253	0.510
	N	14	14	14	14	14	14	14	14	14	14	14	14	14	14
	Regression														
*. Correlation is significant at the 0.05 level .															
**. Correlation is significant at the 0.01 level.															

Table 7: T-test between the control group and the patient group

Statics groups						
CON_PA		N	Mean	±	SD	P VALUE
F.BS.GLUC	CONTROLS	10	87.50	±	5.19	0.002 S
	PATIENTS	28	138.04	±	79.17	
B.UREA	CONTROLS	10	34.70	±	5.85	0.047 S
	PATIENTS	28	56.86	±	55.69	
ALT1	CONTROLS	10	39.20	±	12.15	0.644 NS
	PATIENTS	28	43.11	±	39.43	

AST	**CONTROLS**	10	25.60	±	3.86	0.549 NS
	PATIENTS	28	35.36	±	9.55 SE	
A1K	**CONTROLS**	10	80.30	±	12.89	0.001 S
	PATIENTS	28	119.96	±	52.70	
TBI	**CONTROLS**	10	.55	±	.21	0.414 NS
	PATIENTS	28	.65	±	.37	
DBI	**CONTROLS**	10	.14	±	.03	0.629 NS
	PATIENTS	28	.15	±	.07	
1B1L	**CONTROLS**	10	.40	±	.10	0.716 NS
	PATIENTS	28	.44	±	.34	
CA	**CONTROLS**	10	8.92	±	.33	0.602 NS
	PATIENTS	28	8.72	±	1.19	
NA	**CONTROLS**	10	138.80	±	4.05	0.345 NS
	PATIENTS	28	140.32	±	4.40	
K	**CONTROLS**	10	4.43	±	.33	0.835 NS
	PATIENTS	28	4.35	±	1.18	
CL	**CONTROLS**	10	101.60	±	2.27	0.776 NS
	PATIENTS	28	100.46	±	12.36	
CRE2	**CONTROLS**	10	.79	±	0.17	0.027 S
	PATIENTS	28	2.46	±	0.71 SE	
ALBUMIN	**CONTROLS**	10	4.11	±	0.38	0.703 NS
	PATIENTS	28	4.17	±	0.45	
DHFR	**CONTROLS**	10	20.00	±	1.83	0.002 S
	PATIENTS	28	30.32	±	16.02	
S: SIGNIFICANT BECAUSE P<0.05 **NS: NOT SIGNIFICANT BECAUSE P>0.05**						

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Study The Antibacterial Activity of Aqueous Extract of Lepidium Draba on MRSA Isolated from Children of Tonsillitis

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Abstract

In this work, Staphylococcus aureus is isolated and identified from Iraqi patients suffering from chronic tonsillitis. The bacterial potential to build biofilm is also detected, and the antibacterial efficacy of Lepidium draba aqueous extract on MRSA is ascertained. Eighty-four samples are obtained through swabbing. Thirteen (15.5%) samples produced a negative culture, while 71 (84.5%) samples produced a positive culture. Following microscopic examination and Gram staining, 71 (100%) of the isolates are determined to be Gram-positive bacteria. The results of an API identification of Gram-positive isolates revealed that 30 (42.3%) are S. aureus and 41 (57.7%) are S. pyogens. Two groups of S. aureus isolates are created based on the antibiotic susceptibility results: MRSA 4 (13.3%) and MRSA 26 (86.7%). The results of determining the ability of S. aureus isolates to form biofilms using the test tube method showed that, despite the fact that all isolates are obtained from patients and showed antibiotic resistance, 29 (96.7%) of the isolates formed biofilms of varying thickness, while only 1 (3.3%) did not. Ceftriaxone is the most effective antibiotic against S. aureus, according to a study on the susceptibility of S. aureus isolates to most of the antibiotics used in Iraqi hospitals for the treatment of chronic tonsillitis. The majority of resistant S. aureus isolates that produced the thickest biofilm layer with an inhibition zone (21 mm) are highly inhibited by the crude aqueous extract of L. draba.

Keywords: *Staphylococcus aureus, MRSA, chronic tonsillitis, biofilm formation, antibiotic resistance, Lepidium draba, aqueous extract, antibacterial activity, phytotherapy, Iraqi children*

http://dx.doi.org/10.47832/MinarCongress15-4

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1.1 Introduction

Plants are a good source of natural products, many of which have uses as pharmaceuticals, agrochemicals, flavor and fragrance chemicals, food additives, and pesticides (Fadhil et al., 2013). Due to the high cost of commercial antibiotics treatments and high rate of multidrug resistant microbials, Natural products have been playing a role in health care and disease prevention for a long time (Najim et al., 2020). Lepidium draba is a perennial herb that spreads by seed and horizontal creeping roots. It is often referred to as white top or hoary cress. This plant is indigenous to North America, Africa, especially Algeria, and western Asia, particularly the Middle East and Eastern Europe. L. draba leaf infusion has expectorant and purgative properties. It may be grown in a wide range of disturbed regions, including farmland, rangeland, pastures, roadsides, and iste areas, as well as in a variety of soil types with enough of water. It is shown to do best in riparian or irrigated settings (Bensaid et al., 2018). Inflammation of the tonsils, two soft, oval-shaped tissues at the back of the throat, is known as tonsillitis. Each side has one tonsil. Swollen tonsils, a sore throat, difficulty swallowing, and tenderness in the lymph nodes on both sides of the neck are all signs of tonsillitis. Although circulating viral infections are the most common cause of tonsillitis, bacterial infections like Staphylococcus aureus and Hemophilus influenza can also cause tonsillitis. Streptococcus payogens (Keskin and Oguz, 2019; Munck et al., 2108; Abu-Baker et al., 2018). Gram-positive, catalase-positive cocci belonging to the Staphylococcaceae family include S. aureus. They are facultative, non-motile, spore-forming anaerobes with a diameter of 0.5 to 1.5 µm that typically form in clusters. According to Keneth et al. (2018), this dangerous species of Gram-positive extracellular pathogenic bacteria colonizes, causes a series of infections, and develops a sophisticated virulence mechanism to evade human defenses. Methicillin-resistant S. aureus, or MRSA is the most frequent cause of staph infections, which can spread throughout the human respiratory system and release a number of toxins associated with its pathogenicity, ability, and colonization. Superantigens (TSST) and staphylococcal enterotoxin are the most significant (Filho et al., 2016). Staphylococcal superantigens are useful in treating respiratory conditions, particularly asthma and rhinitis, which can lead to inflammation and the emergence of hyperresponsiveness (Hessam and Elazab, 2013). The role of staphylococcal superantigens is to directly stimulate MHC class II and T cells, which improves CD4 and CD8 T lymphocyte proliferation and activity (Sedighi et al., 2011 and Goran et al., 2020).

lepidium draba

Lepidium draba belongs to the Brassicaceae family of mustards. It has been known by a number of botanical names. It is formerly a member of the Lepidium genus. L. draba can be found in a variety of settings, including rangelands, meadows and pastures, home gardens, national parks, roadsides (very disturbed areas), and isteland. It occurs above and below sea level and at both high and low latitudes (Roughani, 2018). It occurs on arid land as well as high-moisture soils and under irrigation. Although it may sprout in salty soils and thrives in alkaline soils, it can grow in all types of soil. The harmful weed benefits greatly from all of these characteristics and adaptations, which also show how well it can colonize a variety of agri-husbandry systems.. (Radonić et al.,2011 and Mohammadia et al., 2014)

Figure (1-1): Lepidium draba

Taxonomy and Nomenclature

Table 1.1 illustrate the classification of Lepidium draba

Domain: Eukaryota

Kingdom: Plantae

Phylum: Spermatophyta

Subphylum: Angiospermae

Class: Dicotyledonae

Order: Capparidales

Family: Brassicaceae

Genus: Lepidium

Species: draba

Subspecies: Cardaria

Binomial name: Lepidium draba

Common name: Cardaria draba

Medicinal Uses

L. draba has many uses in folk medicine in treatment of inflammation. It is used in Middle east specially in Iraq and Iran for treating coughs and chest congestion, diabetes, detoxify liver and spleen, reduce swelling and clean wounds, because it has antipyretic, analgesic, anti-microbial and antioxidant potent (Bensaid et al., 2018) Excessive use of antibiotics has resulted in the development of multiple drug resistance in numerous pathogens rendering some antibiotics virtually useless. Thus, new drugs are required for the treatment of different infections. Natural products are an important source of new drugs and have served as an optional medicine for the therapy of various diseases for decades(Chyad,2017). Lepidium draba is commonly known as whitetop or hoary cress, is a perennial plant that reproduces by seed and by horizontal creeping roots, iste areas, and is known to prosper in riparian or irrigated areas L.draba leaf and seeds have antimicrobial potential against drug-resistant human pathogen, purgative and expectorant effects (Javad et al.,2015).

Plant Secondary Metabolites

Utilizing plant cell cultures has solved a number of issues with the synthesis of plant secondary metabolites, which are unique sources for medications, food additives, tastes, and other industrial materials (Kabera, 2014; Tiwary, 2015, and Hussein, 2016).

• Alkaloids are initially defined as pharmacologically active nitrogen-containing compounds, but they will inhibit ion channels, block enzymes, or interfere with neurotransmission

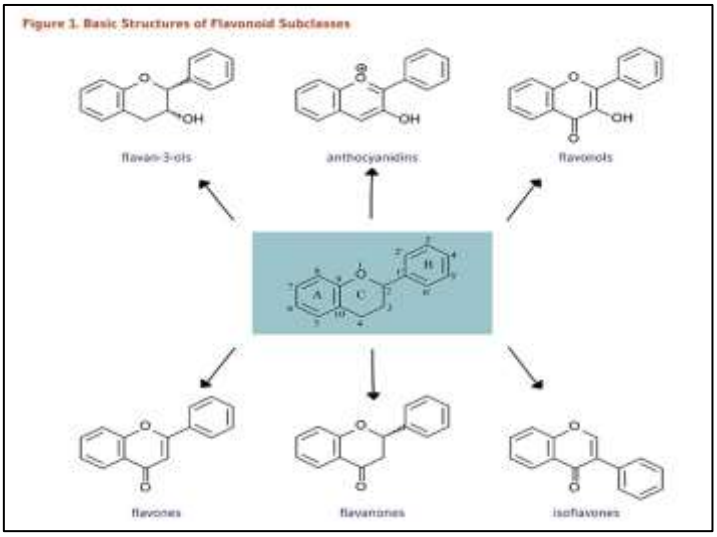
• Phenolic substances hinder cell division, limit development, impede enzyme activity, and obstruct digestion.

• Tannins are a class of natural polyphenols that get their name from the French word "tanin" (tanning material). The phenolic compounds that precipitate proteins are called tannins. They are made up of an extremely diverse collection of polymers and oligomers. They can combine with minerals, cellulose, proteins, and carbohydrates to form complexes.

