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Molecular Detection of Some Virulent Genes of Klebsiella Pneumonia in Pregnant Women with UTI

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Annotation: Background & Aim: Klebsiella pneumoniae and Pseudomonas aeruginosa are responsible for most nosocomial UTI. Over the years, it is reported that the emergence of KD and PA resistance seemed to be rising. Objective: The present study was planned with an aim to delineate few virulent genes in K. pneumoniae among pregnant women. Report of the case and discussion Urinary tract infections (UTIs) are a common bacterial infection during pregnancy.

Materials & methods: A total of 148 urine samples were obtained, which used clinically specimens from Kirkuk Hospital (Kirkuk city) between October 2024 and February 2025. The clinical samples were obtained after recruitment of the patients by the consultant urologist. The colonies of E. coli and *K. pneumoniae* on blood agar and MacConkey agar were identified by the morphological and biochemical characteristics, and then they were cultured and incubated at 37 ◦C for 24 h.

Results: K. pneumoniae was detected in 27 (18.2%) of the 148 urine samples. The isolates exhibited high level resistance to Ampicillin (96.3%), Imipenem (81.5%) on the contrary, sensitivity was observed to Azithromycin (85.2) followed by Tobramycin and Amikacin 96.3% 100%, respectively). Of the 14 representative K. pneumoniae strains subjected to molecular analysis, 12 (85.7%)

harbored the rmpA gene.

Conclusions: Klebsiella pneumoniae was responsible for 18.2% of UTIs, showed high antibiotic resistance and all isolates harbored bssS and most exhibited rmpA genes associated with MDR, XDR and EDR phenotypes.

Keywords: UTI; K. pneumoniae; virulence genes; rmpA.

Introduction

Basis Urinary tract infection (UTI) is the second most common bacterial infection encountered in human being that remain an important public health problem among both male and female of all age group. The reasons for female predominance in UTI are multifactorial and clinical as well as biological factors such anatomical, hormonal, behavioral (eg voiding habits) and physiological/structural characteristics of the female urinary tract like shorter urethra or it contiguity to the anus leading to that affect [1]. Moreover, pregnancy-associated physiologic changes (urinary stasis, ureteral dilatation, and altered immune status) provide further promotion of the infection rendering UTIs a substantial cause of maternal and fetal morbidities when unmanaged. Several microorganisms are known to cause UTIs, including bacteria, fungi, yeasts and viruses [2,3]. Of these, bacteria are the most common pathogens. Escherichia coli is the most frequent pathogen that causes UTIs (the cause of around 75% of infections), most prevalent uropathogens are, Klebsiella species, Proteus spp., Staphylococcus aureus, Enterococcus spp and Pseudomonas aeruginosa [4,5]. Klebsiella pneumoniae, a clinically significant opportunistic pathogen, causes a spectrum of human infections, including pneumonia, septicemicemia, liver abscesses and diarrhea [6]. It is well-recognized as an important nosocomial pathogen, and reported to be associated with increased morbidity and mortality notably among individuals with the immunocompromised patients requiring hospitalization [7]. Recently, K. pneumoniae has emerged as an increasingly important pathogen in community and hospital acquired UTIs. The pathogenesis of K. pneumoniae is predominantly due to multiple virulence factors, such as capsular polysaccharides, endotoxins, siderophores and iron uptake systems, and adhesins that contribute to colonization, evasion of host immunity and disease development. The capsule especially is one of the important virulence factors, that enables the bacterium to escape phagocytosis and host immune responses [8,9]. Capsular typing is highly reproducible and discriminatory [10], and molecular techniques including PCR assays targeting wzy genes have been developed to increase the specificity of capsular typing [11]. Recently, hypervirulent K. pneumoniae (hvKp) has received increased attention for its high virulence. These isolates frequently harbor virulence-associated plasmids containing genes such as the regulator of mucoid phenotype A (rmpA), aerobactin siderophore biosynthesis gene(iucA) among others coding for metabolite transport and iron acquisition [26]. Consequently, these genotypic markers are frequently applied in recent studies with hypervirulent isolates discrimination and screening common virulence factors [12,13]. Nevertheless, the molecular epidemiology of virulence determinants in K. pneumoniae isolates from pregnant women with UTI has been poorly studied, particularly in developing countries, although hypervirulent strains of K. pneumoniae have become an emerging threat worldwide [13]. In addition to these, there are no studies of local molecular epidemiology focused on the frequency of essential virulence genes in these isolates. In this light, the aim of this current investigation was to investigate specific virulence genes of K. pneumoniae in pregnant females with UTIs using molecular detection methods.

