AL-KUFA JOURNAL FOR BIOLOGY
ISSN (Print): 2073-8854 **ISSN (online):** 2311-6544

Original Research Paper

Genetic study of *,magA* , *rmpA,iuc C* and *K2A* produced by *Klebsiella pneumoniae* which isolated from patients with bacterial vaginosis

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Article history
Received: 16 /5 /2025
Revised: 8/ 6//2025
Accepted: 11/6/ /2025

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Abstract: Bacterial vaginosis (BV) is a disease caused by an imbalance of vaginal microecology , Vaginal flora is one of the most important defense mechanisms for reproductive function and maintaining ahealthy environment Healthy vaginal flora is composed of anaerobic bacteria and aerobic bacteria, with Lactobacillus as the dominant microorganism, . Out of a total of 104 patients diagnosed with bacterial vaginosis , Gram-negative bacteria were isolated from 86 specimens (82.69%), while the remaining 18 (17.30%) showed no bacterial growth. Bacterial vaginosis were most prevalent in 3rd trimester of gestational period in compared with other groups of gestational period, 70 (67.3%), and lowest at 1st trimester 10(9.62) . The results of the pure gram-negative isolates indicated that *K. pneumonia* was the most dominate bacteria with 21(20.19%) isolates followed *E. coli* by 19(18.26%) isolates, and lowest *P.aeruginosa* 3(2.88%) . Molecular analysis revealed the presence of key virulence genes, including *magA ,rmpA.iucC* ,ana *K2F* the four genes were found in 90% 0f these isolates .

Keywords: Bacterial vaginaisis ,*Klebsiella Pneumoniae*, *magA*, *rmpA,iucC,K2F*

1. Introduction

Inflammation of the vagina, or vaginalitis, can be caused by a number of things, such as infections, irritants, and hormonal fluctuations. Bacterial vaginosis (BV) is the most common diagnosis among the infectious causes [1] BV is typified by an imbalance in the vagina's natural flora, which results in a decrease in Lactobacillus species and an overgrowth of certain bacteria, including *Gardnerella vaginalis* [2]. Anaerobic bacterial overgrowth is the main cause of bacterial vaginosis. Douching, having several sexual partners, and having a diverse vaginal flora are risk factors [3]. Particularly after sexual activity, patients frequently exhibit a thin, fishy-smelling, grayish-white vaginal discharge. Additionally, there may be discomfort and itching [4]. *k. pneumoniae* infections in women can produce symptoms like discomfort, irregular discharge, and heightened vulnerability to other infections [5]. The possibility of major difficulties, such as pelvic inflammatory illness and unfavorable pregnancy outcomes, is raised by the presence of this pathogen in the vaginal environment. For diagnosis and treatment to be successful, it is essential to comprehend the mechanisms behind Klebsiella infections and their clinical implications [6]. Since *K.pneumoniae* may be linked to symptomatic infections marked by unusual discharge and discomfort, its discovery in the vaginal microbiome presents significant clinical implications [6]. *magA* Encodes a capsular polysaccharide biosynthesis enzyme, aiding in the production of the capsule that helps *K. pneumoniae* evade the host's immune system and enhances its ability to cause disease[7]. *rmpA* Regulates the production of capsular polysaccharides in *K.pneumoniae*, contributing to the bacterium's ability to cause disease by enhancing its virulence[8]. *IucC* Involved in the biosynthesis of the siderophore aerobactin, which helps bacteria acquire iron, crucial for their growth and virulence [9]. *K2AF* associated with the K2 capsule type in *K. pneumoniae*, known to increase the pathogenicity and virulence of the bacterium [10].

2. Materials and Methods

In this research 104 specimens were collected from bacterial vaginosis patients in AL- Najaf Governorate. Iraq, from (September to desember 2024). The types of gram-negative bacteria identified using biochemical testing and vitek 2 system . Specimens were swaps from vagina waman . All specimens were grown on MacConkey agar .PCR testing was performed to detect antibiotic resistance genes and virulence factors of A. K.Pneumonia . In monotypes in order to amplify different parts of genes. The magA , rmpA ,IucC and K2AF from bacteria were selected to be amplified separately using the single-strand PCR technique used in this study.