• Glycosides can be sulfur compounds, alcohol, or phenol. They are characterized by a sugar moiety or component that is unusually bonded to one or more non-sugar fragments. Many plants store chemicals as inactive glycosides, which are activated by the hydrolysis of enzymes. • Saponins are triterpene or steroid glycosides The plant kingdom contains a vast array of biologically active substances, including a third group that some authors refer to as steroidal amines and others as steroidal alkaloids.

• Flavonoids are a broad category of natural phenolic compounds. Flavonoids are found as monomers, dimers, and higher oligomers in the majority of plant tissues, frequently in vacuoles. Flavonoids are a very broad class of substances that serve several purposes.

Every flavonoid has the potential to help plants protect themselves from UV-B radiation. Chalcones, aurones, flavonones, isoflavonoids, flavones, flavonols, leucoanthocyanidin, catechins, and anthocyanins are among the classes of plant metabolites that make up flavonoids (figure 1-1) (Tiwari, 2015).



1.2.6 Tonsil

Tonsil is the small mass of lymphatic tissue, which is settled in the wall of the pharynx at the rear of the throat of humans as shown as Figure (2-1). The **tonsils** have an important function **in the body** for protection against respiratory and gastrointestinal infections. The **tonsil** consists of a network of crypts , which represent a store of fighter cells (Jović et al., 2015; Fagö-Olsen et al., 2019).

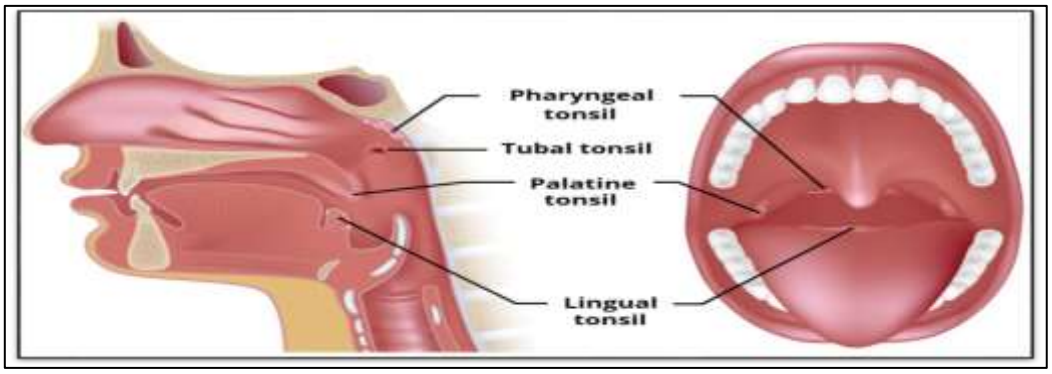


Figure (1-3): Tonsil Location in the human (Wang et al., 2010).

The pharyngeal, tubal, palatine, and lingual tonsils are the four types of tonsils that humans are born with. As the immune system's first line of defense, the tonsils often swell with blood to aid in the body's reaction to common ailments like the common cold. Pathogens can be taken up by the tonsils thanks to the presence of M cells, which are effective antigen-capture cells on their surface. These M cells trigger an immune response by alerting the tonsil's underlying B and T cells to the presence of a pathogen (McClory et al., 2012; Catic et al., 2018).

Tonsillitis : As seen in fig. (2-2), tonsillitis is an inflammation of the tonsils brought on by a bacterial or viral infection. Swollen tonsils, painful throats, trouble swallowing, and enlarged lymph nodes on the sides of the neck are all signs of tonsillitis. It is challenging to distinguish between bacterial and viral tonsillitis, yet doing so is essential to avoiding antibiotic abuse (Keskin and Oguz, 2019). A painful throat affects about 7.5% of people. Tonsillitis is the most prevalent sickness among school-age children, usually occurring during the colder fall or winter months. Each year, has 2% of people seek medical attention for tonsillitis (Bird et al., 2014).-

Figure (1-4): The Tonsillitis (Sommers , 2019)

The presence of tonsillar exudate, painful neck lymph nodes, fever history, age, and lack of cough are the five main criteria that doctors use to diagnose tonsillitis. There are three different types of tonsillitis: acute tonsillitis, which typically lasts three to four days but can last up to two weeks; recurrent tonsillitis, which occurs multiple times in a year; and chronic tonsillitis, which causes persistent sore throats and foul-smelling breath (Pontin et al., 2016; Munck et al., 2018 and Sommer, 2019).

Between 50 and 80 percent of cases of acute tonsillitis are caused by viruses. A group beta-hemolytic streptococci and Haemophilus influenza are the most frequent bacterial germs that cause acute tonsillitis, while rhinovirus, coronavirus, and parainflu-enza virus are the most common causes of viral infections. Acute tonsillitis manifests as fever, malaise, headache, sore throat, joint and muscle discomfort, and difficulty swallowing (Dan et al., 2019). Within 24 hours of taking antibiotics, the chance of transmission can be decreased. Different antibiotic usages have little effect on how long it takes to treat acute tonsillitis. It will take seven to fourteen days for the body to recuperate.

Children under the age of three do not get acute tonsillitis, which is most common in those aged five to fifteen. A child is considered to have recurrent tonsillitis if they experience multiple episodes of acute tonsillitis within a year. Tonsillitis in children may be predicted by a family history of tonsillectomy and atopy (Pontin et al., 2016; Wang et al., 2017; Hathal et al., 2019 and Wu et al., 2021). A three-month history of painful throat, halitosis, odynophagia, perhaps otalgia, and tonsillitis with inflammation—typically accompanied by debris in crypts or tonsillar exudates—is considered chronic tonsillitis. Tonsillitis and softer, cheesy, odorous debris that builds up in tonsillar crypts (cryptic tonsillitis) are two symptoms of chronic tonsillitis. According to Abu Bakar et al. (2018), tonsillitis is characterized by yellow-white, hard calculi that can be spat out or removed from the tonsils using a swab.

Bacterial tonsillitis

Around 15 -30 % of tonsillitis cases result from bacteria, which is more common in children. Its diagnosis is based on primary physical examination of throat followed by a throat culture with antibiotics susceptibility test and complete blood count to determine the severity of inflammation for choosing the correct efficient antibiotic and prevent progression of disease) Dale et al., 2019 and Adler et al 2020)

Bacterial tonsillitis can result from both aerobic and anaerobic bacteria. It is commonly caused by group A beta-hemolytic Streptococcus, Staphylococcus aureus, Streptococcus

pneumoniae, and Haemophilus influenza have also been identified as causative agents. Tuberculosis has also been implicated in recurrent tonsilitis, and clinicians should assess patients' risk (Tamayo et al ., 2016 and poole et al., 2019).

The age and frequent exposure to microbes represent the most effective risk factors of tonsillitis that most often affects children ages 5 to 15 because school-age children are in close contact with their peers and commonly exposed to viruses or bacteria that can cause tonsillitis. Chronic tonsillitis can cause critical intricacy such as difficult breathing during sleep, an infection that spreads deep into surrounding tissue and accumulation of abscess behind a tonsil(Tagini et al., 2017 and Avire et al., 2021) .If tonsillitis caused by streptococcal bacterial infection isn't treated or misusing of antibiotic treatment can cause maximized risk of rare disorders like rheumatic fever can affect various organs, scarlet fever, post-streptococcal glomerulonephritis and arthritis (Watts et al., 2019).

Staphylococcus aureus

Saureus is an opportunistic pathogen that may cause a variety of diseases in human beings The majority of strains of Staphylococcus aureus possess growing resistance to multiple classes of antibiotics. It causes a variety of diseases ranging from mild skin infections, such as pimples, impetigo, folliculitis, carbuncles, scalded skin syndrome and abscesses, though these may be life-threatening, to life-threatening diseases such as pneumonia, meningitis, osteomyelitis, endocarditis, toxic shock syndrome (TSS), bacteremia and sepsis. Its frequency varies from skin, soft tissue, respiratory, bone, joint, endovascular to wound infections. It is one of the five most common sources of nosocomial infection, frequently causing postsurgical wound infections. (Methicillin-resistant Staphylococcus aureus (MRSA) has emerged as a global issue, yet the incidence differs significantly among nations due to SCCmec kinds, bacterial growth rate, and the frequency of toxin and antibiotic resistance genes (David et al., 2006; Eisenstein, 2008; Hansra et al., 2011). Antibiotic-resistant bacteria Innate or intrinsic resistance is the term used to describe the resistance of some bacteria to the effects of specific antibiotics, whereas acquired resistance is the term used to describe the resistance of other bacteria to antibiotic types, which can arise from spontaneous mutation or the acquisition of new genetic information (Naster et al., 2001).

Bacteria may contain the plasmid or undergo mutations that alter the components of their bacterial cell, which would explain their resistance to a particular antibiotic. which carries the genes coded for these resistances or by transposons which are coded for resistance and can move to another plasmid which lacks the resistance trait conjugation, transformation,

or transduction are the three ways that bacterial cells might acquire resistance (Schlessing and Eisenstein, 1999; Hryniewicz et al., 2001).

Antibiotics are categorized based on their chemical makeup or mechanism of action; some, like β-lactams, affect the formation of cell walls, while others, like cephalosporins, affect the integrity of cell membranes (Todar, 2002).

Protein synthesis is inhibited by aminoglycosides, tetracyclines, and macrolides, but nucleic acid synthesis is inhibited by quinolones. Brooks et al., 2004; Todar, 2002). Numerous factors, including the antibiotic's chemical structure, mode of action, duration of use, bacterial species, and capacity to develop a resistance mechanism against the targeted antibiotic, influence the measurement of antibiotic resistance in bacteria (Pitout et al., 1997; Geisinger and Isberg, 2017).

Material and Method

Material

Apparatus and Equipment

Table 2_1

Apparatus and Equipment	Company/Country
Auto vortex	Stuart scientific/UK
Autoclave	KarlKolb/Germany
Automatic pipettes	Brand/Germany
Electric oven	Gallenkamp/England
Hot plate magnetic stirrer	Gallenkamp/England
Incubator	Memmert/Germany
Sensitive balance	Stanton/UK
Shaker incubator	Gallenkamp/England

Chemicals

Table 2_3

Chemicals	Company /Country
Absolute ethanol (99 %)	Schrlo /Spain
HCl	Fluka / Switzerland
Ferric chloride	BDH/England
Lead acetate	
Benedict reagent	
Bismuth sub-nitrate	
potassium iodide	
Sodium hydroxide	

Culture Media

Table 2_3

Media	Company/Country
Mannitol salt agar	Hemadia/ india
Muller – Hinton agar	
Nutrient broth	
Nutrient agar	
Trypton soya broth	
Chromoagar	

Methods

Sterilization methods

Moist heat sterilization

Autoclave is used to sterilize media, buffers and solutions at 121 ºC for (15 Ib/ in^2) for 15 minutes**.**

Dry heat sterilization

Electric oven is used to sterilize glass wares and others by heating at 180 ºC for 2 hours.

