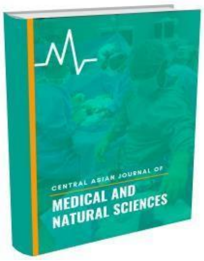

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Article

Virulence Factors of Multidrug Resistant Klebsiella pneumoniae: A Comparative Genomic and Phenotypic Analysis

Rana Jalal Shaker[*1], Asmaa Ahmed Hatem Sultan[2]

1. Assistant Professor Dr. Rana Jalal Shaker Collage of Education for pure Science. Biology Department. University, of Tikrit, Iraq
2. Assistant Professor Dr. Asmaa Ahmed Hatem Sultan., Middle Technical University, Technical Medical Institute, Baghdad, Iraq

* Correspondence: ranajalal@tu.edu.iq

Abstract: Multidrug-resistant (MDR) *Klebsiella pneumoniae* has emerged as a major global health concern due to its high virulence and resistance to antimicrobial treatment. Understanding the genomic and phenotypic characteristics of MDR *K. pneumoniae* and *Klebsiella variicola* is crucial for developing effective treatment strategies. While previous studies have focused on antibiotic resistance, comprehensive genomic and phenotypic analyses of MDR *Klebsiella* strains remain limited. This study aimed to investigate the molecular, genomic, and phenotypic characteristics of MDR *Klebsiella* isolates, focusing on serotype distribution, biofilm formation, and virulence potential. Whole-genome sequencing identified a high prevalence of O1/O2 serotypes (67%) and diverse *K* locus serotypes, with ST15-KL19 being the most frequent. The ST280-KL23 strain exhibited superior biofilm formation, while the KL105-O1/O2v2 serotype demonstrated the highest virulence in the *Galleria mellonella* model. Genomic analysis revealed the presence of key resistance genes, including *blaKPC3* and *blaOXA-48*, contributing to antimicrobial resistance. This study highlights the association between specific serotypes, virulence factors, and resistance genes, emphasizing the role of hypermucoviscosity and biofilm formation in *Klebsiella* pathogenicity. These findings underscore the necessity of continuous molecular surveillance to track emerging MDR strains and inform alternative therapeutic strategies to mitigate public health risks.

Keywords: Klebsiella Variicola, K. Pneumoniae, Virulence, Biofilm, Multidrug Resistance

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1. Introduction

Over Klebsiella parasites. There are gram-negative, opportunistic bacteria that have proven to be a major medical and public health threat. These parasites can colonize various body sites in humans and cause infection. Among these, Klebsiella pneumoniae gained prominence due to its remarkable ability to develop multidrug resistance (MDR), a major challenge in infection control Besides the K**.** [1]. Pneumoniae complex, it includes species closely related species, such as Klebsiella varicola, whose clinical significance is often underestimated due to misdiagnosis.

Klebsiella pneumoniae produces a wide variety of virulence factors that contribute to its survival, immune evasion, and pathogenicity [2]. These include:

Polysaccharide capsules, often referred to as K antigens, serve as crucial virulence factors for bacteria. These capsules play a defensive role by protecting bacteria from the host immune system, particularly by evading phagocytosis and complement-mediated killing. This ability to resist immune-mediated clearance allows bacteria to thrive and causes persistent infections. Another significant component is the Lipopolysaccharide (LPS) O antigen, which is situated in the outer membrane of gram-negative bacteria. This peripheral membrane structure enhances the host defense response and supports antimicrobial activity, contributing to the bacteria's survival and pathogenicity during infection.

Adhesins and fimbriae are surface structures that allow bacterial attachment to cell membranes, thus facilitating colonization and initiating infection. Bacteria are capable, through these structures, of adhering tightly to host tissue, a necessity for the establishment and perpetuation of infections. Siderophore iron-binding determinants are also significant virulence factors. These molecules allow for the release of iron from the host, a compound necessary for bacteria to multiply and perform metabolic functions. By obtaining this essential resource, siderophores enable bacteria to grow and maintain pathogenicity throughout the infection process.

Biofilm formation is an important characteristic that enables the survival and persistence of bacteria, particularly under adverse conditions. For instance, Klebsiella species utilize biofilm formation not just for attachment to surfaces but also as a mechanism to combat environmental stresses and antimicrobial compounds [2].

Biofilm-associated infections pose significant treatment challenges and are thought to account for around 65–80% of all bacterial infections.