Materials & Methods

Specimen Collection

One hundred and forty-eight clinical urine samples were taken from pregnant women attended Kirkuk Hospital/Kirkuk City/Iraq during the period from Oct 2023 to Feb 2024. Sterile urine samples were collected in sterile containers to prevent contamination. The pregnant samples were obtained from women at different ages in whom urinary tract infections was clinically suspected. All of the samples were immediately carried to the microbiology laboratory for further bacteriological tests.

Isolation and Identification of K. pneumoniae

The first isolation of bacterial pathogens was carried out by inoculating urine samples onto blood agar and MacConkey agar, followed by incubation at 37 °C for 24 h. Isolates presumed to be K. pneumoniae were determined by colony morphology, i.e., lactose fermentation on MacConkey agar and typical mucoidness of the colonies. The isolate was further characterised by the conventional biochemical tests like methyl red, citrate utilization test, urease production (or not), Voges–Proskauer reaction, catalase test and oxidase activity with a combination of motility test, KIA (Triple Sugar Iron) and indole. Final identification and confirmation were done by VITEK® 2 system (bioMérieux, France) as per manufacturer's recommendation.

Antimicrobial Susceptibility Testing (AST)

Isolates identified as K. pneumoniae were tested for antimicrobial susceptibility by the disk diffusion (Kirby–Bauer) method on Mueller–Hinton agar, in accordance with the guidelines of Clinical and Laboratory Standards Institute (CLSI, 2020). The following antibiotics (Bioanalyse-USA) were tested: Ceftazidime 30 µg Tobramycin 10 µg Amikacin 30 µg Levofloxacin 5 µg Cefotaxime 30 µg Trimethoprim 1.25 µg Cefixime 5µG Ciprofloxacin Care Quality Commission Nalidixic acid Clindamycin Vancomycin Azithromycin Imipenem Gentamicin Strains were classified as multi-drug resistant (MDR), if resistant to three or more antimicrobial classes.

Molecular Study

Genomic DNA Extraction

Genomic DNA of positive Gram-negative K. pneumoniae isolates was prepared by the instruction from Wizard® Genomic DNA Purification kit (Promega, USA). DNA quality and concentration was measured, and the samples were kept at −20 °C until their subsequent molecular analysis.

Polymerase Chain Reaction (PCR) Assays

The presence of certain virulence-associated genes was detected by standard PCR. The bssS and rmpA genes were amplified by monoplex PCR in a 20–50-µL final reaction volume with 30 pmol/µL each of the primers. The primer sequences, target genes and the predicted amplicon sizes are shown in Table 1.

Table (1) showed the primers used for identification of Klebsiella pneumoniae virulence genes.

Primer	Primer sequence	Length (bp)	Ref.
BssS-F	5- GATTCAATTTTGGCGATTCCTGC-3	225	
BssS-R	5- TAATGAAGTCATTCAGACTCATCC-3		[14]
rmpA-F	5- CATAAGAGTATTGGTTGACAG-3	461	[15]
rmpA-R	5- CTTGCATGAGCCATCTTTCA-3		

Agarose Gel Electrophoresis

Both the presence and size of amplified DNA fragments were checked by agarose gel electrophoresis of PCR products. The gels were stained with a nucleic acid dye and photographed under ultra violet (UV) illumination with a gel documentation system. Fragment lengths were determined with respect to the DNA ladder.