Filtration (membrane sterilization)

Millipore filter unit is used to sterilize the solution has been extracted.

1- **Specimens collection**

2-Identification of Bacterial Isolates

3-Cultural identification

4-Microscopical identification

5- Identification of bacteria by Api Staph Kit

6-Maintenance of bacterial strains

7-Short term storage

8- Medium term storage

9-Plant Collection and Classification

10- **Aqueous Extraction**

11-**Phytochemical Investigation of the Crud Extract (Richird,**

2000)

12- Detection of Tannins tests

13- Detection of polysaccharides

14- Detection of alkaloids (Dragangroff test)

15- **Detection of the Saponins**

16- Detection of Flavonoids

17 -Detection of Polyphenolic Compounds

18- Detection the ability of bacteria for biofilm formation (Test tube method) **(Christensen et al.,1982)**.

19-Antimicrobial Activity of the Crud Extract (Agar Well Diffusion Method)

20Antibiotic sensitivity test **(Atlas et al.,1995).**

Results & Discussion

Phytochemical Tests of Crud Extract.

The results of phytochemical tests are displayed in table (3-1) and revealed that the plant is rich in numerous active constituents that explain why it has been used as a medicinal plant for centuries, even in folk and traditional medicine (Javad et al., 2015). The crude extract produced 7.5 g residue from 50 g of plant powder.

Table (3-1): Results of Phytochemical Tests of Aqueous Crud Extract

Test	Result	Comments
Detection of Tannins	+	White p.p.t.
Detection of polysaccharides	+	Orange-Red p.p.t.
Detection of alkaloids	+	brown p.p.t.
Detection of the saponins	+	Foam formation
Detection of Flavonoids	+	Bright yellow
Detection of Polyphenolic compounds	+	Brown p.p.t

3.1. Isolation and Identification bacteria:

At the Al-Yarmouk Teaching Hospital in Baghdad, 84 samples are obtained from patients by swabbing in order to determine whether bacterial tonsillitis is present or not

between October 1, 2021, and December 31, 2022. Api, morphology, and culture are used to identify each isolate. As indicated in table (3-1), the results indicated that 13 samples had negative cultures and 71 out of 84 samples had positive cultures.

Table (3-2): percentage of positive and negative culture

	Culture	No. of isolates	Percentage (%)
1	**Positive**	71	84.5
2	**Negative**	13	15.5

All 71 (100%) of the isolates are first identified as Gram-positive bacteria based on the microscopical analysis. Table (32) shows the results of the API 20 test for bacterial isolates, which revealed that 30 (42.3%) isolates are identified as S. aureus and 41 (57.7%) isolates as S. pyogens.

Table (3-3): Percentage of Gram-Positive Bacterial Isolates

Bacterial isolates	No. of isolates	Percentage %
Sterptococcus pyogns	41	57.7
Staphylococcus aureus	30	42.3
Total	71	100

Because of its persistence in the interior tissues of the tonsils and resistance to antibiotics, S. aureus is one of the most common pathogens in the pathogenesis of tonsillitis. After many antibiotic treatment failures, tonsillectomy is recommended in situations of recurrent tonsillitis (Cavalcanti et al., 2019).

The bacterium's persistence in this location may be due to its presence in the tonsil's interior tissue. Internal tissue harbors harmful germs, but the tonsillar surface typically displays bacteria that are part of the normal oral microbiota. According to Hamdan-Partida et al. (2018), S. aureus has been found in the tonsils' internal and exterior tissues.

S. aureus is primarily found in the nose of humans. S. aureus is a significant risk factor for nasal infections and is present in the noses of about 30% of healthy people. When

compared to non-carriers, nasal carriers of S. aureus are three times more likely to develop chronic tonsillitis (Heiman et al., 2005).

The highly ordered bacterial populations known as Staphylococcus biofilms are protected by an exopolysaccharide matrix. Due of its superior survival benefits and great resistance to traditional medicines, S. aureus prefers this form over the planktonic one. A number of mechanisms, such as the transcription of genes resistant to antibiotics, the adoption of a less metabolically active state, and the matrix itself, contribute to antibiotic resistance and, in fact, resistance to the host immune system. Biofilms of S. aureus are more closely linked to with tonsil infection that persists throughout time (AlChalabi et al., 2019). Because of its capacity to cause a variety of life-threatening illnesses and its capacity to withstand multiple drugs due to the production of virulence factors, such as adhesions and different exotoxins, which are linked to its pathogenicity, S. aureus is an opportunistic pathogen (Whitby et al., 2011 and AlChalabi et al., 2017). Following culture identification using chromo agar media, S. aureus isolates are split into two groups, as illustrated in figure (3-1): 26 (86.7%) are MRSA and 4 (13.3%) are MSSA.

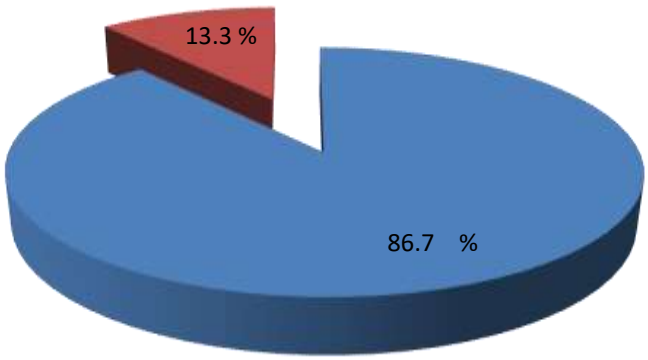


Figure (3-1): Percentage of MRSA

Methicillin-resistant S. aureus (MRSA), which harbors the staphylococcal cassette chromosome mec (SCCmec), has become a leading cause of hospitalacquired infections worldwide, accounting for >60% of S. aureus isolates in United States hospitals (Bart et al.,2012).

Biofilm formation

The ability of pathogenic S. aureus isolates from children with chronic tonsillitis to produce biofilms is determined using the test tube method. According to the results displayed in table (3-3), a significant proportion of S. aureus isolates exhibited the ability to form

biofilm. As seen in figure (3-1), 29 (96.7%) S. aureus isolates create biofilm of varying thicknesses, whereas only 1 (3.3%) isolates are unable to do so.

Table 3-4: percentage of biofilm formation by S. aureus isolates

	S. aureus isolates	No.	Percentage %
1	**Biofilm Former**	29	96.7
2	**Biofilm Non-former**	1	3.3

Figure (3-2): Biofilm production by S.aureus isolates

According to Nitsche-Schmitz et al. (2007), microorganisms vary in their capacity to form biofilms, and the thickness of these biofilms varies depending on a number of conditions, including temperature, pH, and the type and genus of the producing bacteria. The S. aureus isolates produced varying thicknesses of biofilm. The initial stage of biofilm formation is bacterial adherence followed by surface colonization. Under specific conditions, biofilm is formed from spherical micro-colonies coated with extracellular polysaccharide material (Olson et al., 2002; Anderson et al., 2007 and AlChalabi et al., 2019). By creating a resting reservoir of pathogens, biofilm enables bacteria to persist for extended periods of time; regrowth of bacteria from this reservoir may be the cause of relapse infection (Deleo et al., 2010).

Both live tissues and artificial devices can form biofilm, which can result in persistent and frequently incurable infections. The most prevalent opportunistic pathogens, S. aureus, are able to create infections with a greater ability to build biofilms, which allows them to get a safe haven and withstand removal by the host's innate response. By concentrating nutrients and shielding the cells from surfactants, biocides, and phagocytic cells, the biofilm matrix alters the environment around adherent cells (Skiest et al., 2007; Nathan et al., 2011). When an organism is embedded in a biofilm, antibiotics are ineffective. Compared to plank tonic cells, which exhibit greater tolerance to antimicrobial treatments, biofilm cells have unique characteristics. biofilm that S.aureus significantly causes antibiotic resistance by preventing

antibiotics from entering, resulting in an increasingly severe case (Yan et al., 2016; Suvi et al., 2017).

Antibiotics Sensitivity

Antibiotics are effective medications that either kill or stop the growth of germs in order to treat bacterial infections.

Certain microorganisms (bacteria and fungi) naturally produce biological chemicals called antibiotics that either stop the growth of other microorganisms or kill them (David, 2013).

Antibiotic-resistant bacterial growth is a worldwide phenomenon that, as a significant concern, is a sign of evolutionary processes during antibiotic treatment. Antibiotic treatment may selectively enable bacterial strains with genetically or physiologically increased resistance to high antibiotic dosages. In some circumstances, it may lead to the preferential proliferation of resistant bacteria, while the antibiotic suppresses susceptible germs (Rhee and Gardiner, 2004; Ocampo et al., 2014). The usual disk diffusion method is used to determine the antibiotic sensitivity of S. aureus isolates, and the results are compared with NCCLs. Tables (3-4) and Figure 3-2 presented the results, which indicated that Ceftriaxone (6.7%) is the more effective antibiotic for S. aureus strains. The isolates exhibited a very high proportion of resistance (100%) to Amoxicillin, Penicillin, Cefotaxime, Gentamicin, and Rifampin. The percentages for Methicillin and Azithromycin are 86.7 and 73.3%, respectively. The percentage of S. aureus strains that are resistant to amoxicillin (72.2%) and kanamycin (68.5%) varied.

Table 3-5: The percentage of antibiotics resistance of S. aureus isolates

Antibiotics	Resistance	
	No.	Percentage %
Amoxicillin	30	100
Amoxicillin + Clavulanic acid	6	20
Cefotaxime	30	100
Ceftriaxone	2	6.7
Azithromycin	22	73.3
Gentamicin	30	100
Methicillin	26	86.7
Penicillin G	30	100
Rifampin	30	100

There are several kinds of antibiotics; some are broad spectrum, meaning they work on both Gram-positive and Gram-negative bacteria, while others are limited spectrum, meaning they only work on a certain kind of microbe (Tan et al., 2015). Innate or intrinsic resistance is the term used to describe bacteria that are naturally resistant to the effects of a particular antibiotic, whereas acquired resistance is used to describe bacteria that are resistant to antibiotic classes and can result from either spontaneous mutation or the acquisition of new genetic information (Gill et al., 2015).