The hypermucoviscosity phenotype, commonly associated with hypervirulent bacterial strains, further heightens their resistance to phagocytosis and antibiotics. This phenotype is linked to severe infections, as it significantly increases bacterial fitness and virulence, making infections caused by such strains more dangerous and difficult to treat. The acquisition of the MDR mechanism has enhanced the clinical impact of K. pneumoniae. Resistance to antibiotics, especially β-lactams, fluoroquinolones, and aminoglycosides, has been extensively documented. Although carbapenems and cephamycin are relatively efficacious, emerging resistance to these antibiotics has been reported, further limiting treatment options [3].

The coexistence of immune determinants and infection complicates the management of Klebsiella -associated infections. Genetic factors such as plasmids and transposons play an important role in the spread of resistance genes, and this interaction with commonly pathogenic chemicals underscores the importance of understanding the genetic basis of resistance and downstream of the pathogen to emphasize effective therapeutic strategies. Whole genome sequencing (WGS) of Klebsiella spp. Genomic analysis reveals significant variation in the capsular polysaccharide (K) and lipopolysaccharide (O) loci, which are important for immune response and pathogenesis. Some of the common sequences (STs) identified worldwide, such as ST15, ST147, and ST258, are associated with resistance and increased virulence Vehicle virulence develops [4].

Phenotypic analyzes complement genomic research by providing insights into the functional expression of virulence factors. For example, biofilm formation is a well-established phenotypic trait that enhances bacterial survival on biotic and abiotic surfaces. Biofilm formation in Klebsiella species varies greatly among species, with some exhibiting high biofilm-forming capacity. These changes highlight the need for targeted therapeutic interventions [5]. Hypermucoviscosity, another phenotypic characteristic, is frequently associated with infectious disease.

Those organisms with this phenotype are dynamic colonies that promote immune resistance. Hyper mucosadhesion phenotype is typically screened using the cord test, which recognizes the formation of a cord-like structure in colonies when stretched with a loop. The Galleria mellonella larval model has gained popularity as a practical model for the assessment of Klebsiella spp. The model has several benefits, such as inexpensiveness, simplicity, and relevance to human disease. Infection with highly virulent Klebsiella isolates causes high mortality of ticks, and thus the model serves as a good tool for studying pathogens [6].

Here, the virulence of Klebsiella isolates was tested in the G. mellonella model. Trees with certain serotypes and hypermucoviscosity phenotypes were found to have enhanced virulence, emphasizing the contribution of these characteristics to disease severity Such observations stress the need for detection of virulent virus for prevention oral and prioritize infection prevention and control. The discoveries of this think about demonstrate that Klebsiella spp Observing of hereditary and phenotypic variety is basic to understanding their advancement and the study of disease transmission atomic observation not as it were making a difference recognize rising clones with expanded resistance but too gives unused restorative techniques [7].

2. Materials and Methods

2.1. Klebsiella spp Strains and Development Conditions

Analysts examined drugresistant strains of Klebsiella to get it their harm and their separation A add up to of 21 tests were assessed counting 19 Klebsiella pneumoniae strains and 2 Klebsiella varicola strainsThese samples were chosen because they differed in their resistance to antibiotics, which is important to understand their potential for infection [8]. The bacteria were termed multidrug resistant (MDR) because they were able to survive against a wide range of antibiotics. This condition can cause serious complications in the treatment of infection. To obtain reliable results, the bacteria are grown on a special nutrient plate called Trypticase Soy Agar (TSA) at 37 °C for 24 h This condition is optimal for their growth. TSA was chosen because it supports the growth of a wide range of bacteria, especially Klebsiella, without the risk of altering their symptoms [5]. The bacteria were specifically selected based on antibiotic resistance and carrying of various genes that contribute to treatment resistance This is important given the growing concern about antibiotic resistance in clinical settings. By focusing on bacteria with different resistance levels, the researchers aimed to identify important genes and traits associated with harmful MDR Klebsiella strains The study could help to we have understood how these viruses affect public health and how to effectively combat those diseases

2.2. Whole Genome Sequencing

Entirety Genome Sequencing WGS was done on all the Klebsiella tests to urge nitty gritty hereditary data DNA was taken from each test measured and after that sequenced employing a strategy that peruses 250 base sets at a time This method produced highquality genome information which was utilized to analyze single nucleotide polymorphisms SNPs within the primary genome SNPs offer assistance us get it hereditary contrasts and the connections among the tests The genome information were submitted to open databases PRJNA1023834 for advance ponder and future utilize making beyond any doubt that total hereditary data is accessible for comparisons and following wellbeing patterns [9].

