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Molecular detection of *klebicin* gene and antibacterial resistance patterns in *Klebsiella pneumoniae* clinical isolates in Diwaniyah,Iraq.

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Abstract:

Background*: klebsiella pneumoniae* is an important opportunistic pathogen that has been associated with various hospital-acquired infection and has demonstrated increasing resistance to antibacterials .the purpose of the current study was to examine the phenotypic and molecular characteristics of k.pneumoniae isolate, with special reference to antibacterial resistance and the present of the *klebicin gene.*

Objective: the research for detecting *klebicin gene* in isolates using PCR-technique.

Methods: 150 clinical samples were collected from patients at the Maternity and Children's Hospital and Al-Diwaniyah Teaching Hospital. After performing standard sample culture and biochemical testing, 14 samples were found to contain *Klebsiella pneumoniae*. Following the use of Vitec2 technology, NON-MDR, MDR, and XDR strains were identified. PCR testing was then performed to identify the *klebicin gene* in the clinical samples from Al-Diwaniyah city to help determine the causes of bacterial resistance and virulence factors.

Results: The isolates have been show different levels of susceptibility to antibacterial. They were quite resistant to β-lactam antibacterials, such as third- and fourth-generation cephalosporins and fluoroquinolones. Some isolates were found to be multidrug-resistant (MDR) or extensively drug-resistant (XDR), but none were found to be pan drug-resistant. PCR examination showed that all of the isolates (100%) had the *klebicin gene*, which means that it was found in a lot of the strains that were investigated.

Conclusion: The results show that a lot of k.pneumoniae clinical isolates are resistant to antibacterial and that they all have *klebicin gene*.The fact that k.pneumoniae has both resistance phenotypes and bacteriocin-associsted genes shows that it can adapt to clinical setting. This shows how important it is to keep an eye on resistant strains and use antibacterials wisely to stop them from spreading.

Keywords: klebsiella pneumonia ;Antibacterial resistance ;MDR ;XDR ;Klebicin gene;PCR.

Introduction: klebsiella pneumoniae is major opportunistic bacteria that causes serious infection in hospitals and communities. It is amajor causes of many common infection,such as pneumonia ,urinary tract infection, wound infection ,and septicemia, especially in those who are already sick in the hospital. *K. pneumoniae* has become one of the most common causes of healthcare-associated infections around the world because it can survive in many different situations and live in the human body.(1)(2)

K. pneumoniae has become a major worldwide health issue in the last few years because new strains are quickly becoming resistant to many drugs. Clinical isolates of these bacteria demonstrate resistance to multiple types of antibacterials, including beta-lactams, fluoroquinolones, and aminoglycosides, principally due to the synthesis of broad-spectrum beta-lactamases and carbapenems. The expansion of *K. pneumoniae* that is resistant to carbapenems has been linked to greater death rates, longer hospital stays, and higher healthcare expenses. The World Health Organization has put carbapenem-resistant *K. pneumoniae* on its list of top priority pathogens. This shows how important it is to keep an eye on it and come up with innovative ways to control it. (3)(4)(5)

Klebsiella pneumoniae not only shows a lot of resistance to antibacterials, but it also has a number of virulence factors that help it avoid the immune system, colonize hosts, and cause disease. Identified virulence factors include polysaccharides that protect against phagocytosis, cilia that help bacteria attach to host tissues, siderophores that help bacteria get iron, and biofilms that help bacteria stay alive in clinical settings. The parameters mentioned above make it easier for K. pneumoniae to infect, stay in the body, and spread.. (6)(7)
Bacteriocins are important virulence factors *in K.pneumoniae* because they help the bacteria compete with other bacteria and stay alive in the host. Bacteriocins are antibacterial peptides that bacteria make to stop closely related species from growing. This gives them an edge in colonization and infection. *K. pneumoniae* makes a bacteriocin called *Klebcin*, which is known to be a major virulence factor that helps the bacterium survive by stopping other microorganisms from growing in its environment. This competitive edge may indirectly make *K. pneumoniae* more dangerous and better at keeping bacterial infections going.(8)(9)
Identifying and defining virulence genes is essential for comprehending the pathogenicity of clinical isolates. The polymerase chain reaction (PCR) is an exceptionally sensitive and precise molecular method .the extensive application in finiding bacterial genes linked to virulence and disease is especially noteworthy. PCR facilitates the swift verification of virulence facters and enhances conventional techniques ,including culture based and other automated technologies. The amalgamation of molecular and typologicsl methodological yields amore thorough evaluation of clinical pathogens ,exemplified as *k. pneumoniae* .(10)