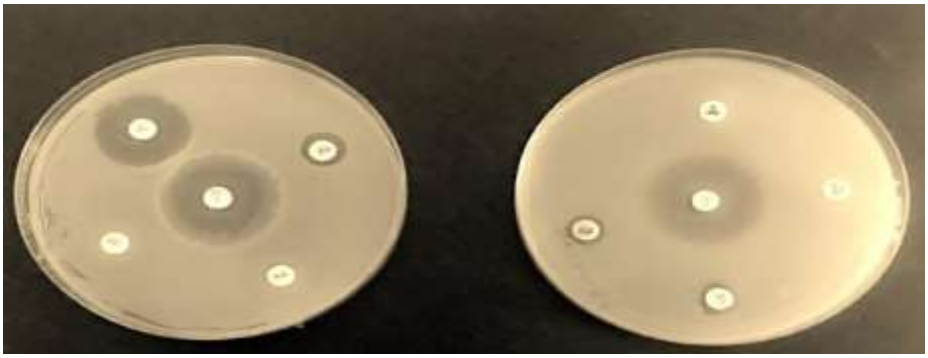


Figure (3-2): Antibiotic sensitivity test of S. aureus

Determination of antibacterial activity of L. draba aqueous extracts against MRSA

By using the agar well diffusion method with varying extract concentrations, the antibacterial activity of L. draba's aqueous extract against MRSA that is isolated from patients with chronic tonsillitis is ascertained. As seen in Fig. (3-3), the results demonstrated that only crude aqueous extract exhibited antibacterial activity against the more effective biofilm-forming MRSA isolate with an inhibition zone (21 mm).

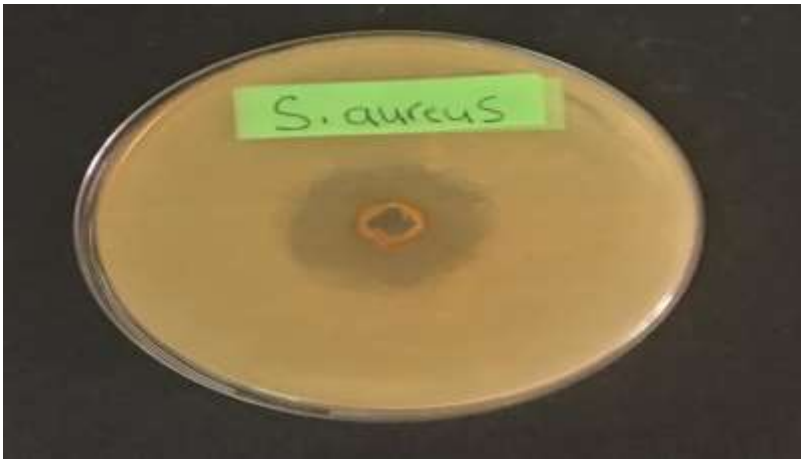


Figure (3-3): Antibacterial activity of aqueous extract of L. draba against

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Molecular Characterization of Bacteria Isolated From Tumors Formed on The Myrtus Communis L Stems and Its Biological Action

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Abstract

An endophytic bacteria isolated from tumors formed on the *Myrtus communis* L. stems on solidified Agrobacterium Mannitol Medium (AM) medium. The novel strain of the bacteria isolate grown are in white color after 48 hours, and take a circular shape slightly convex, have sticky appearance, showing production of mucus substance, Gram negative and positive for oxidase and catalase test. The bacteria are resistance to the antibiotics 30µg/ml Metronidazole, 10µg/ml Ampiclin sulbactum and sensitive to the other. The National Center for Biotechnology Information (NCBI) global database is used to analyze the nitrogenous bases of the 16S rRNA gene in accordance with the isolate's molecular diagnostic utilizing DNA sequencing., the results appeared 99% similarity the isolate of this study to the genus *Pantoea* are found in gene bank, therefore its recorded for the first times at *Pantoea* sp. RAZ strain in NCBI, and success of inoculation by direct injection, as the results showed higher rates of tumor formation by inoculation with bacterial suspension at 24 hours of age than by inoculation with bacterial suspension at 48 hours of age, at rates of 70% and 50%, respectively

Key words: *Myrtus communis L., Pantoea sp. RAZ, 16s rRNA.*

http://dx.doi.org/10.47832/MinarCongress15-5

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Introduction

Gavini is the first person who defines the genus Pantoea, which includes a remarkably diverse group of bacteria (Gavini *et al*., 1988). The family Enterobacteriaceae includes this genus. and currently includes 19 species which are: *Pantoea eucalyptii, P. agglomerans, P. vagans, P. conspicua, P. deleyi, P. anthophila, P. brenneri, P. ananatis, P. allii, P. stewartii, P. cypripedii, P. calida, P. gavinae, P. dispersa, P. séptica, P. wallisii, P. eucrina, P. rodasii, and P. rwandensis* . discovered in buckwheat seeds, plant surfaces, human iste, and other environments. (Asai *et al*., 2019*).* The genus *Pantoea* is global, non-encapsulated, Gram-negative, and incapable of sporulation.

A number of *Pantoea* species are regarded as plant growth-promoting bacteria (PGPB) by both direct and indirect means. The direct processes use bacterial characteristics that directly promote plant growth, such as nitrogen fixation, ammonia and phosphorous solubilization, phytohormone synthesis, and bacterial siderophores' sequestration of iron. (kim *et al.*,2015. ; Cherif.silini *et al.*,2019). Conversely, the indirect processes include bacterial characteristics that produce antibiotics, enzymes that break down plant walls, siderophores, competition, hydrogen cyanide, and induced systemic resistance, all of which inhibit plant pathogenic organisms (fungi and bacteria). (Thissera *et al.*, 2020).

Genetic transformation means the insertion of a foreign gene with a desirable trait into the bacteria genome (nuclear or cytoplasmic*)* (Wilson and Coverley, 2017). Transduction has now become a very important method of gene transfer and of interest to obtain gene expression for desired traits. The process of transformation requires three essentials: the desired gene, the target into which the gene will be inserted into its genome, and the gene vector for the success of the process (Garcia *et al.*, 2012).

The concepts of genetic transformation express either the manipulation of the genetic material by including it in the recipient cells, as happens in the fusion of cells or somatic hybridization, and this is known as genetic manipulation, or those in which certain sequences of the genetic material of a particular organism are cut and included in an appropriate location in the genome of the recipient cell in another organism and expression for its qualities that are not originally present in it and it is called genetic engineering (Varasteh-shams *et al*., 2020). These transformations included genetic engineering techniques, DNA reorganization, tissue culture, isolation and fusion of protoplasts with the aim of obtaining genetically modified plants or acquired plants with a group of soma clonal variation, or the use of *Agrobacterium* and its strains as natural vectors (Keshavareddy *et al.*, 2018).

Several studies deal with the scientific explanation of the steps of infection of plants and the formation of coronary tumors on them by bacteria *Agrobacterium .tumefaciens* (Tzfira and Citovsky, 2008). The first evidence of interaction between the bacterium and the host plant appears in the association between the two cells bacteria and plant. Once the bacterium enters, the disease genes (*vir* genes) begin to express themselves sequentially in order to control the transmission of a piece of DNA from the bacterium plasmid (Ti-plasmid) to the nucleus of the plant host cell, to then induce the plant cells to divide randomly to form the so-called coronal tumors (Gustavo *et al.*, 1998).

There are two ways to transfer genes:

1- Vertical genes transfer: It is the process of transferring genes from parents to children (Husnik and McCutcheon, 2018).

2- Horizontal genes transfer: is the process of transferring genes between different species and genera which is provides the possibility of forming new genetic unions on local strains (Soucy *et al.*, 2015).

There are three ways of horizontal gene transfer, they are: Transformation, Transduction and Conjugation (Lacroix and Citovsky, 2018*)*

An evergreen shrub of the *Myrtaceae* family, myrtle (*Myrtus communis* L.) is widely distributed in the Mediterranean region in impromptu bush-cover formations. There are two subspecies of myrtle: *communis* and *tarentina*. Both of the subspecies may belong to the melanocarpa (bluish-black peel) based on the color of the ripe fruit. (Usai *et al.*, 2020). Although myrtle is a quite rustic plant that can withstand harsh weather conditions, it is susceptible to cold winds. (Medda and Mulas, 2021). Various parts of myrtle, especially the berries, leaves, flowers, and essential oils, have been widely used in traditional medicine to treat a variety of conditions, including cough, gastrointestinal issues (such as stomach ulcers, diarrhea, and hemorrhoids), urinary disorders (such as urethritis), and skin conditions (such as reddened skin). They have also been used to inactivate microorganisms and promote wound healing (Alipour *et al.*, 2014 ; Giampieri *et al.*,2020).

Biomass from the myrtle plant is used as a raw material in the food, cosmetic, pharmaceutical, and decorative sectors. The most economically significant plant parts are the leaves and fruits. Essential oil glands are found in leaves, which are utilized to extract aroma. (Mulas and Melis, 2011 ; Usai *et al.*, 2020).

Aims of this study

Aims of this study to isolated the bacteria from myrtle stems tumors and Identification of isolated bacteria by microbiological, biochemical and molecular biology test then investigation its biological activity.

Materials and Methods

Isolation of microorganisms from tumors that developed on myrtle plant stems

The tumors crown form on the stems of the *myrtus communis* L. plants grown near the Department of Civil Engineering / Colloege of Engineering/ University of Mosul/Iraq (Figure,1) are excised with avoided any cut or wound.

Figure (1): Tumors grown on the Myrtle stem

It are ished with running water for 30 minutes, and superficially sterilized by immersed it in a solution of 96% ethyl alcohol for two minutes, then in 3% sodium hypochlorite (NaOCl) for 15 minutes, then ished with sterile water four times (minute/time) and dried using sterile filter paper*(*AL-zaidy, 2014). Then it is placed on the surface of 30 ml of Nutrient Agar (NA) medium and incubated at (28 ± 2) for 24 hours to ensure the efficiency of its surface sterilized, then selected 5 pieces of it and crushed well in 3.0 ml of liquid Agrobacterium Mannitol Medium (AM) medium (Murugesan *et al.,* 2010) used a sterile glass rod. Several decimal dilutions are prepared from suspension, 0.1 ml of the last three dilutions are taken and spread on the surface of solid AM medium separately by used L shape sterile glass, and incubated for 48 hours at 28 ±2°C. After growth, one colony is taken for each dilution and cultured on the surface of medium in 9.0 cm plastic dishes by plotting method (Prajapati *et*

al.,2018) to obtain single, pure colonies and incubated the same conditions above. The plates are transferred and kept in the refrigerator at 4C° until used.

Identification of isolated bacteria

- **Morphological characterization**

The colony characteristics are determined by observing the colonies on AM plates that grow for 48 hours at 28 ℃. Microscopic observation of the isolated is done using gram stain technique.

- **Catalase test**

A young colony of bacteria is placed on a glass slide with a drop of 3% hydrogen peroxide solution H_2O_2, and the appearance of gas bubbles indicate the positive result of the test and the ability of the bacteria to produce the enzyme *catalase* that breaks down H_2O_2 into H_2O and O_2, thus releasing oxygen gas (Chhetri *et al.*, 2019) .

- **Oxidase test**

Placed young colony of pure bacteria on a filter paper moistened with drops of the oxidase reagent. The appearance of the violet or purple color indicates the ability of the bacteria to produce the enzyme *Cytochrom oxidase*, which oxidizes the reagent to the product indophenol (Wadhwa *et al.*, 2017).