2.3. In Silico Typing

The genomes of Klebsiella microbes were analyzed utilizing computational devices for critical hereditary data such as species sort grouping ST surface proteins and qualities related with illness resistance Set up databases were utilized to accurately recognize and relegate species arrangements which made a difference to get it their hereditary foundation Surface proteins such as O and K antigens have moreover been distinguished making a difference to characterize the viral phenotype The consider too looked for particular qualities that are hurtful to microscopic organisms such as yersiniabactin colibactin and aerobactin These qualities are basic for the disease To get it anti-microbial resistance a scoring framework was utilized to classify microscopic organisms based on the nearness of resistance qualities Scores extended from to 5 with demonstrating no resistance and higher scores showing more extreme resistance such as the capacity to break down carbapenems or resistance to colistin This comprehensive genomic investigation given important experiences esteem on qualities contributing to Klebsiella hurt and sedate resistance Got that to oversee diseases And vital for control of the contamination.

2.4. Phylogenetic Analysis

The scientists analyzed the relationships between Klebsiella strains by comparing their essential genes, which all produce the same essential genes, using a tool called the Rory Pipeline to compare genes the series of the ho. By sequencing the genes, they created a tree-like diagram that can show how bacterial species are related to genetic relationships. This image was created by another tool called FastTree, which helped them identify similarities or differences between the bacteria. Using FigTree software, they were able to clearly demonstrate genetic diversity and relationships among Klebsiella species. This has helped them collect the bacteria and understand their evolutionary history, including how traits such as antibiotic resistance can spread across species [10].

2.5. Hypermucoviscosity Phenotype

The hypermucoviscosity HMV characteristic was tried employing a straightforward strategy called the string test This test checks on the off chance that Klebsiella microscopic organisms produce thick sticky fabric Within the test we develop the bacteria on a extraordinary sort of agar and after that extend them with a clean apparatus In case the microscopic organisms make sticky strings longer than 5 mm it implies they have HMV These strings are made of materials that make the microscopic organisms thicker [10]. The string test is a straightforward way to discover out on the off chance that Klebsiella microbes have this characteristic which is related to their capacity to cause more genuine diseases A positive result from the string test appears that the microbes have characteristics that make them more unsafe making a difference us get it how genuine Klebsiella contaminations [10].

2.6. Biofilm Formation

To memorize how microbes make biofilms we developed them in a extraordinary fluid with included sugar To begin with we cleaned the microbes and made beyond any doubt each sort had the same number of cells coming to 1 million cells in each milliliter At that point we included 200 microliters of these microscopic organisms to wells in a 96well plate each filled with the sugar fluid We kept the plates at 37 degrees Celsius for 24 hours so the microbes seem adhere and frame biofilms We too had a well with fair the fluid and no microbes to see in case the fluid itself would make any signals After recoloring the biofilms with a color called gem violet we measured how much light was retained at a particular wavelength We rehashed this test at slightest three times on diverse days with six rehashes each time to guarantee our comes about were rectify This strategy made a difference us discover out which microscopic organisms strains are great at shaping biofilms which is imperative for understanding how Klebsiella microbes can cause diseases and survive in clinics.

2.7. Biofilm Biomass Quantification

Biofilm biomass was measured employing a strategy with precious stone violet color After 24 hours the biofilms were washed with a salt arrangement to evacuate any free cells At that point the biofilms were settled with methanol and airdried Another precious stone violet color was included to recolor the biofilms for 5 minutes The biofilms were washed twice with sterile water to induce freed of additional color After that 200 microliters of acidic corrosive was included to the recolored biofilms The color concentrated was measured at 570 nm employing a microplate peruser This test was rehashed at slightest three times on diverse days with six tests each time to guarantee the comes about were dependable This strategy made a difference degree the biofilm biomass clearly and compare the biofilmforming capacity of diverse Klebsiella segregates.