Ethical Approval

The protocol was approved by the local ethics committee. All were informed about the objectives and design of the study, had given written consent for the use of their samples. The patient information was kept confidential during the whole study.

Statistical Analysis

Analytical aspect of the software was employed for statistical analysis. Results were condensed using descriptive statistics. Univariate and multivariate analyses were utilized to assess the association between variables. P value <0.05 was considered as statistically significant at 95% confident level.

Results

Samples distribution

A total of 148 urine samples collected from pregnant females with symptoms of UTIs were examined (Table 2). Among these, 27 (18.2%) were positive for the growth of K. pneumoniae in blood agar and MacConkey agar plates whereas 121 (81.8%) samples were negative. K. pneumoniae is a rod shaped, Gram-negative bacteria that grow as mucoid lactose-fermenting colonies on MacConkey agar (Figure 1). Biochemical tests were positive for catalase, Voges–Prskauer, citrate utilisation and urease test but negative for oxidase, indole, methyl red and motility test (Figure 2).

Table (2): Table (2): Distribution of samples according to their results

	No. (%) **+ve**	No. (%) **-ve**	Total No.(%)
Isolates	27(18.2%)	121(81.8%)	148 (100.0%)

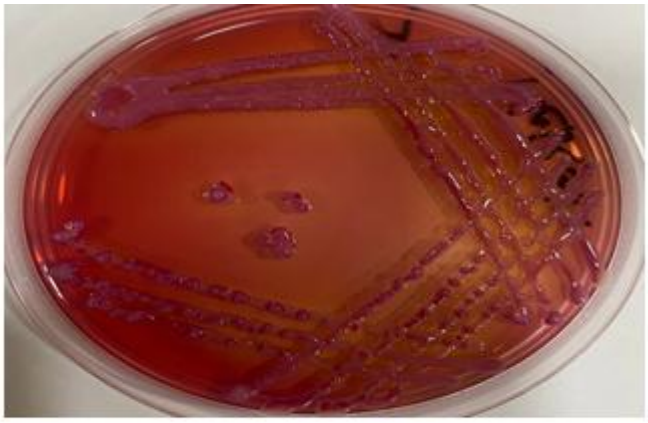

Figure (1): *K pneumoniae* colonies on MacConky agar.

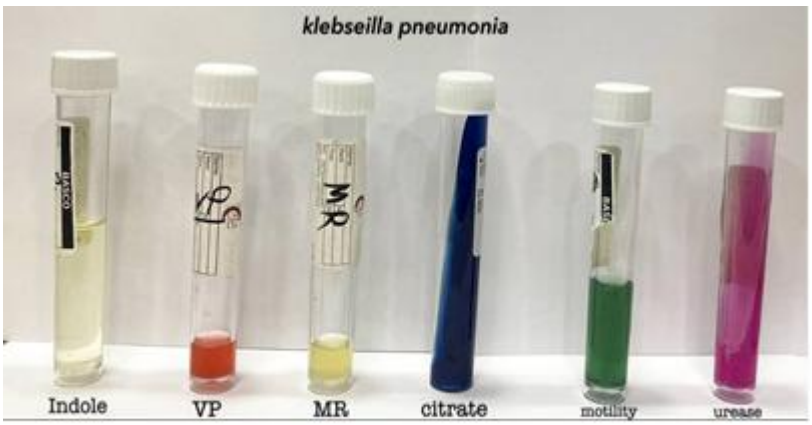

Figure (2): the biochemical tests for *K. pneumoniae* isolates.

Antibiotic susceptibility test

Antibiotic susceptibility testing revealed high resistance of K. pneumoniae isolates to Ampicillin and Imipenem, reaching 96.3% and 81.5%, respectively. Conversely, the isolates were highly sensitive to Azithromycin (85.2%), Tobramycin (96.3%), and Amikacin (100%) (Table 3).