- **Antibiotic sensitivity test**

The sensitivity of bacteria isolated is tested with different types of antibiotics in 9 cm petri dishes containing AM solid medium and added the discs to the surface medium after being sterilized. The antibiotics select in this study are tested in (Table, 1).

Table (1): The antibiotics used and their final concentrations

Antibiotic	Final concentration (µg ml^{-1})
Metronidazole	30
Ampicllin sulbactum	10
Piperacillin	30
Metronidazole	30

Tetracycline	10
Gentamicin	10
Ticarcillin acid	10
Ceftriaxone	10
Levofloxacin	5

The antibiotics in different concentrations provided from (CLSI, 2018) on the discs form are added to the surface medium after being sterilized. Separately by spreading 0.1 ml of the bacterial suspension on all medium using a sterile L. shape, the dishes are closed with their lids and placed in a growth incubator at 28 C° in dark conditions for 48 hours (Soriful *et al*., 2010), then the samples are observed to identify the response that the bacteria showed towards antibiotics are being tested.

Preparation of the bacterial suspension

The bacterial suspension is prepared by transferred one full lobe of the isolated bacteria grown on the solid AM medium to flask containing 20 ml of liquid AM medium, and incubated in the shaker incubator at darkness, at 28°C for 24 and 48 hours and rotation speed of 120 cycle min^{-1}. Then the bacterial suspension are harvested in a refrigerated centrifuge for 10 minutes at 600 rpm^{-1} , the filtrate is excluded and one ml of liquid AM medium is add to precipitated bacteria to obtain the bacterial inoculum .

Identification of isolated bacteria by 16s rRNA gene amplification

Genomic DNA of isolated bacteria is extracted using a genomic DNA purification kit (Geneaid, Korea) according to the manufacturer's instructionsNanodrop (spectrophotometer, Biodrop, England) is used to quantify its concentration at the two wavelengths of 280 and 206 nm. (Dhahi *et al*., 2011). Separated DNA samples are electrophoresed in a 1% agarose gel using Safe Red stain (Safe Red stain Dye, Korea) while being shocked by electricity at 80 volts for an hour. (Wang *et al.,* 1996).

Molecular identification of the bacteria

PCR amplification of the 16s rRNA gene universal primers :

27F: AGAGTTTGATCMTGGCTCAG

1522R: AAGGAGGTGATCCARCCGCA

PCR mixture, amplification condition and PCR products sequencing are conducted according to (Wang *et al*.,1996). Safe red stain is used to visualize the amplification products on a 1.0% w/v agarose gel.

Determination of nucleotide sequencing of amplified pieces using DNA sequencing technique

The sequence of the nitrogenous bases of the amplified DNA is determined by sent the PCR products with 16S rRNA gene primers to read the sequence of the gene using the 3130 Genetic Analyzer device (supplied by the Japanese company Hitachi) located in the city of Erbil. BLAST software is used to examine the results after the gene-specific sequences are compared to those listed in the National Center for Biotechnology Information (NCBI).

Surface sterilization of Broccoli (*Brassica oleracea* var.italica) seed and production of the seedlings.

Broccoli seeds are surface sterilized by immersing them in 70% ethyl Alcohol solution for two minutes, followed by immersion in 2% NaOCl solution for five minutes. The seeds are ished with sterile distilled water 3-4 times/min conditions (Sultan and Mohammed, 2020), dried by used sterile filter papers, then planted on MS (Murashige and Skoog, 1962) and incubated at 24 ± 2 C° in dark condition and transfer when its germination after two days to light condition with 16 hours daily.

Inoculation by direct injection technique of Broccoli hypocotyl seedlings with bacteria isolates

Broccoli seedling with age of 15 day isolated and inoculated at two different locations by direct injection using a minute needle (Insulin syringe) a sterile end submerged separately in the bacterial culture for 24 and 48 hours (Al-Mallah and Mohammed, 2012). Samples are instilled upright in 30 ml of MS solid medium (at the rate of 2-3 pieces each). For the purpose of comparison, the same plant part are inoculated with sterile distilled water. All of the aforementioned samples are maintained in the growth chamber at a temperature of 24 ± 2 °C and 400 lux of light.

Results and Discussion

Cultural characters of isolated bacteria

The colonies isolate bacteria which grow on solid medium appeared to have sticky appearance, showing production of mucus substance. After two days of incubated (Figure, 2). This result similar to (Singh *et al*., 2008) report which characters of colonies of bacteria.

Figure (2): Isolated bacteria from the myrtle stem tumors grown on the AM medium after 48 hour of incubation

Biochemical characters of isolated bacteria

The results of biochemical tests of isolated bacteria showed **positive** for the catalase test, as indicated by the formed gas bubbles on the glass slide used in the examination after adding young colonies of them to a 3% solution of hydrogen peroxide H_2O_2, which indicates its ability to produce the enzyme catalase that reduces H_2O_2 and releases oxygen . **Positive** for the oxidase test, as the appearance of the violet color indicates the ability of the bacteria to produce the enzyme Cytochrome Oxidase after transferring a young colony of bacteria to the surface of a filter paper saturated with the oxidase reagent . Although the Gram's staining result is negative (Figure , 3).

Figure (3) : Isolated bacteria under the light microscope stained with Gram stain

Resistance and sensitivity to antibiotics

The outcomes of isolated bacteria cultured on solid AM media containing various antibiotic types demonstrated the bacterium's capacity to withstand to Metronidazole 30 µg ml $^{-1}$ and Ampicilin sulbactum 10 µg ml $^{-1}$ (Figure, 4), whlie sensitive to Piperacillin30µg ml $^{-1}$, Metronidazole 30 µg ml $^{-1}$, Tetracycline 10 µg ml $^{-1}$, Gentamicin 10 µg ml^{-1} , Ticarcillinclavunnic acid 10 µg ml $^{-1}$, Ceftriaxone 10 µg ml $^{-1}$and Levofloxacin 5 µg ml $^{-1}$ (Table, 2) .

Table (2) : Antibiotic test sensitivity and resistance

Antibiotic	Final concentration (µg ml -1)	Resistance
Metronidazole	30	R
Ampiclin sulbactum	10	R
Piperacillin	30	S
Metronidazole	30	S
Tetracycline	10	S
Ticarcillin Clavunnic acid	10	S
Ceftriaxone	10	S
Levofloxacin	5	S

Figure (4): Antibiotic test sensitivity and resistance

The ability of bacteria to adapt to survive in the presence of numerous antibiotics produced by fungi and actinomycins in the soil may be the cause of their antibiotic resistance. This adaptation encouraged the bacteria to develop specialized self-mechanisms to sustain their growth and reproduction, which in turn increased their resistance to antibiotics. (Zhang *et al.*, 2018). The process of halting the inhibition of the protein these antibiotics stimulate inside the bacterial cell may be explained by the sensitivity of bacteria to other antibiotics. (Cochrane and Lohans, 2020) .

Molecular analysis of microorganisms isolated from Myrtle stem tumors using the PCR method

Which divided to:

A. Concentration and purity of chromosomal DNA:

According to the findings, the extracted chromosomal DNA had a concentration of 321.9 ng µl-1 and a purity of 1.8.. UV rays of the gel piece reveals that the chromosomal DNA is electrophorese. The molecular weight is large in terms of its proximity to the agarose gel pits in which the sample is placed before migration, and it is pure in terms of its uninterrupted (Figure, 5).

Figure (5) Electrophoresis of the chromosomal DNA isolated in 1.0% agarose.

Polymerase chain reaction technique specialized in sequencing the chromosomal DNA using the 16s rRNA gene

When the chromosomal DNA amplified product is electrophoresed in a 1% agarose gel using the 16s rRNA gene specific primer, the results revealed the separation of a single band with a molecular weight of 1495 bp (Figure 6), which is comparable to the molecular weight of the particular primer used in this investigation.

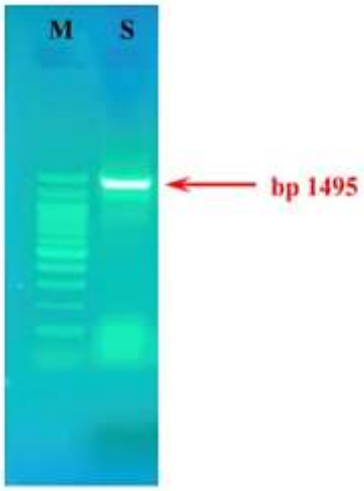

Figure (6): DNA extracted from the investigated bacteria and amplified the 16s rRNA gene by PCR is electrophoresed in 1.0% agarose. (M= Ladder , S= Sample)

The appearance of the band with this molecular weight corresponds to the value of identical sequences in the sequence of nitrogenous bases present in the template chromosomal DNA, which can be duplicated when using its primer (Bustin , 2002).

The findings of the DNA Blast program's analysis of the nucleotide sequence of the 16S rRNA gene revealed a 96.73% similarity between this sequence and the sequences of the Pantoea sp. genus that are registered with the NCBI Gene Bank. The study is able to register it

as a new strain for the first time and is given the name ***Pantoea* sp. RAZ** after transmitting the results of the nitrogenous base sequence analysis to the NCBI gene bank. (Figure, 7). From the potential to identify genetic differences among the isolates under examination using the researcher's method of examining nitrogenous base sequences (Grison *et al.*, 2015) Using DNA sequencing methods for the 16S rRNA region, the researchers isolated 56 rhizobia bacterial isolates from the leguminous host *Anthyllis vulneraria* in southern France.

