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Identification of *Klebsiella* Species From Pregnant Women Suffered From Urinary Tract Infection in Thi-Qar Province.

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Abstract

Urinary tract infections (UTIs) are among the most common bacterial infections in pregnant women and are often caused by opportunistic uropathogens such as *Klebsiella pneumoniae*. This study aimed to isolate and identify uropathogenic *K. pneumoniae* from pregnant women with UTIs in Thi-Qar Province, Iraq, and to determine their antibiotic susceptibility patterns. A total of 22.72% of the isolates were identified as *K. pneumoniae* using the VITEK 2 automated system. The highest infection rate was recorded among women aged 18–27 years-old . Notably, the third trimester showed the highest prevalence of infection (50%). Urban residents exhibited a higher rate of *K. pneumoniae* isolation compared to rural residents. Regarding antibiotic susceptibility, high resistance rates were observed against Amoxicillin (50%) and Fosfomycin (40%), while strong sensitivity was recorded for Gentamicin (100%), Amikacin (90%), and Imipenem (80%).These findings highlight the importance of early detection using reliable automated systems such as VITEK 2 and support the need for proper antibiotic selection to manage UTIs effectively and reduce the spread of resistant strains.

Keywords:***Klebsiella pneumoniae*, urinary tract infection, pregnant women, VITEK 2, antibiotic resistance**.

الملخص

تعد التهابات المسالك البولية (UTIs) من أكثر أنواع العدوى البكتيرية شيوعًا لدى النساء الحوامل، وغالبًا ما تُسببها مسببات أمراضية انتهازية مثل *Klebsiella pneumoniae*. هدفت هذه الدراسة إلى عزل وتشخيص *K. pneumoniae* الممرضة للمسالك البولية من نساء حوامل مصابات بعدوى المسالك البولية في محافظة ذي قار، العراق، وتحديد نمط مقاومتها للمضادات الحيوية. تم الكشف عن *K. pneumoniae* في 22.72% من العينات باستخدام نظام VITEK 2 الآلي. سُجلت أعلى نسبة إصابة بين النساء في الفئة العمرية 18–27 سنة، كما لوحظ أن الثلث الثالث من الحمل كان الأعلى من حيث معدل الإصابة (50%). أظهرت النساء المقيمات في المناطق الحضرية معدل عزل أعلى للبكتيريا مقارنةً بنظيراتهن في المناطق الريفية. أما فيما يخص مقاومة المضادات الحيوية، فقد سُجلت معدلات مقاومة عالية ضد الأموكسيسيلين (50%) والفوسفومايسين (40%)، في حين ظهرت حساسية عالية تجاه الجنتامايسين (100%)، والأميكاسين (90%)، والإيميبينيم (80%). تؤكد هذه النتائج على أهمية التشخيص المبكر باستخدام أنظمة آلية موثوقة مثل VITEK 2، والحاجة إلى اختيار المضادات الحيوية المناسبة للحد من انتشار السلالات المقاومة وتحقيق فعالية علاجية أعلى.

الكلمات المفتاحية: **الكلبسيلا الرئوية، عدوى المسالك البولية، النساء الحوامل، جهاز VITEK 2، مقاومة المضادات الحيوية**

Introduction

Urinary tract infections (UTIs) are a highly prevalent condition that can affect men and women of all ages [1]. Women are more likely than males to suffer from (UTIs). The majority of female urinary tract infections are asymptomatic. Although over 60% of women get UTIs at some point in their life, 10% of women may experience UTIs annually [2]. Bacteria, fungi, yeasts, and viruses are some of the microorganisms that can cause urinary tract infections, the most common bacterial infection in pregnancy, and have been estimated to affect 2% to 15% of the pregnant population [3,4]. Bacteria that belong to the Enterobacteriaceae family are found in the

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digestive tracts of both humans and animals. This heterologous family includes Echerichia coli, Salmonella, Shigella, Enterobacter, klebsiella and proteous spp. Echerichia coli is the cause of 85-90 of urinary tract infections [5]. Infections produced by K. pneumoniae are more common than those caused by other Gram-negative opportunistic pathogens for a number of reasons, including bacteria's ability to tolerate starvation [6]. The rod-shaped, non-motile, Gram-negative bacteria in the Klebsiella genus usually express two antigens on their cell surface capsular polysaccharide (K antigen) and lipopolysaccharide (O antigen) [7, 8]. It has a width of (0.3-2) µm and a length of (0.6-6) µm. Because it is a facultative anaerobe, *K. pneumoniae* can survive in both anaerobic (without oxygen) and aerobic (with oxygen) conditions. It ferments lactose, is catalase-positive, and is cytochrome oxidase-negative biochemically [9].