Table (3): sensitivity test for antibiotic

Antibiotics	Sensitive %	Intermediate %	Resistant %	P value
Ampicillin	3.7	0.0	96.3	0.001
Vancomycin	74.1	3.7	22.2	
Clindamycin	59.3	0.0	40.7	
Trimethoprim	70.4	7.4	22.2	
Ceftazidime	37.0	7.4	55.6	
Cefotaxime	51.9	3.7	43.4	
Cefepime	74.1	3.7	22.2	
Gentamicin	85.2	0.0	14.8	
Imipenem	14.8	3.7	81.5	
Nalidixic acid	63.0	11.1	25.9	
Ciprofloxacin	55.6	3.7	40.3	
Levofloxacin	81.5	3.7	15.2	
Azithromycin	85.2	3.7	11.1	
Amikacin	100.0	0.0	0.0	
Tobramycin	96.3	0.0	3.7	

Recently MDR K. pneumonia has been reported all over the world as serious pathogens of women with UTI infection in both hospital and community (16-17). In the present study, a relatively high MDR resistance rate among UTI patients by K. pneumonia (18-19) was also seen that reflects increased worries of repeated failures in UTIs treatment. Unregulated use and abuse of variety of antibiotics would also have contributed to the generation of high levels of bacterial resistance. Secondly, it could be because there are few laboratories where resistance bacteria can be detected in our city. Ciprofloxacin resistant was 33.3% for Quinolones antibiotics, respectively. Al-Obadi.(20) also reported that 20 % of the isolates were impenetrable to Ciprofloxacin. Data was somewhat contradictory compared to the findings of Ali et al., (21) and. A total of 72.22% Klebsiella isolates were resistant to Ciprofloxacin as reported by them. The development of multidrug resistance (MDR) in K. pneumoniae infections is a rising issue with great complexity and it is increasingly complicated to manage UTIs that are becoming costlier and time-consuming, which further narrows the choices for the local agents used in infection treatment. The K.pneumoniae infections have been reported across different outbreaks. A factor in the international dissemination of multidrug resistance. The finding was in line with

similar studies on the resistance of K. pneumoniae reported by the study of Jassim et al. [22], and Guragain et al. [23] The same results were obtained in Al-Saeedi and Raheema [24] and Lob et al. [25] It could be argued the low rates of resistance for imipenem and nitrofurantoin as found in this study may not reflect on the inappropriate utilization of these antibiotics in Kirkuk hospital.

Genetic study

In the present investigation among 14 K. pneumoniae isolate, rmpA was detected as virulence gene in 12(85.7%) K. pneumoniae isolates depicted in figure (3,4).

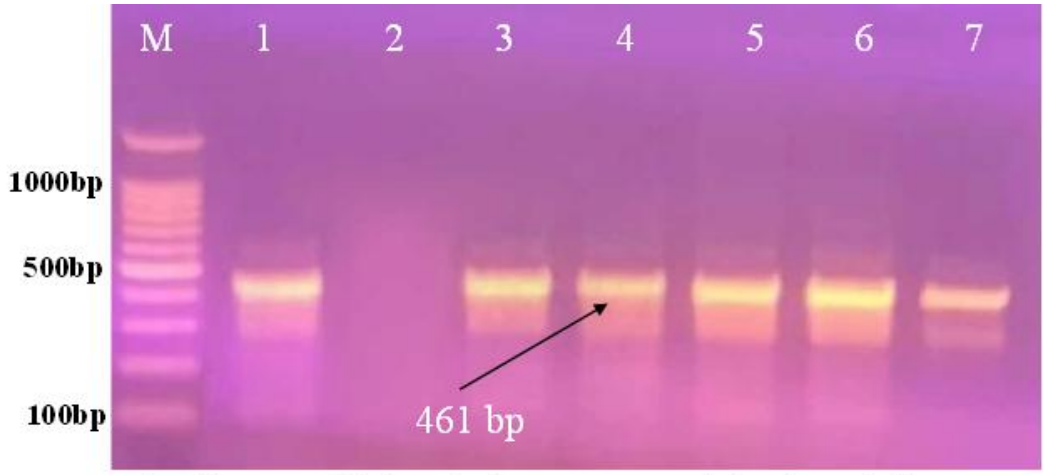

Figure (3): PCR amplification of 461bp rmpA gene; Lane (M) 100bp DNA ladder, Lane 1-7 positive sample on a 1.5% agarose gel electrophoresis.