Aim of the study

The present study aimed to investigate the antibacterial resistance. Patterns of clinical *klebsiella pneumoniae* isolates and to perform PCR_based molecular detection of the *klebicin virulance gene* . Understanding the distribution of virulance _associated factors alongside antibacterial resistance

profiles may contribute to improved epidemiological surveillance and provide valuable insights for infection control and clinical management strategies.

Materials and methods:

Methods:

sample collection

This study was set up as a cross section study in alab, using clinical sample taken from pateint who came to hospital during the study period . I has been collected 150 clinical sample from different places where found ,such as urine and respiratory specimens. Sample were collected in sterile conditions and processed immediately following standard microbiological procedures. The microbiology lab goy all of the samples so that they could be isolated and identified.

Identifying bacterial isolates: Clinical samples were grown on the right selective and differential media, like MacConkey agar, and kept at 37 °C for 18 to 24 hours in an aerobic environment. Were used colony morphology, Gram staining, and standard biochemical tests to find suspected Klebsiella colonies. The VITEK 2 automated identification system confirmed the final species-level identification according to the manufacturer's instructions.

This study was structured as a laboratory-based cross-sectional inquiry utilizing clinical specimens obtained from patients visiting the hospital during the study period. The VITEK 2 system was used to test the antibacterial susceptibility of all confirmed *K. pneumoniae* isolates. The susceptibility profiles were interpreted according to the guidelines of the Clinical and Laboratory Standards Institute (CLSI). The tested antibacterials represented different antibacterial classes commonly used in clinical practice. isolates were categorized into three groups: susceptible, intermediate, or resistant. Multidrug resistance (MDR) meant that the isolate was resistant to at least one agent in three or more antibacterial categories.m according to the manufacturer’s instructions.

DNA extraction and PCR detection of the *Klebicin gene*

Using a standard bacterial DNA extraction protocol and following the manufacturer's instructions, genomic DNA was taken from confirmed *Klebsiella pneumoniae* isolates. Polymerase chain reaction (PCR) with specific primers was used to find the *Klebicin* virulence gene at the molecular level. The PCR reaction was done in a total volume of 25µl under optimization cycling conditions, included an initial denaturation, followed by repeated cycles of denaturation, annealing at 57°c, and extension. Agarose gel electrophoresis was used to separate the amplified PCR products(294 bp), and ultraviolet light was used to see them to make sure the target gene was present.

Results:

Out of 150 clinical specimens collected during the study period, 14 isolates (14%) were identified as *Klebsiella pneumoniae.*

The distribution of *Klebsiella pneumoniae* isolates according to specimen type was as follows:

12 isolates (85.7%) were recovered from urine samples.2 isolates (14.3%) were isolated from lower respiratory tract specimens (bronchial/tracheal aspirates).

All isolates showed characteristic growth on MacConkey agar as large, mucoid, pink colonies, indicating lactose fermentation On blood agar, the isolates produced non-hemolytic colonies.

Microscopic examination revealed that all isolates were Gram-negative bacilli.

Table (1): Biochemical Tests for Characterization of K. pneumoniae

Biochemical test	Catalase	Oxidase	Indole	MR	VP	Citrate	Urease	Motility
k.pneumoniae	+	-	-	-	+	+	+	-

Identification using the VITEK 2 automated system confirmed all isolates as *Klebsiella pneumoniae* with a high level of confidence based on automated biochemical analysis.

Antibacterial susceptibility testing of *K.pneumoniae* isolates was performed using the VITEK 2 automated system. The isolates exhibited diverse antibacterial susceptibility profiles, ranging from fully susceptible phenotypes to multidrug-resistant and extensively drug-resistant patterns.