The optimum temperature for the growth of Gram-negative bacteria with a conspicuous polysaccharide capsule that encases the whole cell surface and is rich in glucuronic and pyruvic acids was 12-43°C, with the optimum temperature being 37°C [10].

K. pneumoniae is divided into two important phenotypes hypervirulent *K. pneumoniae* (hvKp) and classical *K. pneumoniae* (cKp) [11, 12]. *Klebsiella* includes several virulence features with other infections, including its production of enterotoxins such as lipopolysaccharide, adhesion factors, capsule antigens, and drug resistance.[13]. Siderophores, biofilm, and fimbriae are the virulence factors in hvKp [14, 15]. It can result in serious infections affecting multiple organs, involving the lungs, bloodstream, urinary tract, and wounds, risking patient safety, especially in susceptible groups like the elderly and people with weakened immune systems [1 6, 17].

Klebsiella pneumoniae related infections, skin and soft tissue infections, and isolates of *K. pneumoniae* can also result in endophthalmitis, kidney, lung, and neck abscesses, and severe skin and soft tissue infections (myositis, necrotizing fasciitis, and cellulitis) [18]. There are few studies about the role of *klebsiella pneumonia*e as causative agents for pregnant women UTI in Thi-Qar provanice therefor aim of this study to investigate this role: Isolation and identification of *Klebsiella* species from pregnancy women in Thi-Qar province by culturing, Morphological characteristics and confirmed diagnosis by VITEK II system and antimicrobial susceptibility test for main *Klebsiella* species isolated from pregnancy women**.**

Materials and method

Preparation of different media

Blood Agar Medium

Human blood was introduced into a blood agar medium that had been produced

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following the manufacturer instructions. The medium was then sterilized using an autoclave at atemperature of 121°C for duration of 15 minutes and then cooled down to 50°C [19].

MacConkey Agar Mediuma

For preparation MacConkey agar medium which is used to identify members of the *Enterobacteriaceae* family, 51.53 grams of its powder were dissolved in 1 liter of distilled water, autoclaving it for 15 minutes at 121°C, and then pouring it into sterile Petri dishes and letting it cool to room temperature [20]

Samples Collection

Between September 2024 and January 2025, a total of 150 urine samples were aseptically collected from pregnant women with clinically diagnosed urinary tract infections (UTIs) at various hospitals and private clinics in Thi-Qar province, including Al-Shatrah General Hospital, Bint Al-Huda Maternity and Children Hospital, and Al-Hussein Teaching Hospital. The patients' ages ranged from 18 to 45 years. Clinical histories and demographic data—such as name, age, and gestational month—were directly obtained from the patients and recorded systematically.

Isolation of *Klebsiella pneumoniae*.

Urine samples were directly streaked onto MacConkey agar and blood agar plates using a sterile platinum loop. The plates were incubated at 37°C for 24 hours. Pink, lactose-fermenting colonies indicative of *Klebsiella* species were selected and sub-cultured onto fresh MacConkey agar to obtain pure colonies via repeated streaking [21].

Gram stain method

Before being studied under oil immersion (100X), a small piece of a bacterial colony was spread out on a clean slide with a drop of regular saline, fixed with heat, and then stained with crystal violet, iodine, alcohol, and safranine [22].

Identification Using the VITEK II System.

Bacterial species identification and antimicrobial susceptibility testing were performed using the VITEK2 Compact system (bioMérieux, France), following the manufacturer's instructions and previously described methods [23,24]. From overnight cultures, bacterial colonies were suspended in 3.0 ml of sterile saline and adjusted to a turbidity of (0.5) McFarland using a DensiChek turbidity meter. The suspension was then inoculated into the GN-ID card and loaded into the VITEK 2 system chamber along with the specimen tubes.

statistical analysis

The data of the current study were statistically analyzed using SPSS (Statistical Package for the Social Sciences) version 25. Graphs were generated using Graph Pad

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Prism version 10. A significance level of 0.05 was used, and a p-value less than 0.05 was considered statistically significant.