3. Results and Discussion

Type of bacteria and period of gestation

The results of this research appaered that 104 specimens were collected from patients suffering from bacterial vaginosis, 86 (82.69 %) specimens had been given positive growth of gram negative bacteria while 18(17.30 %) of specimens showed no growth of gram negative bacteria,The results of the pure gram-negative isolates indicated that K. pneumonia was the most dominate bacteria with 21(20.19%) isolates followed E. coli by 19(18.26%) isolates, and lowest P.aeruginosa 3(2.88%) as shown in table (1) .Concerning the period of gestation, the present results show most patients with bacterial vaginosis in 3rd trimester of gestational period in compared with other groups of gestational period, 70 (67.3%), and lowest at 1st trimester 10(9.62) was shown in table (2).

Table 1: prevalence of gram negative bacteria isolated from bacterial vaginosis .

Bacterial species (Gram Negative)	**Number (percentage %)**	**P value**
Klebsiella Pneumonia	21 (20.19)	0.001 ¥ S
E. coli	19 (18.27)	
Enterobacter aerogens	8 (7.69)	
Proteus mirabilis	12 (11.54)	
Pseudomonas fluorescens	4 (3.84)	
Pseudomonas aeruginosa	3 (2.88)	
Unidentified bacteria	5 (4.81)	
Total	72 (69.23)	

n: number of cases; ¥: Chi-square test; **S**: significant at $p < 0.05$

Table 2: Distribution of bacterial vaginosis according to gestational period

Gestational period	**bacterial vaginosis infection** ***n* =104** **Number (percentage%)**	***P***
1st trimester	10 (9.62)	**0.001** **¥** **S**
2nd trimester	24 (23.08)	
3rd trimester	70 (67.30)	

SD: standard deviation; n: number of cases; ¥: chi-square test; S: significant at $P < 0.05$.

A study showed that 6 women (36.4%) were in the second trimester, while 20 women (45.5%) were in the third trimester. This finding is consistent with the results of my research.as cited in source [11].

Virulence Factors of Klebsiella.Pneumoniae

Detection of antibiotic resistance genes (magA ,rpmA,iucC and K2A), the amplification results of PCR study for magA (1283bp) , rmpA (536bp) ,iucC (944) and K2F(543) Specific PCR primer was used for the detection of magA gene. The results showed that 9 (90%) K. pneumonia of investigate isolates contained magA gene in (1,2,3,4,5,6,7,8 and 9) . As shown in figure (1) .

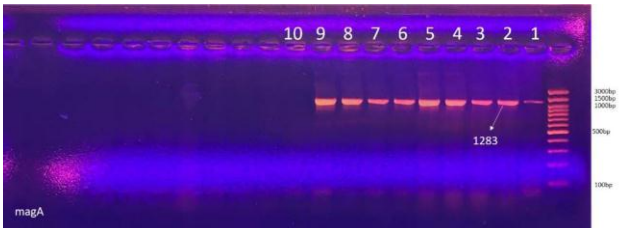

Fig 1: The PCR product of gene i magA n 1.5% agarose gel electrophoresis, voltage (70 V), time (45 minute) and 5 µL of PCR product load1ed in each well. Lane M: DNA Ladder (3000bp) Lanes 1,2,3,4,5,6,7,8,9 : PCR product (positive case band 1283 bp).

The proportion of rmb A genes was 90% of what could be relied upon a positive indicator of the presence and spread of the rmb A gene, according to the results of the this study, the results showed that the resistance gene was produced from the isolates (2,3,5,6,7,8and 9), as shown in the figure (2) .

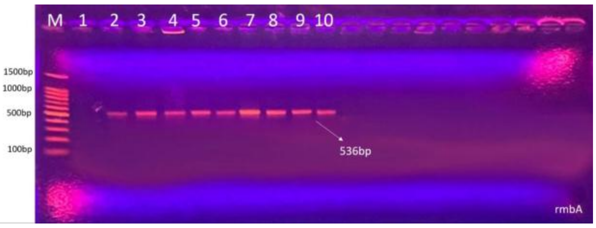

Fig 2: The PCR product of *rmbA* gene in 1.5% agarose gel electrophoresis, voltage (70 V), time (45 minute) and 5 µL of PCR product load1ed in each well. Lane M: DNA Ladder (3000bp) Lanes 2,3,4,5,6,7,8,9,10 : PCR product (positive case band 536 bp).