Overall, a high level of susceptibility was observed to carbapenems (imipenem and meropenem) among several isolates. Aminoglycosides, particularly amikacin and gentamicin, also demonstrated good activity against the majority of isolates. In addition, most isolates were susceptible to tigecycline and colistin.

In contrast, high resistance rates were detected against ampicillin/sulbactam, third-generation cephalosporins (cefotaxime, ceftazidime, and ceftriaxone), and fluoroquinolones, particularly ciprofloxacin, in a subset of isolates. Resistance to trimethoprim/sulfamethoxazole was also frequently observed.

Based on the antibacterial susceptibility profiles, several isolates met the criteria for multidrug resistance (MDR), while at least one isolate exhibited resistance to multiple antibacterial classes, including β-lactams, carbapenems, aminoglycosides, and fluoroquinolones, consistent with an extensively drug-resistant (XDR) phenotype.,Non-MDR: 4/14 (28.6%),MDR: 5/14 (35.7%),XDR: 5/14 (35.7%),PDR: 0

Table 2. Resistance patterns of *Klebsiella pneumoniae* isolates

(n=14)

Sample No.	Resistance	pattern Classification
Sample 1	Susceptible to most tested antibacterials	Non-MDR
Sample 2	Susceptible to most tested antibacterials	Non-MDR
Sample 3	Resistant to ≥3 antimicrobial classes	MDR
Sample 4	Resistant to ≥3 antimicrobial classes	MDR
Sample 5	Resistant to ≥3 antimicrobial classes	MDR
Sample 6	Resistant to ≥3 antimicrobial classes	MDR
Sample 7	Carbapenem -resistant with resistance to multiple classes	XDR
Sample 8	Carbapenem -resistant with resistance to multiple classes	XDR
Sample 9	Carbapenem -resistant with resistance to multiple classes	XDR
Sample 10	Carbapenem -resistant with resistance to multiple classes	XDR
Sample 11	Susceptible to most tested antibacterials	Non-MDR
Sample 12	Susceptible to most tested antibacterials	Non-MDR
Sample 13	Resistant to ≥3 antimicrobial classes	MDR
Sample 14	Carbapenem -resistant with resistance to multiple classes	XDR

PCR amplification of the *klebicin gene* in *Klebsiella pneumoniae* isolates revealed a clear and specific amplification product of approximately 294 bp. Agarose gel electrophoresis showed distinct bands at the expected molecular size in all tested samples (lanes 1–14). No absence of amplification or non-specific bands was observed. These results indicate that all examined K. pneumoniae isolates (100%) were positive for the *klebicin gene.*