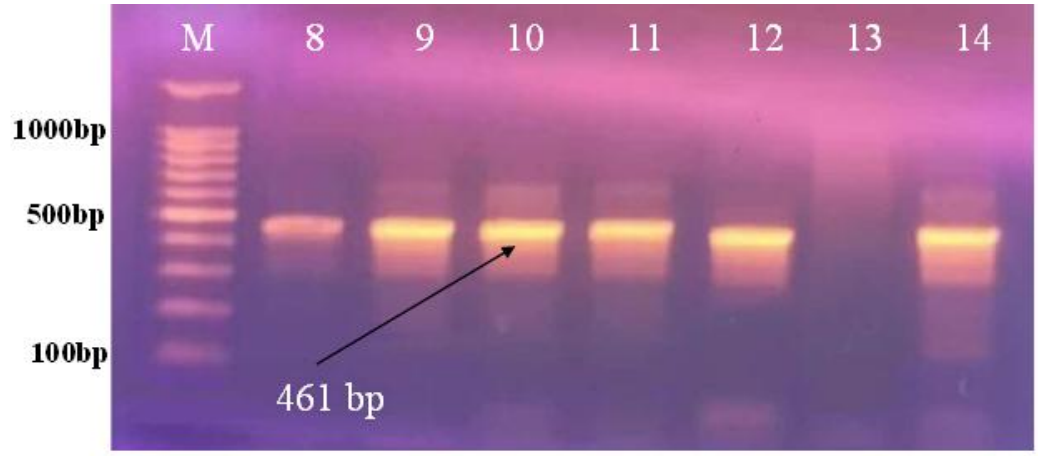

Figure (4): PCR amplification of 461bp rmpA gene; Lane (M) 100bp DNA ladder, Lane 1-7 positive sample on a 1.5% agarose gel electrophoresis.

rmpA virulence gene was identified in 12/14 (85.7%) of K. pneumoniae isolates studied, and suggested a high prevalence of the virulence factor among tested strains in this study. The rmpA gene modulates capsular polysaccharide synthesis and is linked to hypermucoviscosity that is important for escape of the bacterium from hosts' immune reactions as well as contributing to pathogenicity in invasive infections [26]. Many researches all over the world have revealed that rmpA is an important virulence marker in K. pneumoniae. For instance, in clinical isolates from cerebrospinal fluid, rmpA was detected on around 69.7% of isolates and this gene was associated with phenotypes correspondent to higher virulence [27]. Moreover, plasmid-borne rmpA was demonstrated to be associated with the coexistence of other virulence genes, thus increasing pathogenicity [28]. Of great significance, region-specific studies in Iraq indicate that

virulence-associated genes especially rmpA occur at a high rate in clinical K. pneumoniae isolates. rmpA was detected in 62 % of isolates from patients with diabetic foot ulcers in Najaf Governorate illustrating its importance within local clinical settings [29]. Another study in western Iraq showed that rmpA was highly predominant among clinical isolates indicating its role in pathogenesis and antibiotic resistance profile [30]. Since 85.7% of our isolates carried rmpA, we would speculate that the ANL strain was largely predominant in our study population--this could also explain even greater severity and treatment complexity caused by these bacteria particularly where they were resistant to antibiotics. This observation highlights the relevance of molecular monitoring of virulence genes in clinical practice. Nevertheless, the discrepancies in rmpA prevalence between studies also indicate differences of genetic background and epidemiological profiles among regional K. pneumoniae populations, which should be carefully examined with larger sample sizes [31].