2.8. Enumeration of Bacteria in Biofilms

To discover out how numerous microbes can develop from the biofilm we to begin with scratched the biofilm off the wells of a 96well plate without utilizing any microscopic organisms At that point we blended the biofilm cells in a saltwater arrangement to form a uniform blend Another we weakened this blend ten times making distinctive qualities from 10000 to 1000000000 We took 10 microliters from each weakening and put it on extraordinary plates called trypticase soy agar or TSA and kept them at 37 degrees Celsius for 24 hours After that we checked how numerous colonies shaped which appeared how many live microscopic organisms were within the biofilm We did this prepare at slightest three times to create beyond any doubt the comes about were accurate and dependable Checking the colonies made a difference us get it the nearness of live microscopic organisms within the biofilm counting Klebsiella species.

2.9. In Vivo Virulence Assay

The potency of the Klebsiella bacterium was tested using Galleria mellonella larvae, which is a common strain used to study the virulence of the bacterium. In this experiment, samples of various viruses were prepared and stored at specific concentrations before being inoculated into the mosquitoes. Each larva was given 5 microliters of virus, and the control group received a virus-free saline solution. The mosquitoes were observed for three days to assess survival rate and check their health daily. The researchers recorded things like how active the larvae were, whether they were cocooned, and color change. Larvae that were less active, took longer to pupate, and showed greater color change were in poorer health, indicating greater bacterial injury Survival and health outcomes helped identify less dangerous strains greater than. To ensure consistency of results, experiments were performed in triplicate, and statistical analysis was used to detect significant differences in survival rates This model helped to understand the injurious nature of Klebsiella isolates and underestimate their infectiousness.

2.10. Statistical Analysis

Statistical analysis for in vivo models was performed using one-way ANOVA, nonparametric tests, and Kaplan-Meier survival curves. Differences at $P < 0.05$ were considered statistically significant..

3. Results

Chronic In this study, we looked at 21 out of 102 Klebsiella samples that had been tested for resistance genes. These 21 strains were analyzed using whole genome sequencing (WGS), which helped us understand their genetics and how they spread. Two of the samples, originally thought to be Klebsiella pneumoniae, were found to actually be Klebsiella varicola, but we kept them in the study because they are similar. This shows that current testing methods can be misleading. The genome sizes of the 21 samples varied from 54 to 59 megabases. We also looked for links between these samples, their resistance types, and specific traits. We identified different groups of the bacteria, with ST15 being the most common (19% of samples), followed by ST11 (14%), and others like ST17, ST147, ST454, ST10, ST14, ST45, ST277, ST280, ST307, and ST294 (about 5% each). This shows that these isolates have a lot of genetic variety, which can affect their resistance and ability to cause disease. We found that some isolates had certain types of proteins (O antigens) linked to highly toxic Klebsiella pneumoniae strains, showing their role in causing disease. Four isolates had strong resistance genes, including blaKPC3 and blaOXA 48, which are related to resistance against carbapenems, a type of strong antibiotic. Some isolates that were part of ST454 and ST277 showed resistance to rifampicin and carbapenems, even with a mild resistance score.

The study also determined an association between certain genetic characteristics and pathogenicity-enhancing factors. For instance, certain virulent strains carry genes that facilitate iron uptake, formation of protective capsules, and adhesion to surfaces. These correlations are quite pronounced in sequence types ST15 and ST454, which are characterized by genes associated with infection and robust immune responses. In general, the study highlights the necessity of rigorous genetic analysis in deciphering the mechanisms by which drug-resistant Klebsiella pneumoniae leads to disease. The findings also indicate that conclusions obtained from these genetic tests can predict the efficacy of bacterial resistance to treatment and direct the selection of more effective treatment.

Table 1. MIC Profiles of Multidrug-Resistant *Klebsiella pneumoniae* Strains (1162281 vs. JH1).