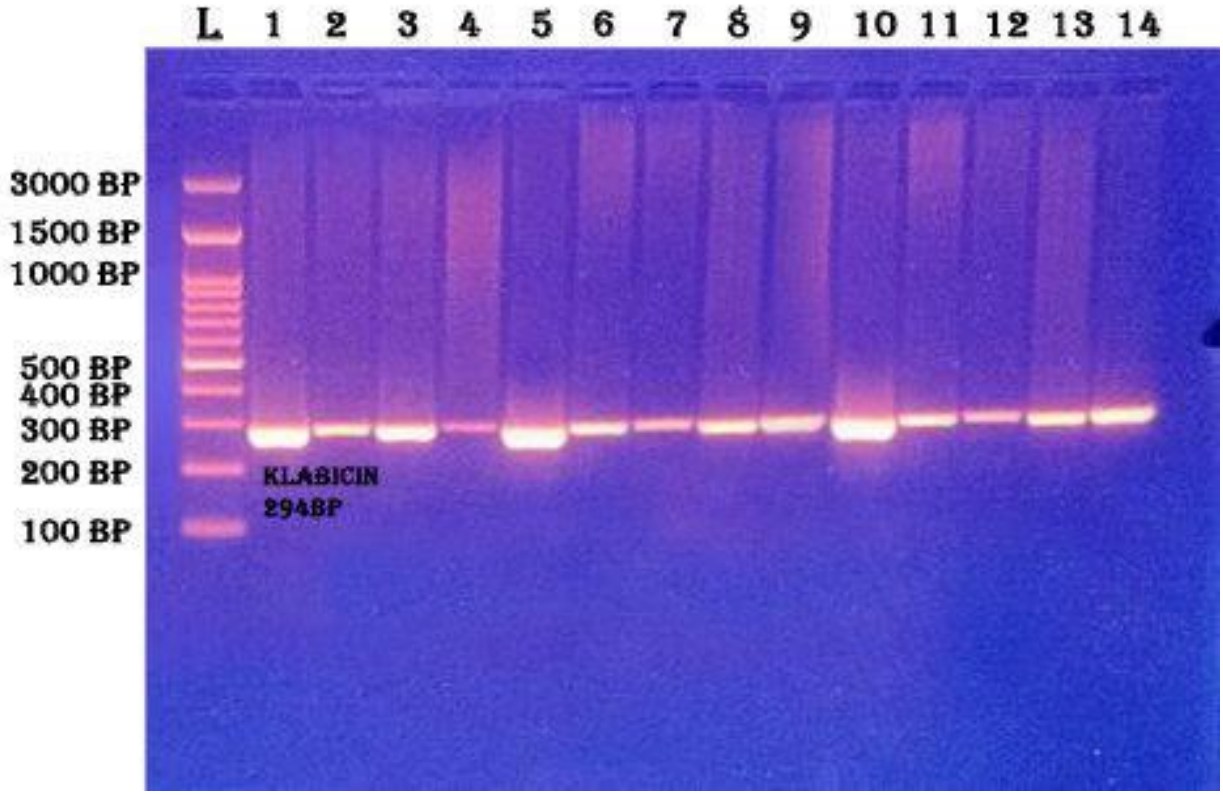

Figure1: garose gel electrophoresis of PCR products showing amplification of *the klebicin* gene (294 bp) in *Klebsiella pneumoniae* isolates. Lane L represents the DNA molecular weight marker (ladder). Lanes 1–14 represent K. pneumoniae isolates, all of which show a distinct band at approximately 294 bp, indicating positive amplification of *the klebicin gene*

Discussion

The isolation of *Klebsiella pneumoniae* signifies its importance as an opportunistic pathogen. On MacConkey medium, big mucoid lactose-fermenting colonies grew, which is a typical sign of this pathogen. This is due to the fact that *K. pneumoniae* has a large polysaccharide capsule that makes it very dangerous and able to live for a long time in the host and in the hospital. This is clear from earlier reports that show this pathogen has similar traits Comparison with Local Iraqi Research by Al-Azawi .(11)

These findings are consistent with those reported by Al-Azawi at the College of Medicine at the University of Al-Qadisiyah , Iraq, who documented a high prevalence of K. pneumoniae in clinical specimens, especially in urinary tract infections. The concordance between the present study and that of Al-Azawi underscores the sustained clinical significance of K. pneumoniae as a uropathogen in Iraq healthcare settings,highlighting it is persistence as a nosocomial threat in the Al-Qadisiyah region of Iraq.(12)