Pantoea sp. strain RAZ 16S ribosomal RNA gene, partial sequence

GenBank: OQ300077.1

FASTA Graphics

Go to:

```
LOCUS       OQ300077                906 bp    DNA     linear   BCT 27-JAN-
2023
DEFINITION  Pantoea sp. strain RAZ 16S ribosomal RNA gene, partial
sequence.
ACCESSION   OQ300077
VERSION     OQ300077.1
KEYWORDS    .
SOURCE      Pantoea sp.
  ORGANISM  Pantoea sp.
            Bacteria; Proteobacteria; Gammaproteobacteria;
Enterobacterales;
            Erwiniaceae; Pantoea.
REFERENCE   1  (bases 1 to 906)
  AUTHORS   Fathel,R.M., Mohammed,A.A. and Qaddawi,Z.T.
  TITLE     Direct Submission
  JOURNAL   Submitted (22-JAN-2023) Sciences, University of Mosul, Al
Majmoaa,
            Mosul, Mosul 43, Iraq
COMMENT     ##Assembly-Data-START##
            Sequencing Technology :: Sanger dideoxy sequencing
            ##Assembly-Data-END##
FEATURES             Location/Qualifiers
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                     /mol_type="genomic DNA"
                     /strain="RAZ"
                     /host="Myrtus"
                     /db_xref="taxon:69393"
     rRNA            <1..>906
                     /product="16S ribosomal RNA"
ORIGIN
        1 tctgcgggta gcacaggaga gcttcgctct ttgcggtgac gagttggcgg acgggtgagt
       61 aatgtctggg aaactgcccg atggaggggg ataactactg gaaacggtag ctaataccgc
      121 ataacgtcgc aagaccaaag tgggggacct tcgggcctca caccatcgga tgtgcccaga
      181 tgggattagc tagtaggcgg ggtaatggcc cacctaggcg acgatcccta gctggtctga
      241 gaggatgacc agccacactg gaactgagac acggtccaga ctcctacggg aggcagcagt
      301 ggggaatatt gcacaatggg cgcaagcctg atgcagccat gccgcgtgta tgaagaaggc
      361 cttcgggttg taaagtactt tcagcgggga ggaaggcgat gcggttaata accgcgtcga
      421 ttgacgttac ccgcagaaga agcaccggct aactccgtgc cagcagccgc ggtaatacgg
      481 agggtgcaag cgttaatcgg aattactggg cgtaaagcgc acgcaggcgg tctgttaagt
      541 cagatgtgaa atccccgggc tcaacccggg aactgcattt gaaactggca ggcttgagtc
      601 tcgtagaggg gggtagaatt ccaggtgtag cggtgaaatg cgtagagatc tggaggaata
      661 ccggtggcga angcggcccc ctggacgaag actgacgctc aggtgcgaaa gcgtgggagc
      721 aaacaggatt anataccctg tagtccacgc ctaacgatgt cgacttggag gttgtgccct
      781 tgagcgtggc ttccggactc acgcgttaag tcacgcctgg ggagtacggc cgcgaaactc
      841 aatgatgacg ggggccccac agcgtgagca tggtatattc agcacgcgag cttactactc
      901 tcgtca
```

Figure (7): Register the novel Pantoea sp. RAZ in NCBI

Tumor formation on the stems of broccoli (*Brassica oleracea* Var. italica) seedlings caused by a changed stem caused by the bacteria *Pantoea* sp. RAZ

The results indicate that the ability of bacteria to stimulate the initiation of tumors on the stems of broccoli seedlings (Table, 3).

Bacteria suspension with 24 hours in induced the tumors production with 70% after 21 days, and the average number is 2, its shape is green oval on the stems (Figure, 8- B), while the percentage of its formation after inoculation with the bacterial inoculum 48 hours reached 50%, with an average of 1.6 after 28 days (Figure, 8- C). Compared with control seedlings (un inoculated) that did not form any knots on their stems (Figure. 8-A).

Table (3): The development of tumors on the stems of broccoli (Brassica oleracea Var. italica) seedlings due to the transformation of the stem by the bacteria Pantoea sp. RAZ

Suspension age of Pantoea sp. RAZ (hour)	No. of Inoculated / Responsive	Tumor production (%)	Average of initiated tumor/stem
24	20/14	70	2
48	20/10	50	1.6
D. water (Control)	20/0	0	0

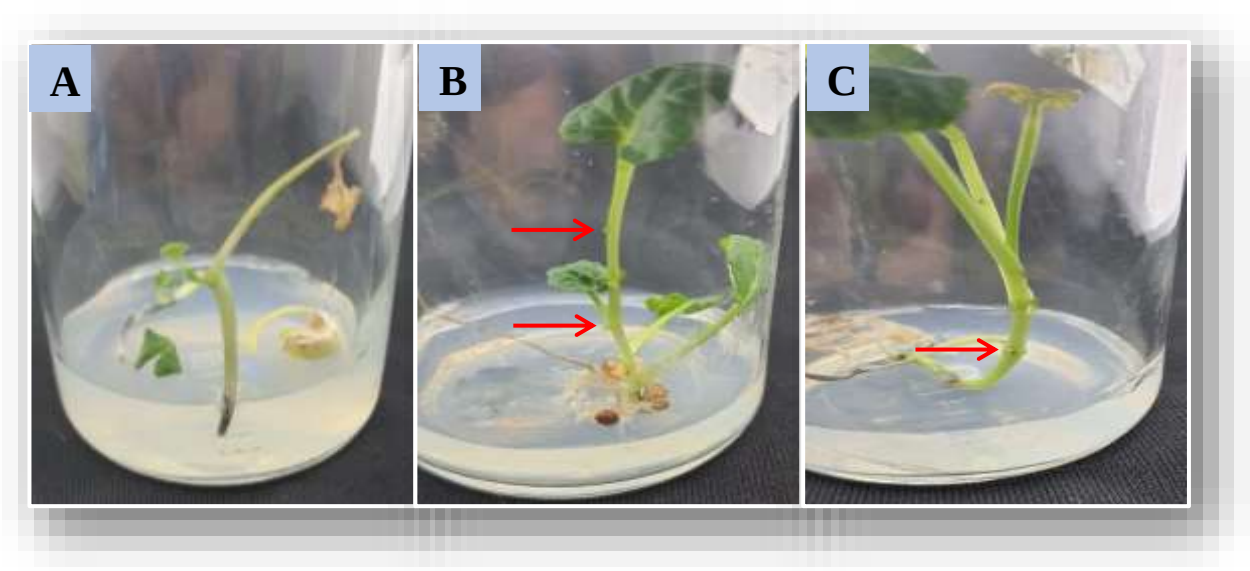

Figure (8): Growth of tumors on the stems of broccoli (Brassica oleracea Var. italica) seedlings injected with the bacteria *Pantoea* sp. RAZ

A- Control

B- Tumor formed on stem inoculated with *Pantoea* sp. ZAZ Suspension with 24 hour

C- Tumor formed on stem inoculated with *Pantoea* sp. RAZ Suspension with 48 hours.

By adopting a plasmid-borne pathogenicity island, *Pantoea* agglomerans has changed from a commensals bacterium linked to numerous plants to a host-specific gall-forming pathogen, Along with genes for type III effector proteins, the manufacture of the phytohormones cytokinin and indole-3-acetic acid, numerous distinct insertion sequences, and pseudogenes, this pathogenicity island is home to the hrp/hrc gene cluster. (Barash and Manulis- Sasson, 2007).

A hormonal imbalance that causes an increase in the quantity (hyperplasia) and size (hypertrophy) of cells is what defines the formation of a plant tumor. Generally speaking, IAA and CKs released by the pathogen during infection are responsible for gall induction caused by phytopathogenic bacteria. (Morris *et al.*,1986).

A unique framework for comprehending the appearance of novel plant diseases or variations of pre-existing ones is offered by Pag and Pab.. We postulate that they might have acquired the pathogenicity traits not through slow adaptive evolution but rather through ‘quantum leaps’ that enabled them to transfer complete virulence systems in the course of one or a few steps (Mecsas *et al*.,1996).

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Susceptibility of UTI Pathogens Against Copper Oxide Nanoparticles Biosynthesized By Pantoea and E. Coli Mixture

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Khamael Lutfi Shakir [3]

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Abstract

In the recent years, many of antibiotics loosed their ability to treat some bacteria as a result to the development of resistance. The study's goals are to use a mixture of extract *E. coli* and *Pantoea* to prepare the Copper oxide nanoparticles (CuO-NPs) and their use as antibacterial agents against multidrug-resistant *Enterococcus faecalis*, *Staphylococcus aureus*, *Acinetobacter baumanii*, and *Pseudomonas aeruginosa* that are obtained from MSc students in the biology department at Baghdad University, which are isolated by students from various clinical sources. CuO NPs are biosynthesized using 1 gram of copper chloride, which is added to 10 mL of the mixed supernatant of *E. coli* and *Pantoea.* The CuO NPs biosynthesized are identified using techniques such as UV-VIS, AFM, and FESEM. The result showed that the wavelength of CuO NPs is 284nm, the average diameter of CuO NPs is 52.09 nm, and the FE-SEM image displays a Spherical nano-cluster and a range of sizes from 40 to 60 nm. Four dilutions of CuO NPs are used (1/20, 1/40, 1/80, and 1/160) to determine antibacterial effects. *Pseudomonas aeruginosa* seems more sensitive than others, while *Acinetobacter baumanii* is more resistant.

Key Words: *Biosynthesized, Copper Oxide Nanoparticles (Cuo Nps), Mixed Extract e. Coli and Pantoea, Enterococcus Faecalis, Staphylococcus Aureus, Acinetobacter Baumanii, and Pseudomonas Aeruginosa.*

http://dx.doi.org/10.47832/MinarCongress15-6

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Introduction

The distribution of multi-resistant microorganisms to antibiotics is leading to a general health issue around the globe. These elevate the demand for increasing antibiotic doses, which cause toxic effects. Alternative therapies for microbial infection are researched, and special nanomaterials are used as novel factors with antimicrobial effects. Nanoparticles possess several distinctive chemical and physical properties, generally attributable to their proportionality between volume and surface area. [1–3].

Copper oxide nanoparticles represent promising candidates depending on their antimicrobial traits. The copper antimicrobial traits have been known for centuries against humans. One of the uses is the sterilization of water by copper sulfate and infection treatment as early as 2400 BC [4,5].

Recently, CuO NPs seem to have generated attention to their distinctive diamagnetic, electrical, biological, photocatalytic, and optical properties among other nanoparticles [6].

Bacteria that resist most antibiotics are prevalent strains, leading to a rise in mortality and morbidity around the world. This has enhanced the creation of antimicrobial compound with novel properties, including nanoparticles (NPs)[7].

Cooper oxide nanoparticles are observed to be toxic to E. coli, which disrupts membranes by the ROS production (Reactive Oxygen Species) that damages bacteria. It has antimicrobial factors that inhibit the growth of bacteria that have multidrug resistance and inhibit biofilms [8].

There are several methods for CuO NPs preparation, including chemical and physical procedures. The different methods employed for the synthesis of CuO nanoparticles enable the production with different shape and sizes [9].

The benefits of biological properties, such as bacteria, fungi, plants, and their extracts, for CuO NPs synthesis represent an interesting area for researchers [10].

Green Methods” mean using biological organisms or their extract or system for NPs manufacturing. Green methods” are now increasingly favored for providing a cost-effective and sustainable alternative by decreasing pollutant generation during the production of CuO NPs [8].

Researchers have mentioned that most of the articles prepared CuO NPs using methods that depend on biological organisms. The most abundant used method is “green synthesis” by utilizing plant extracts. Many methods also employed bacterial and fungal cultures, as well as products of their extracts and extracellular matrix [11].

Studies have mentioned a more pronounced CuO NPs antibacterial effect synthesized by the “green pathway.” Sabeena et al. reported a more pronounced CuO NPs cytotoxic effect against MCF-7 cancer cells [12].