Results and Discussion

The *Klebsiella pneumoniae* isolates in this study exhibited characteristic morphological features on culture media. On blood agar, the colonies appeared large, mucoid, white to light gray in color, and showed no hemolysis, indicating a non-hemolytic nature (Figur1). When cultured on MacConkey agar, the isolates formed large, pink, and mucoid colonies due to lactose fermentation and acid production. The mucoid appearance was attributed to capsular polysaccharide production, giving the colonies a shiny and sticky texture (Figure 2). These phenotypic features were consistent with previous studies [25,26]. A total of 44 Gram-negative bacterial isolates were recovered from pregnant women with urinary tract infections (UTIs). Among these, *Escherichia coli* was the most prevalent species (19/44, 43.18%), followed by *Klebsiella pneumoniae* (10/44, 22.73%) and *Pseudomonas aeruginosa* (4/44, 9%), while other bacterial species represented 2.27% each. Statistical analysis revealed significant differences in species distribution ($P<0.05$), suggesting a diverse profile of uropathogens (Figure 3).

These results are in agreement with findings from Wasit Province, Iraq [27], where *Klebsiella* spp. was also reported as the second most frequent isolate (22.07%). Similarly, the results correspond with a study from Edna Adan Hospital, Hargeisa, Somaliland [28], in which E. coli and *K. pneumoniae* accounted for 45.6% and 20.3%, respectively. Comparable results were also observed in Ankara [29], where *Klebsiella* species represented 20% of infections among pregnant women. However, the current findings differ from those reported in Bangladesh [30], where *E. coli* (60%) predominated, followed by *K. pneumoniae* (16.67%). While this study disagrees with the study reported in Baghdad by [31], they found *E. coli* (34%) and *Klebsiella* spp. (14.6%). Additionally, the current results disagree with another study conducted in the United Arab Emirates (UAE) [32] found that *E. coli* accounted for 30.9%, while *K. pneumoniae* represented 13.1% of the isolates. Furthermore, the results contrast with findings from Afikpo, Ebonyi State, Nigeria [33], where E. coli (11.7%) and *K. pneumoniae* (5.8%) were recorded. These variations across regions may reflect differences in sample size, population characteristics, hygiene practices, or local antimicrobial usage. Regarding age-related distribution, although the age group of 18–27 years showed the highest prevalence of *K. pneumoniae* (60%), the differences among age groups were not statistically significant ($P>0.05$), presented in Table (1). These

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findings are consistent with a study conducted in India [34] .which differ with the findings of [35], who reported the highest infection rate among individuals aged 26–30 years (17.4%). In terms of trimester distribution, *K. pneumoniae* was most prevalent in the third trimester (5/10, 50%), followed by the first (30%) and second (20%) trimesters, with no statistically significant differences (P>0.05), table (3). This trend aligns with reports in Damt, Yemen [36], which found the highest prevalence of *K. pneumoniae* infections during the third trimester. The increased susceptibility during this period may be linked to physiological and changes in late pregnancy that favor bacterial colonization and urinary stasis. Additionally, analysis of residency revealed a higher prevalence in urban areas (60%) compared to rural areas (40%), but without statistical significance (P>0.05), presented in Table (2). The current findings agree with the results [37], from Al-Yemen, which showed that the isolation rate of *Klebsiella* was 74.74% in urban areas and 25.26% in rural areas. This pattern contrasts sharply with the findings [36] in Damt, Yemen; they reported an urban isolation rate of 28% and a rural rate of 72%. The antimicrobial susceptibility testing of *K. pneumoniae* isolates using the VITEK 2 system revealed variable resistance patterns among the tested antibiotics. A high resistance rate was observed for Amoxicillin (50%) and Fosfomycin (40%), while moderate resistance rates were recorded for Cefazolin, Cefuroxime, and Cefuroxime axetil (30% each). Resistance to Piperacillin, Imipenem, Nitrofurantoin, Ceftazidime, Ceftriaxone, Ertapenem, Cefepime, and was recorded at 20%, suggesting a potential decrease in the efficacy of these agents. In contrast, complete sensitivity (100%) was observed with Gentamicin, while sensitivity rates (90%) were noted for Amikacin, Meropenem, Ciprofloxacin, and Trimethoprim. A slightly lower sensitivity (80%) was observed for Ceftazidime, Nitrofurantoin, Ceftriaxone, Ertapenem, Piperacillin, Imipenem, and, as presented in Table (4). These findings align with a study conducted in Damt District, Yemen [36], which also reported 100% sensitivity of *K. pneumoniae* to both Gentamicin and Amikacin. Similarly, our results are in agreement with a study from Bangladesh [38], which reported 91.7% sensitivity to Gentamicin. The present study further confirms the high effectiveness of Amikacin (90%) against *K. pneumoniae* isolates from pregnant women, supporting the findings of [39] who documented a high level of sensitivity of *K. pneumoniae* isolates to aminoglycosides, particularly Amikacin., The findings of the current study do not align with those reported by [40] in Saudi Arabia, where a higher resistance rate to Ampicillin (82.8%) was observed, while the susceptibility to Gentamicin was notably