The biochemical test results from this study match what we already know about the biochemical profile of *klebsiella pneumonia*. The isolates were Gram-negative rods that didnot move, had no oxidase, and had positive catalase. This confirmed that they belonged to the Enterobacteriaceae family. The negative indole and methyl red (MR) reactions mean that tryptophan is not being broken down and mixed-acid fermentation is not happening. The positive Voges–Proskauer (VP) reaction confirms that acetoin is being made, which is a well-known metabolic marker for K. pneumoniae.. (4) Urease, citrate, and carbohydrates All strains showed positive test results for using citrate as a carbon source and making urease. These biochemical characteristics are acknowledged as reliable markers for differentiating K. pneumoniae from other related Gram-negative bacteria. Also, the strains were non-motile, which set them apart from other motile coliform bacteria, such as Escherichia coli. (13) The consistency of cultural traits and biochemical test outcomes with previous Iraqi and international research substantiates the continued application of conventional culturing and biochemical testing techniques for bacterial isolates in standard clinical microbiology laboratories. The current analysis's agreement with earlier studies highlights the importance of traditional diagnostic methods, especially in settings with limited resources where modern diagnostic tools may not be available. The isolates showed different levels of susceptibility to antibacterial. They were quite resistant to β-lactam antibacterials, such as third- and fourth-generation cephalosporins and fluoroquinolones. Some isolates were found to be multidrug-resistant (MDR) or extensively drug-resistant (XDR), but none were found to be pandrug-resistant. PCR examination showed that all of the isolates (100%) had the *klebicin gene*, which means that it was found in a lot of the strains that were investigated. The continued presence of K. pneumoniae isolates underscores the imperative for surveillance in resource-limited settings.The current study found a high number of multidrug-resistant (MDR) and extensively drug-resistant (XDR) *K..pneumoniae* isolates in clinical samples, especially urine samples. This trend shows that K. pneumoniae is becoming more resistant to antibacterials around the world, making infections caused by this pathogen harder to treat and control. K. pneumoniae is a major opportunistic pathogen that causes illnesses in people who are already sick and has higher resistance rates to many types of antibacterials around the world. (12The increased resistance to broad-spectrum β-lactam antibacterials, which includes third- and fourth-generation cephalosporins and extended-spectrum penicillins, is consistent with known resistance mechanisms, such as the creation of extended-spectrum β-lactamases (ESBLs) and carbapenemase enzymes. These enzymes break down β-lactam antibacterials, which greatly lowers their ability to treat infections. Also, carbapenem resistance, which is often made easier by carbapenemases like KPC, NDM, VIM, and OXA-48, is a big problem for treatment and is becoming more common in clinical isolates.. (14)(15) Even though aminoglycosides (like amikacin and gentamicin), tigecycline, and colistin worked very well against many isolates in our study, it has been shown that these last-line drugs are becoming less effective against more and more bacteria. Surveillance data and systematic reviews show that carbapenem-resistant K. pneumoniae is becoming more resistant to tigecycline and colistin. This makes it harder to treat infections because there arenot many antibacterials that work well against them. (16) Research in Iraq and adjacent areas indicates a rising prevalence of MDR and XDR phenotypes in K. pneumoniae isolates, especially within healthcare facilities. These patterns are presumably influenced

by antibacterial abuse, insufficient antibacterial stewardship initiatives, and horizontal gene transfer of resistance determinants among bacterial populations.

(17)(18). *Klebsiella pneumoniae* infection is a global infection and causes high mortality rates due to its high resistance to antibacterials, in addition to the blood, urinary and respiratory diseases it causes. Therefore, it has become important to find appropriate solutions (19)

anticipated molecular size of 294 bp, signifying the presence of the *klebicin gene* in all examined isolates.All 14 of the *K.pneumoniae* isolates (100%) tested positive for the *klebicin gene*. The PCR results showed that all of the samples had the same band size and intensity. This means that the *klebicin gene* was spread out among the isolates. There were no amplifications or bands that weren't specific. The fact that the *klebicin gene* was found in all of the *K. pneumoniae* isolates in this study suggests that this gene may be a common adaptation trait across clinical strains. Bacteriocins,like klebicin _like peptides,are antibacterial agents made by ribosomes that are necessary for bacteria to be competitive and survive in the environment Riley and Wertz say that bacteriocins made by Enterobacteriaceae are very important for competition because they keep closely related bacteria from growing ,which helps craete and keep niches in complex microbial communities .Bacteriocin genes are often regarded as elements of accessory genome,potentially enhancing bacterial adaptability without being essential for survival in all situations.The prevalence of the *klebicin gene* identified in this study corroborates the hypothesis that bacteriocins confer selective advantages in environment marked by elevated microbial density and competition ,such as hospitals.Riley and Wertz contend that bacteriocin producation can influence population structure and dominance within bacterial communities,thereby underscoring the potential importance of *klebicin gene* in the ecological success and pathogenicity of k.pneumoniae . (20)(21)

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