Material and method

Bacterial Sample collection

1.1 Bacteria used for nanoparticle formation:

Escherichia coli and Pantoea are obtained from the clinical laboratory of Al-Yarmook Hospital/Baghdad isolated from urinary tract infection cases, and diagnosed by VITEK -2 Compact system

1.2 Test Isolates:

Four isolates from urinary tract infection cases are used as test isolates obtained from the biology department of the College of Science, Baghdad University. The isolates belonged to Gram-positive (Staphylococcus aureus and Enterococcus faecalis) and to Gram-negative (Acinetobacter baumanii and Pseudomonas aeruginosa)

The isolates are reidentified by being cultivated on Nutrient agar, MUG Nutrient agar, MacConkey Agar, EMB agar, and Blood agar and then incubated at 37°C for 24 h. After that, bacteria are identified using the Gram stain method and microscopic examination. Biochemical tests, including catalase, indole, methyl red, and lactose fermentation, are conducted to continue identification. Moreover, isolate identification is confirmed by the VITEK 2 compact system.

Antibiotic resistance test

The antibiotic resistance test for isolates is performed by the VITEK-2 compact system to confirm that the isolates are multidrug resistant. The Antibiotic Susceptibility Card Types used for Enterococcus faecalis are AST—P516 and AST—P515 for Staphylococcus aureus, while AST—N010 is used for Gram-negative bacteria (Acinetobacter baumanii and Pseudomonas aeruginosa).

Biosynthesis of Copper oxide Nanoparticles (CuO NPs) using a mixture of E. coli and Pantoea supernatants

The first step in nanoparticles preparation is preparing eight flasks (4 for E. coli and 4 for Pantoea); the first flask for each bacterium is incubated for 24, 48, 72, and 96 hr. Each flask contains 150 ml of nutrient broth. The supernatant of two bacteria (300 ml) is mixed, and then 30 g of copper chloride is added. The flask is placed intermittently in an Ultrasonic Bath (Sonicator) for 10 minutes (at interval periods) and then for 24 hours in the shaker in a dark condition. After 24 hours of shaking, the solution in the flask is poured into plain tubes, about 5 ml in each tube; the tubes are placed in a centrifuge at 5000g for 15 minutes, and ished with absolute ethanol; this is repeated twice. The solution is placed in a petri dish and left to dry in an incubator [13]. The prepared nanoparticles underwent several tests to check their characteristics.

Characterization of Copper oxide NPs

Cobalt oxide Nanoparticles are characterized by several techniques like UV-Visible, FTIR, AFM, and FESEM. UV-Visible is done by using (Shimadzu UV-1800- Vis Spectrophotometer, AFM (Atomic force microscopy) is used to find out the average diameter of Copper oxide Nanoparticles.

Study CuO nanoparticles' antibacterial effect on UTI pathogens

Stock solution of CuO NPs prepared by suspending 500 mg of the prepared nanoparticles in 5 mL of D.D.W (100mg/mL). Then Serial dilutions are prepared from CuO NPs stock solution (1/20, 1/40, 1/80, and 1/160), and 100µl from each dilution of suspended nanoparticles are added to the wells in media (Muller Hinton agar) inoculated by spreading method with test isolates then determine its antibacterial effect by well diffusion method that appears as clear zone around the well. Each test is done in triplicate to have more accurate values.

Results and discussion

Escherichia coli and Pantoea detection confirmation

Confirmatory tests are used for Escherichia coli and Pantoea obtained from the clinical laboratory of Al-Yarmook Hospital/Baghdad, isolates identification based on the cultural characteristics of three different selective and differential media for E. coli. Isolates appeared on MacConkey agar as pink colonies, on EMB medium, as green metallic sheen, and on

MUG Nutrient agar as fluorescence colonies under the UV illuminator system; Pantoea is cultured in the same culture media used for E. coli, it appeared as pink colonies on MacConkey agar, in EMB it appears as pale to brownish colonies without green metallic sheen, MUG nutrient agar gave the fluorescence colonies properties under the UV illuminator for E.coli only, so Pantoea grow without any fluorescence under U.V. illuminator system as shown in Fig.1

A B

Figure 1: Preliminary identification of bacteria. A: E. coli, B:Pantoea

CuO Nanoparticle preparation

After the incubation period (24, 48, 72, and 96)hr. for E. coli and Pantoea, the supernatant for both is obtained by centrifugation (5000g, 10 min); the volume of each is 150 ml; the two supernatants are collected together and then mixed with 30 g $CuCl_2$ for 24 hr. with agitation at room temperature in dark condition. The supernatant is centrifuged at 5000g for 15 min, then the precipitate is collected and ished with ethanol 2 times and then dried in an incubator at 40 °C. The dried precipitate yield that is obtained increased with the incubation period (Table 1).

Table 1- CuO nanoparticles obtained in different incubation periods

Incubation period (hr)	CuO nanoparticle (gm)
24	0.875
48	1.232
72	1.68
96	1.886

Characterization of CuO Nanoparticles

UV-visible spectroscopy

CuO NPs Synthesis using the extract of E. coli and Pantoea is characterized by scanning a UV-Visible spectrophotometer (Shimadzu, Japan) in Figure 2 to detect the maximum absorption. The result showed that the absorbance of CuO nanoparticles shows an absorption peak between 200 and 300 nm, i.e., 284 nm, which can be attributed to the characteristic absorption of CuO NPs**.** [14].

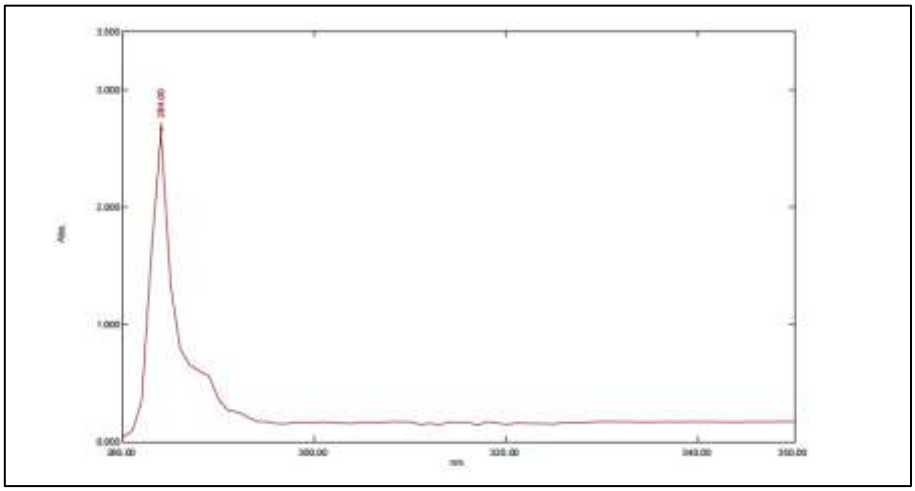

Figure 2: The UV-VIS of CuO NPs

Atomic force microscopy (AFM):

The surface shape formation of the CuO NPs is studied by atomic force microscopy to show that CuO NPs have 2D and 3D. (Figure 3). AFM images show that the biosynthesized CuO NPs had an average diameter of 52.09 nm (Table 2), which is also measured by AFM. This result agrees with [15].

Table 2: Average diameter of CuO nanoparticle

Avg. Diameter:52.09 nm	**<=10% Diameter:10.00 nm**
<=50% Diameter:35.00 nm	**<=90% Diameter:55.00 nm**

Field emission scanning electron microscopy (FE-SEM) analysis:

The properties (morphological) of the biosynthesized CuO nanoparticles are tested using the FE-SEM technique. As shown in Figure 4, the prepared CuO nanoparticles sample exhibited particles that have a spherical shape and plate-like structures. It is worth mentioning that the average diameter of the copper oxide nanoparticles ranges in size from 40 to 60 nm using Image J software. Selvaraj revealed the monoclinic crystalline structure of the prepared CuO samples. CuO samples appeared as spindle-shaped with a size distribution ranging from 70 nm to 90 nm.[16].

Figure 3. Atomic force microscopy (AFM) of CuO NPs biosynthesized, 2D and 3D topology

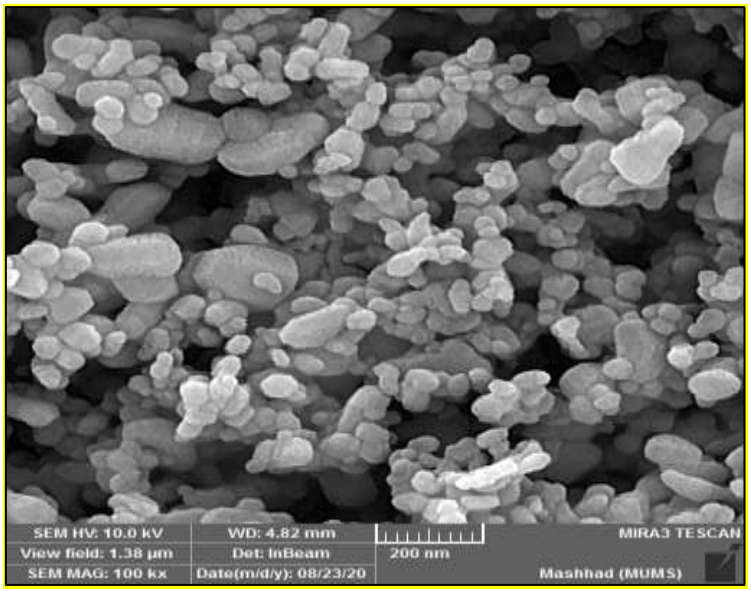

Figure 4 FE-SEM image of the bio-synthesized CuO nanoparticles

Test isolates

Results of preliminary culturing for isolates agreed then with the identification result of the VITEK 2 compact system. Staphylococcus aureus appears as golden colonies on the Mannitol salt agar medium that differentiate it from other Staphylococcus species. Enterococcus faecalis form smooth, nontransparent, white or creamy, spherical colonies on common nutrient agar plates. Pseudomonas aeruginosa is characterized by pyocyanin production in media; Acinetobacter baumanii is detected primarily by CHROMagar^{TM}Acinetobacter that appeared as red convex colony instead of blue colony for other Gram-negative bacteria (figure 5)

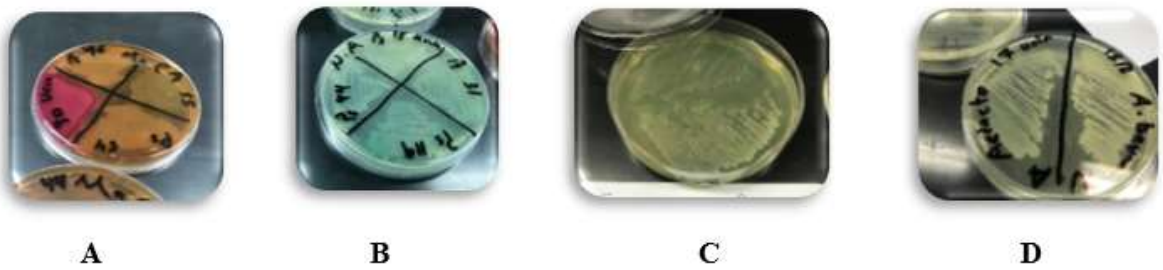

Figure 5: Test isolates on different media. A: Staphylococcus aureus, B: Pseudomonas aeruginosa, C: Enterococcus faecalis, D: Acinetobacter baumanii

Antibacterial effect of prepared CuO nanoparticle

The WHO declared the resistance of some bacterial isolates a public health crisis, making it a life-threatening modality to treat the increasing burden of disease [17]

Multi-drug resistant isolates (Enterococcus faecalis, Staphylococcus aureus, Acinetobacter baumanii, and Pseudomonas aeruginosa) are determined according to antibiotic susceptibility test by VITEK-2 compact system (AST—P516, AST—P515 and AST—N010).