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lower (34.1%) compared to the present study results. However, our results contrast with a study conducted in Karnataka, India [41], which reported significantly lower sensitivity rates to Gentamicin (53.8%), Ciprofloxacin (53.8), and Nitrofurantoin (30.7%). These discrepancies may be attributed to differences in geographic regions, antibiotic usage patterns, or strain-specific resistance mechanisms.

Conclusion

Klebsiella pneumoniae was identified as a common etiological agent of urinary tract infections (UTIs) among pregnant women, with the highest prevalence observed in the 18–27 age group, in urban areas, and during the third trimester of pregnancy. The VITEK 2 system demonstrated high efficiency in the rapid phenotypic identification of *K. pneumoniae* isolates and in accurately determining their antimicrobial susceptibility profiles. Antimicrobial susceptibility testing revealed a concerning level of resistance to commonly used antibiotics such as Amoxicillin (50%) and Fosfomycin (40%), whereas high sensitivity was recorded for Gentamicin (100%), as well as for Amikacin, Meropenem, and Trimethoprim (90%).

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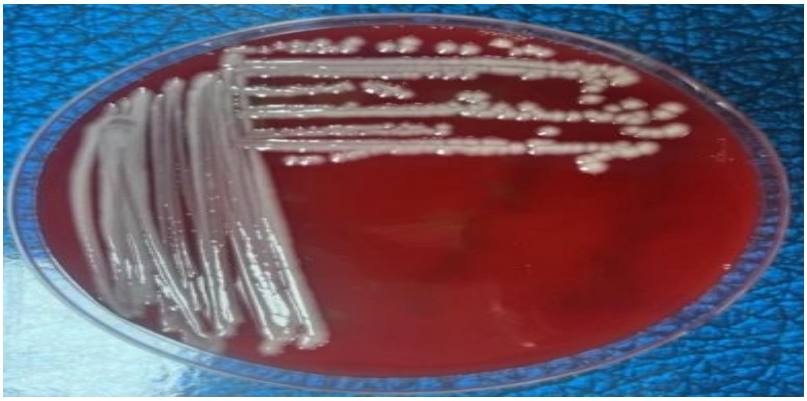
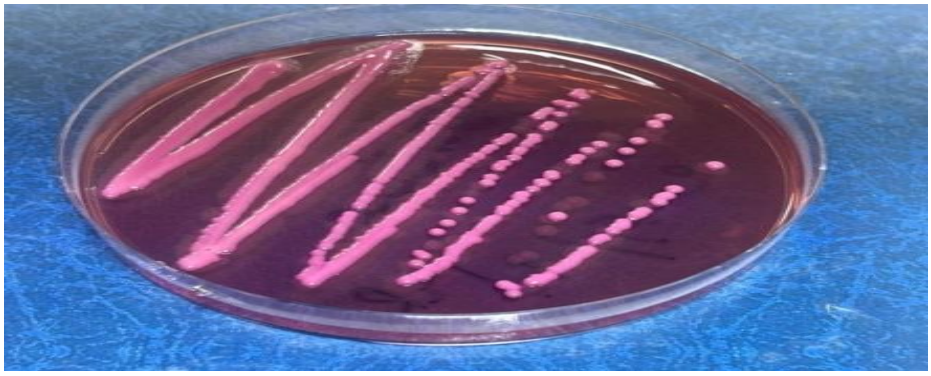