The results showed that 9 isolates (90%) of K. pneumonia were possessed iucC gene in isolates (1,2,3,6,7,8 and 9) show in figure (3).

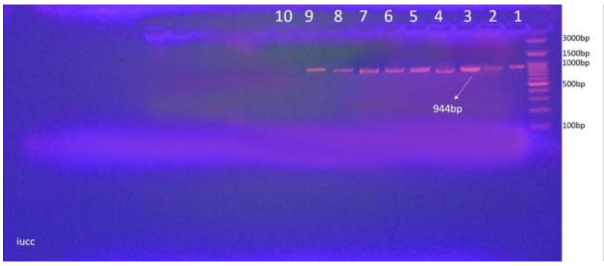

Fig 3: The PCR product of gene in *iucC* 1.5% agarose gel electrophoresis, voltage (70 V), time (45 minute) and 5 µL of PCR product load1ed in each well. Lane M: DNA Ladder (3000bp) Lanes 1,2,3,4,5,6,7,8,9 : PCR product (positive case band 944 bp.

The results showed that 9 isolates (90%) of K. pneumoniae were possessed K2A gene in isolates (1,2,3,4,5,7,8,9 and 10) as shown in figure (4).

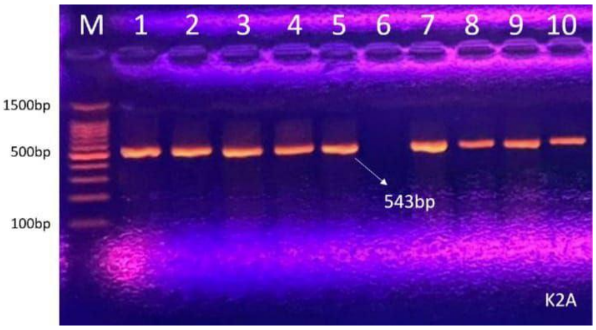

Fig 4: The PCR product of gene in *K2A* 1.5% agarose gel electrophoresis, voltage (70 V), time (45 minute) and 5 µL of PCR product load1ed in each well. Lane M: DNA Ladder (3000bp) Lanes 1,2,3,4,5,7,8,9,10 : PCR product (positive case band 543 bp).

The magA gene stands, which is linked to increased virulence in K.pneumoniae, particularly in hypermucoid strains. This gene plays a role in the production of the polysaccharide capsule, enhancing bacterial resistance to phagocytosis and contributing to its ability to cause infections [12].

The presence of rmpA is linked to the production of capsular polysaccharides, which play a significant role in the virulence of these bacteria.The product of the rmpA gene is involved in regulating the synthesis of the polysaccharide capsule specifically, type I capsule that surrounds bacterial cells[13]. The iucC gene is part of the aerobactin biosynthesis system, which plays a crucial role in iron acquisition for bacterial pathogens. It is involved in the synthesis of aerobactin, a siderophore that enhances bacterial survival in iron-limited environments, contributing to virulence in strains like E.coli and K.pneumoniae [14].

The K2A gene in K. pneumoniae is associated with capsular polysaccharide synthesis, contributing to the bacterium's virulence and resistance to host immune defenses. Studies have shown that K2A is one of the key capsular genes linked to hypervirulent strains of K. pneumoniae, which are often implicated in severe infections [15].

Conclusion

Out of 104 specimens 86 (82.69 %) specimens had been given positive growth of gram negative bacteria while 18 (17.30 %) of specimens showed no growth of gram negative bacteria. Pregnant women in the 3rd trimester of pregnancy suffering from bacterial vaginosis higher rate than from 1 trimester and 2 trimester .The results of the gram-negative isolates indicated that K.Pneumoniea was the most dominate bacteria. Among the virulence factors of K.Pneumoniae , the most frequently isolated bacterium from bacterial vaginosis patients, the four genes were found in 90% 0f these isolates .

Ethics

This paper was approved by the ethical committee in University of kufa , al-Najaf city- Iraq in 2025 .

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