These resistant isolates are used to evaluate the antibacterial activity of CuO nanoparticles. The results showed (Table 3) that Pseudomonas aeruginosa is more susceptible to CuO nanoparticles with the highest concentration (1/20), with an inhibition zone average of 28 mm than Staphylococcus aureus (23 mm), Enterococcus faecalis (19 mm), and Acinetobacter baumanii (16 mm).

Table 3: Inhibition of CuO nanoparticle dilutions on different test isolates

	Inhibition of CuO nanoparticle dilutions (mm)															
Test isolates	**1/20**			**mean**	**1/40**			**mean**	**1/80**			**mean**	**1/160**			**mean**
Enterococcus faecalis	22	20	20	21	19	19	18	19	11	12	12	12	10	10	12	11
Pseudomonas aeruginosa	29	27	28	28	22	22	23	22	17	17	17	17	13	13	14	13
Staphylococcus aureus	23	23	23	23	20	19	19	19	18	19	18	18	12	12	13	12
Acinetobacter baumanii	15	17	16	16	12	15	14	14	10	12	12	11	0	1	0	0

In the dilution 1/40, Pseudomonas aeruginosa is more susceptible to (22 mm) CuO nanoparticles than others . In this dilution, Enterococcus faecalis is more susceptible (19 mm) than Staphylococcus aureus (19 mm), while Acinetobacter baumanii is still more resistant than others (14 mm).

In the dilution 1/80, Staphylococcus aureus (18 mm) is more susceptible to CuO nanoparticles than Pseudomonas aeruginosa (17 mm) and more than Enterococcus faecalis (12 mm) and Acinetobacter baumanii (11 mm).

At the highest dilution (1/160), a slight effect is seen in three of four test isolates, and they are susceptible to CuO nanoparticles: Pseudomonas aeruginosa (13 mm), Staphylococcus aureus (12 mm), and Enterococcus faecalis (11 mm). However, with Acinetobacter baumanii, no inhibition zone is determined in the 1/160 dilution (Figure 6).

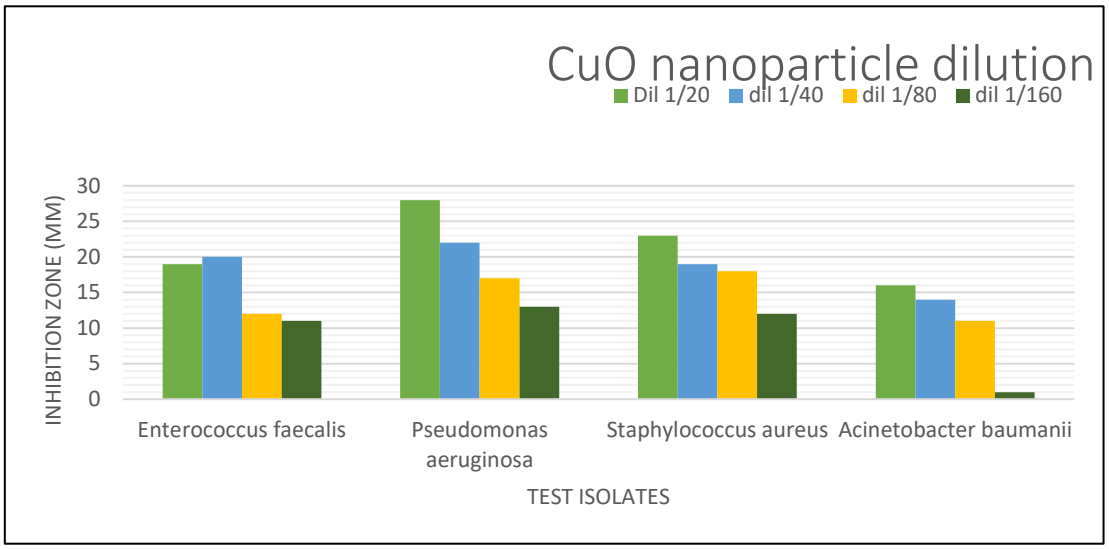

Figure 6: Effect of different CuO nanoparticles on some UTI pathogens

The effect of CuO nanoparticles on Enterococcus faecalis seems to be divided into two categories: the first one is with dilutions 1/20 and 1/40, which have closely related inhibition zones (19 mm and 20 mm, respectively); the second one drops with dilutions 1/80 and 1/160, which also have closely related inhibition zones (12 mm and 11 mm, respectively).

Pseudomonas aeruginosa is affected by CuO nanoparticles. Gradually, the effect decreases with the increased dilution.

The effect on Staphylococcus aureus seems closely related in the highest three concentrations, 1/20, 1/40, and 1/80 (23 mm, 19 mm, and 18 mm, respectively), and dropped in the dilution 1/160 (12 mm).

Acinetobacter baumanii showed that as the highest resistance among test isolates, the dilution 1/20 led to an inhibition zone closely related to the inhibition zones at dilution 1/160 for other test bacteria. Furthermore, the dilution of 1/160 has no effect.

Khairy and Coworkers mentioned that the green synthesized copper oxide nanoparticles have an antibacterial effect against some bacteria, including methicillin-resistant Staphylococcus aureus (MRSA), Escherichia coli, Pseudomonas aeruginosa, Acinetobacter spp., Klebsiella pneumoniae, and Stenotrophomonas maltophilia.

The minimum inhibitory concentration (MIC) of CuO NPs ranged from 62.5 to 125 µg/ml [18]

While Rand mentioned that the antimicrobial activity of CuO NPs showed decreases in bacterial growth of pathogenic K. pneumonia (0.52 ± 0.04 cell/ml) than control (1.60 ± 0.01 cell/ml). As S. aureus appeared, the number of bacterial growth is also affected, (0.79 ± 0.07 cell/ml) compared with the control (1.90 ± 0.01 cell/ml). CuO NPs enhancing antimicrobial activity, the percentage of resistant is decreasing from 66.6% to 22.2% the same results appeared with S. aureus sensitivity test showed resistant percentage is decreased from 55.5% to 33.3% at 24 hours [19]

Colnclusions

From the results, it can be concluded that the prepared nanoparticle is more effective against gram-positive bacteria than gram-negative bacteria. Acinetobacter appeared as the most resistant bacteria.

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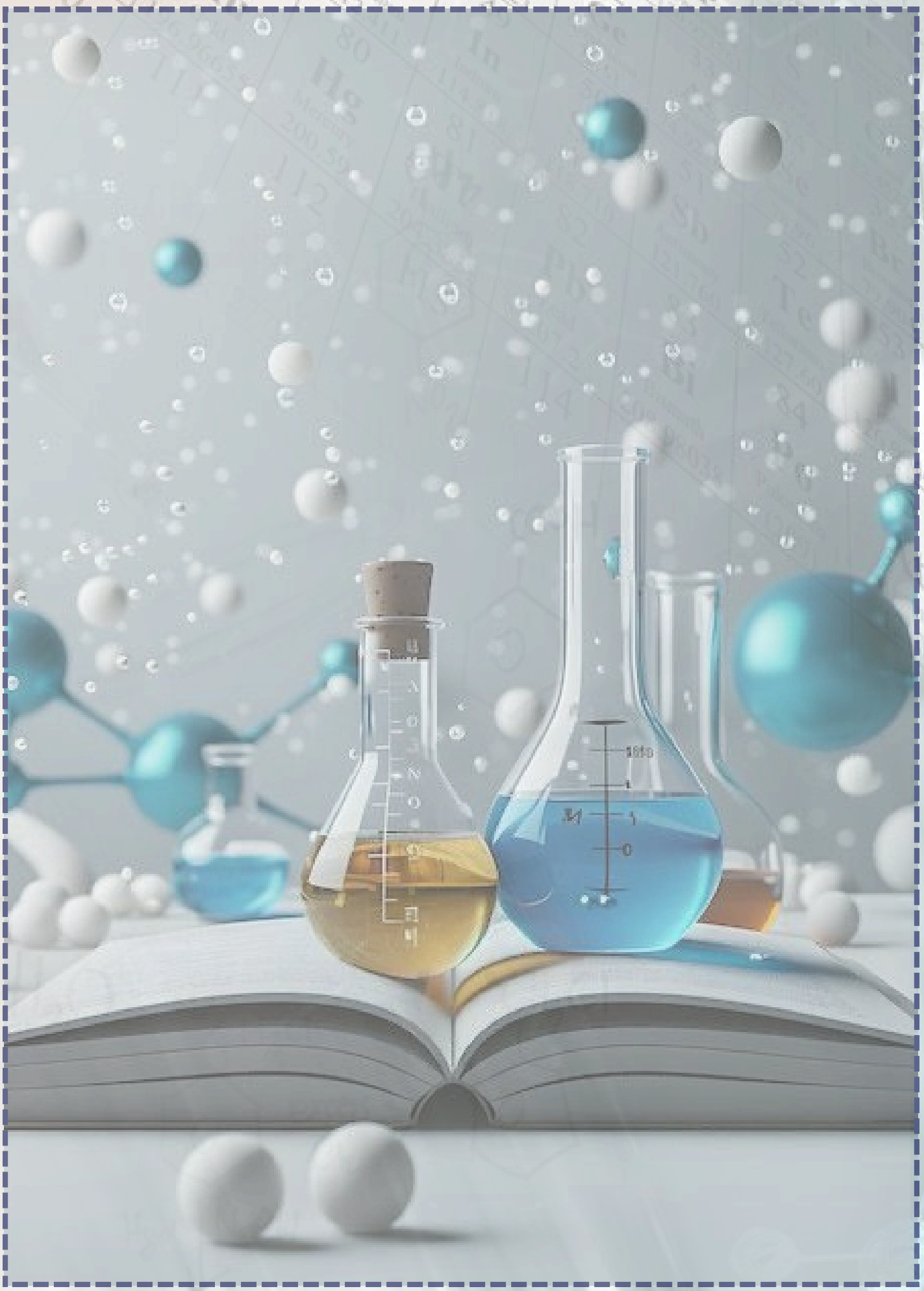

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XV. International Scientific Congress of Pure, Applied and Technological Sciences

ISBN 978-625937770-4

Rimar Academy
Publishing House