Antibiotic	Class	Strain 1162281 MIC (μg/ml)	Strain JH1 MIC (μg/ml)	Resistance Level
Gentamicin	Aminoglycoside	16	2	Higher in 1162281
Chloramphenicol	Chloramphenicol	>64	8	Higher in 1162281
Trimethoprim	Diaminopyrimidine	>64	1	Higher in 1162281
Ciprofloxacin	Quinolone	64	0.03	Higher in 1162281
Levofloxacin	Quinolone	64	≤0.06	Higher in 1162281
Moxifloxacin	Quinolone	64	0.06	Higher in 1162281
Norfloxacin	Quinolone	>64	0.125	Higher in 1162281
Ofloxacin	Quinolone	64	0.125	Higher in 1162281
Azithromycin	Macrolide	32	16	Higher in 1162281
Telithromycin	Macrolide	64	64	Equal
Erythromycin	Macrolide	>64	>64	Equal
Tetracycline	Tetracycline	8	4	Higher in 1162281
Cefotaxime	Cephalosporin	2	0.125	Higher in 1162281
Ceftriaxone	Cephalosporin	8	0.125	Higher in 1162281
Ceftazidime	Cephalosporin	>64	0.25	Higher in 1162281
Cephalexin	Cephalosporin	16	4	Higher in 1162281
Cefuroxime	Cephalosporin	32	8	Higher in 1162281
Imipenem	Carbapenem	0.25	0.5	Higher in JH1
Meropenem	Carbapenem	≤0.06	≤0.03	Higher in 1162281
Fusidic Acid	Fusidane	>64	>64	Equal
Vancomycin	Glycopeptide	>64	>64	Equal
Polymyxin B	Polymyxin	1	4	Higher in JH1

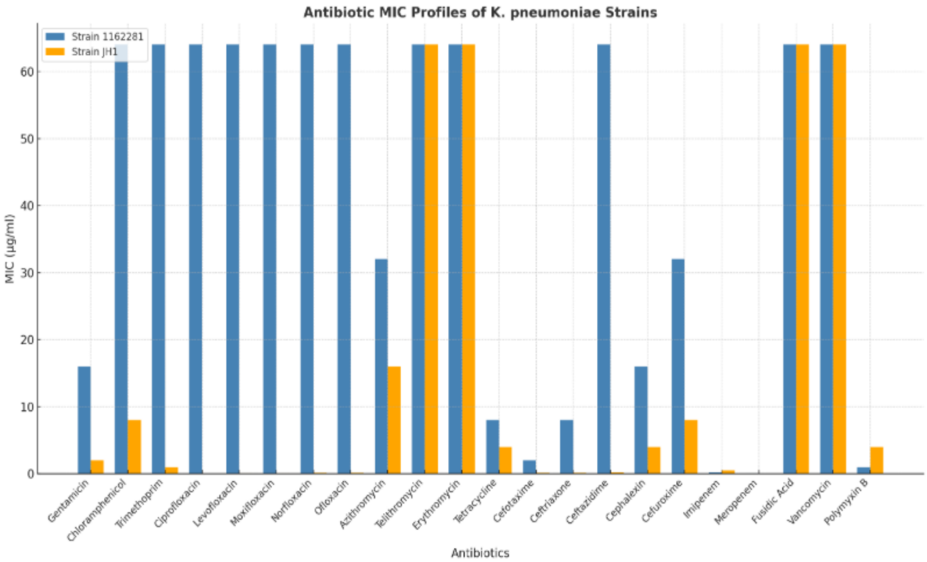
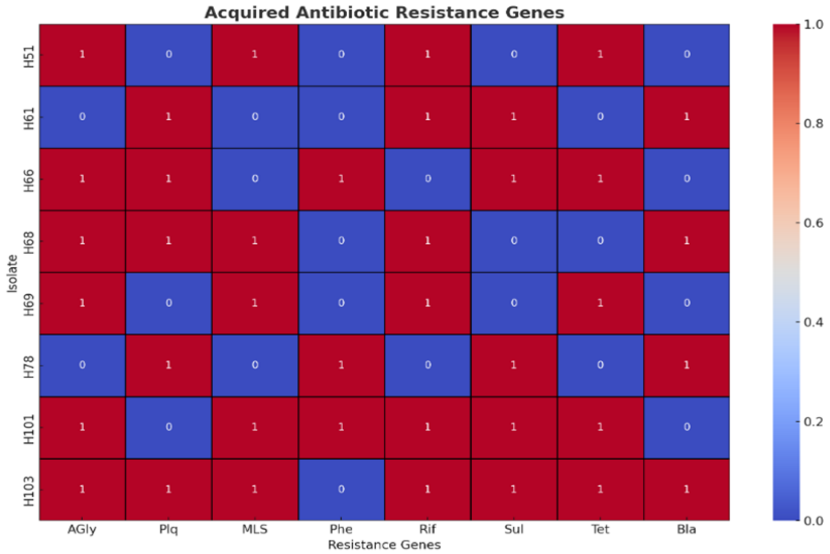


Figure 1. Antibiotic MIC profiles of k.pneumonae strain.