Correlation of the rmpA Virulence Gene with Antimicrobial Resistance

Nasal and throat swabs were collected from 27Klebsiella pneumoniae isolates, among which antimicrobial resistance was highly frequent. Of these isolates, 15 (55.6%) were MDR, 9 (33.3%) XDR and 3 (11.1%) EDR Highlights Here some highlights: most of the isolates showed a high degree in resistance patterns. Of the 14 isolates, 4 were chosen for molecular identification of the rmpA virulence gene. rmpA was detected in 12 (85.7%) isolates, suggesting a high prevalence for this significant virulence factor between our isolates.

Correlation of rmpA and resistance patterns

As presented in Table 4 the prevalence of rmpA gene was different among resistance groups. The gene was found in 11/15 (78.6%) MDR, 9/9 (64.3%) XDR and 2/3 EDR isolates. This observation indicates the association of rmpA to MDR and XDR isolates, rather than with EDR strains. The relatively high frequency of rmpA carriage in MDR and XDR isolates suggests that there may be many strains which have both virulence and resistance properties, resulting in more serious as well as resistant infections. In contrast, the decreased prevalence of rmpA in EDR isolates could be indicative of a biological trade-off between maintaining virulence factors and acquiring multidrug resistance determinants. Highly resistant strains might put survival under antimicrobial pressure ahead of expression of classical virulence properties, such as hyperproduction of the capsular polysaccharide in a capsule-mediated form (namely by rmpA). In general, as shown in Table (4), a diverse correlation between rmpA and antimicrobial resistance is recognized, and MDR or XDR isolates are heavily enriched for hypervirulent characteristic which become less prevalent in extreme drug-resistant strains. This observation has a high clinical relevance, since strains harboring both rmpA and multiple resistance genes are considered an extremely high-risk group in relation to severity of infection and the outcome of treatment.

Table (4): Association between resistance patterns and rmpA gene among *Klebsiella pneumoniae* isolates

Resistance pattern	No. of isolates (n=27)	rmpA positive (n=14)
MDR	15	11 (78.6%)
XDR	9	9 (64.3%)
EDR	3	2 (14.3%)
Total	**27**	**12 (85.7%)**

Molecular epidemiologic research has also demonstrated an association between virulence genes and antimicrobial resistance phenotypes. For some of these chromosomally located virulence determinants, such as rmpA, there is evidence that they are on the same mobile genetic element as resistance genes and have cointegrated into the same plasmid or chromosome structure by linkage in specific regions of the genome, although its frequency varies among different populations and geographic areas [32]. Although global reports are consistent with variable

prevalence of rmpA, among MDR isolates at different geographical locations mixed prevalence is present. The rmpA was high in CSF and other clinical isolates, corresponding with high virulence strains in some studies, but not always directly correlate to resistance phenotypes in the all the reported info demonstrating the variance inter-studies [29]. In the same way, monitoring in heterogenous clinical settings showed significant associations between virulence genes (such as rmpA) and resistance profiles including resistant strains that were able to maintain their expression of virulence genes [33]. The present findings demonstrate that the carriage of rmpA is common in MDR and XDR K. pneumoniae isolates, which may possibly increase infection severity while residing with resistance genes. However, the reduced frequency of rmpA in EDR isolates may represent genomic trade-offs wherein highly resistant strains favor evolutionary survival strategies involved with resistance over specific virulence mechanisms. This intricate relationship highlights the necessity for concomitant tracking of virulence and resistance traits in order to help elucidate the contributions that pathogenic K. pneumoniae strains have on patient outcome while also aiding in treatment options.

Conclusions

In the present study, urine from UTI cases was found to be positive for Klebsiella pneumoniae in 18.2% cases substantiating its importance as a significant urine pathogen. Antibiotic susceptibility testing revealed a high presence of resistance to ampicillin and imipenem, but isolates generally remained susceptible to amikacin, tobramycin, and azithromycin. Molecular analysis revealed 100% expression of the bssS gene and high prevalence of rmpA at 85.7%, indicating their pathogenicity potential. A strong relationship was noted between bssS and rmpA and the presence of virulence genes in combination with antimicrobial resistance; all MDR, XDR, and EDR isolates had bssS while most had rmpA highlighting the difficultly in treating highly virulent resistant K. pneumoniae infections.

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