Figure (1) Klebsiella pneumoniae colonies on blood agar after 24hr.of incubation at 37ºC.



Figure(2):Klebsiella pneumoniae colonies on MacConkey agar after 24hr.of incubation at 37ºC

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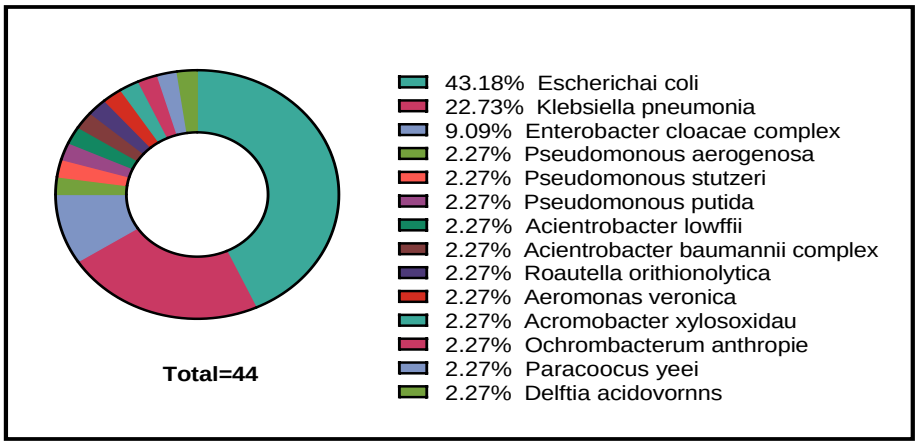

Figure(3): Types of Gram negative bacteria isolated from pregnant women in (44 isolated)

Table (1): The prevalence of *K. pneumonia*e according to the age groups

	Klebsiella pneumoniae		*P-value*
Age groups	No	%	0.150
18-27 years	6	60	
28-37 years	3	30	
38-45 years	1	10	
Total	10	100	

Calculated X^2 = 3.800, tabledX^2= 5.991, DF=2

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Table (2): The distribution of *K. pneumoniae* infections between Urban and Rural area

Residency	No. of positive samples	%	
Urban	6	60	OR: 1. CI= 0.5251 to 3.138 at 95% significance level 244
Rural	4	40	
Total	10	100	
P-value	0.527		

Table (3): The prevalence of *K. pneumoniae* in UTI pregnant women according to the trimester

Trimester	**No. of positive cases**	**%**	**P-value**
First trimester	3	30	0.497
Second trimester	2	20	
Third trimester	5	50	
Total	10	100	
Calculated X^2= 1.4, tabledX^2= 5.991, DF=2			

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Table (4): Antimicrobial Susceptibility of *K. pneumoniae*.

Antibiotic	Resistant		Intermediate		Sensitive	
	No	%	No	%	No	%
Amoxicillin	5	50	0	0	5	50
Fosfomycin	4	40	0	0	6	60
Cefazolin	3	30	0	0	7	70
Cefuroxime	3	30	0	0	7	70
Cefuroxime axeil	3	30	0	0	7	70
Piperacillin	2	20	0	0	8	80
Ceftazdime	2	20	0	0	8	80
Ceftaxone	2	20	0	0	8	80
Ertapenem	2	20	0	0	8	80
Nitrofuraution	2	20	8	80	0	0
Cefepime	1	10	0	0	9	90
Imipenem	2	20	0	0	8	80
Meropenem	1	10	0	0	9	90
Ciprofloxacin	1	10	0	0	9	90
Trimethoprim	1	10	0	0	9	90
Amikacin	0	0	1	10	9	90
Gentamycin	0	0	0	0	10	100