Isolate	AGly	Plq	MLS	Phe	Rif	Sul	Tet	Bla
H51	1	0	1	0	1	0	1	0
H61	0	1	0	0	1	1	0	1
H66	1	1	0	1	0	1	1	0
H68	1	1	1	0	1	0	0	1
H69	1	0	1	0	1	0	1	0
H78	0	1	0	1	0	1	0	1
H101	1	0	1	1	1	1	1	0
H103	1	1	1	0	1	1	1	1

Figure 2. Heatmap Acquired Antibiotic resistance genes.

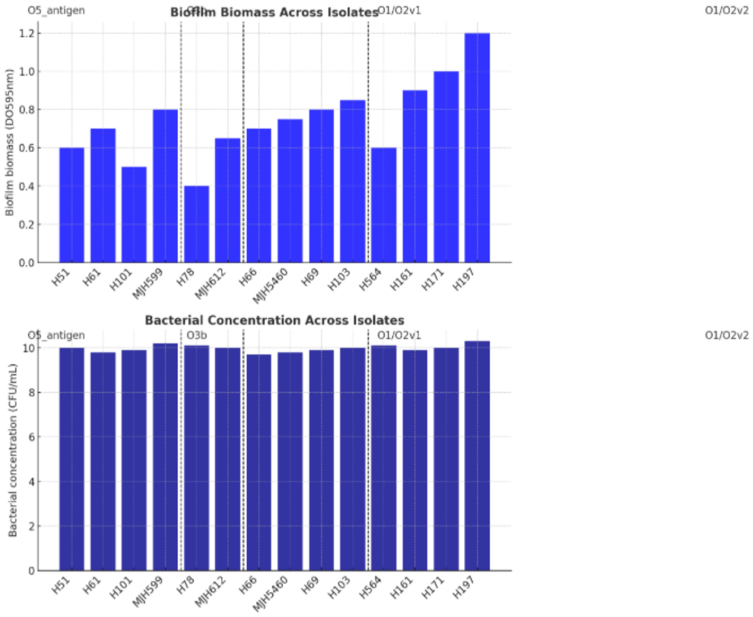


Figure 3. ANOVA analysis.

The two charts compare distinctive sorts of microbes based on how much biofilm they create and how numerous microbes there are The primary chart appears that a few microbes like H171 and H197 make a part of biofilm whereas others like H101 and MJH599 make much less The moment chart appears that the number of microscopic organisms is decently comparative over all sorts with as it were slight contrasts This implies that having a part of microscopic organisms doesnt continuously cruel they will deliver a part of biofilm Other things like particular chemicals the microscopic organisms discharge their qualities and the environment they are in moreover play a part in how biofilms frame These discoveries propose that there are other critical components in biofilm arrangement other than fair the number of microbes Understanding typically imperative since biofilms can cause enduring wellbeing issues and make it harder to treat diseases with anti-microbials Klebsiella microbes particularly K pneumoniae and K variicola are genuine wellbeing dangers since they can cause genuine diseases and gotten to be safe to anti-microbials To handle this issue we require a wellrounded arrange that incorporates teaching the open Campaigns to raise mindfulness instructive programs and particular activities can offer assistance control the spread of Klebsiella microscopic organisms diminish anti-microbial resistance and make strides wellbeing results Information is key in battling diseases Instructing individuals approximately Klebsiella contaminations makes a difference them take steps to avoid the spread of these germs One of the finest ways to do typically by promoting good cleanliness like washing hands appropriately keeping shared spaces clean and taking after nourishment security rules These hones are straightforward but viable in bringing down diseases within the community and facilitating the burden on healthcare frameworks [11].

Antimicrobial stewardship is exceptionally imperative within the battle against anti-microbial resistance and teaching the open is key to making it work Klebsiella microscopic organisms can effortlessly ended up safe to numerous anti-microbials making contaminations harder to treat By instructing individuals around the dangers of misusing antibioticslike not taking the total measurements utilizing them without a medicine or pushing specialists for superfluous prescriptionswe can empower dependable utilize and offer assistance keep medications compelling Getting communities involved is crucial for any open wellbeing program Working in conjunction with neighborhood bunches schools and healthcare suppliers can spread mindfulness and make instructive programs more viable For occurrence schools can hold workshops to assist kids learn around cleanliness and contamination avoidance making long lasting solid propensities Also community wellbeing centers can run sessions for grown-ups to clear up misguided judgments almost anti-microbials and share commonsense tips for remaining solid Healthcare specialists moreover have an critical part in teaching the community They can offer assistance translate scientific data into easytounderstand messages approximately anticipating and treating Klebsiella contaminations Preparing programs for healthcare experts can allow them the aptitudes required to associate with distinctive groups of onlookers guaranteeing that imperative data comes to as numerous individuals as conceivable.

4. Discussion

In todays advanced world media and innovation give imperative instruments for teaching the open Social media websites and versatile apps can spread data rapidly to numerous individuals These stages can share things like infographics recordings and articles approximately Klebsiella contaminations in ways that are simple for diverse bunches of individuals to get it counting in different dialects For case brief recordings appearing how to wash hands appropriately or clarifying the perils of abusing anti-microbials can lock in individuals superior than conventional strategies Portable wellbeing apps can moreover make a huge contrast They can remind individuals to require their pharmaceutical track their side effects or give accommodating data making a difference people oversee their wellbeing way better Moreover intuitively stages permit clients to inquire questions and get individual exhortation from healthcare specialists In any case open instruction faces a few challenges One major issue is deception which can spread rapidly online Tending to myths almost Klebsiella diseases and anti-microbials requires collaboration from open wellbeing authorities media and teachers Making exact and curiously substance and redressing wrong data is vital Another challenge is coming to individuals who may not have simple get to healthcare or education Its fundamental to form programs that consider people groups social foundations dialects and budgetary circumstances Community wellbeing specialists and nearby pioneers can offer assistance plan and execute these programs to way better interface with particular bunches Public education is important, but healthcare professionals have an important role to play in combating Klebsiella infection.

It is necessary that these practitioners be updated on the newest diagnostic methods, therapeutic approaches, and preventative interventions to provide beneficial assistance. There should be an ongoing education module to keep clinicians up to date on emerging risk factors and optimal practices. Such training might cover the diagnosis and treatment of Klebsiella infections, antibiotic use, and clinical infection control practices. By disseminating this information to medical practitioners, we can enhance screening, enhance patient care, and limit the spread of antibiotic-resistant.

5. Conclusion

Bacterial serotype analysis and the respective characteristics provided ample information on virulence, virulence, and antimicrobial resistance patterns In this research, we found that O1/O2 serotypes were prevalent in tested isolates in 2010, and accounted for 67% of total cases (14 of 21 extracted) Below is a chart illustrating that, thus these serotypes are prevalent in the community and the hospital. These variations reinforce the notion that O1/O2 serotypes are an important area to study and research for understanding Klebsiella infections.

Apart from the number of O1/O2 serotypes, the research demonstrated vast diversity among K locus (KL) serotypes, which are very important for capsular polysaccharide genes synthesis. This diversity is indicative of the genetic and phenotypic diversity typical for the Klebsiella genus, as it is noted. Among the recognized K locus serotypes, the most frequent was K19, present in 4 of 21 isolates and bearing a close relation to the O1/O2v2 serotype. The presence of the K19 repeat in O1/O2v2 identifies a promising correlation that would be worth exploring in greater depth, especially its significance for immune evasion and toxicity. Capsule polymer, whose locus is K, is very important in shielding viruses against the host immune system, thereby helping them survive in the host and aiding in the elimination of infectious pathogens. In this study, we found that the K19-related isolates have unique phenotypic characteristics, namely evasion of host defenses and through additional studies that may improve the virulence of the pathogen It may become evident whether the predominance is due to selective pressure in specific regions or is a global trend in Klebsiella populations worldwide. One particularly notable isolate in the O1/O2v2 serotype group, ST10-KL151 K. varicola (strain H97), displayed a broad set of virulence genes Known to encode factors such as siderophores, adhesins, and immune modulators, these genes play an important role in enhancing bacterial survival and virulence Despite high virulence gene content, strain H97 showed no direct correlation with biofilm-forming capacity, hypermucosa viscosity, or resistance scores This finding crosses the traditional assumption that multiple virulence genes need to be expressed down to a more virulent phenotype is not challenged.

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