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Virulence Factors of Multidrug Resistant Klebsiella Pneumoniae: a Comparative Genomic and Phenotypic Analysis

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Abstract: Multidrug-resistant (MDR) Klebsiella spp. poses a significant challenge to hospitals worldwide. The aim of this study was to investigate the molecular and phenotypic characteristics of MDR Klebsiella pneumoniae and Klebsiella varicola isolates. Whole-genome sequencing (WGS) using in vitro assays was used to assess biofilm formation and in vivo pathogenicity assessment using a Galleria mellonella larval model

Our findings revealed a high prevalence of O1/O2 serotypes (67%), consistent with previous global data. Different K locus serotypes were identified, with ST15-KL19 being the most common (19%) and most closely associated with O1/O2v2 serotype Notably, a widespread Klebsiella varicola strain of O1/O2v2 serotype (ST10-KL151) was responsible the system went into bacterial genes . Significant differences in biofilm formation capacity were observed among species. One K. pneumoniae strain, ST280-KL23, showed more positive biofilms than the other nine isolates. In terms of infection, KL105-O1/O2v2 serotype exhibited the highest virulence, causing the most significant mortality in the Galleria mellonella sample

These data: MDR Klebsiellas pp. The study highlights the critical importance of continued molecular surveillance to identify key drug pathogens and alternative therapeutic strategies. Klebsiella parasites. There is a need to develop effective treatments for this multidrug-resistant pathogen and minimize public health impacts.

Keywords: Klebsiella variicola, K. pneumoniae, Virulence, Biofilm, Multidrug resistance

Introduction:-

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.**(Abdel-Wahab F,2013)** pneumoniae complex, it includes species closely related species, such as Klebsiella varicola, whose clinical significance is often underestimated due to misdiagnosis.

Key Virulence Factors in *Klebsiella* spp.

Klebsiella pneumoniae produces a wide variety of virulence factors that contribute to its survival, immune evasion, and pathogenicity**(Gorrie CL, Mirčeta M,2022)** These include:

1. **Polysaccharide Capsule (K Antigen):** Capsule is an important virulence factor that protects bacteria from host immune responses, especially phagocytosis and complement-mediated killing
2. **Lipopolysaccharide (LPS) O antigen**: This component of the peripheral membrane enhances host defense and antimicrobial activity.
3. **Adhesins and fimbriae:** These surface structures facilitate bacterial membrane attachment and establishment of infection.
4. **Iron binding siderophores:** Siderophores enable bacteria to secrete iron from the host, and are required for bacterial growth and metabolism during infection
5. **Biofilm formation:** The ability to generate biofilms is an important virulence trait, enabling Klebsiella spp**(Gorrie CL, Mirčeta M,2022)** . For adherence to surfaces and resistance to environmental stressors and pesticides. Biofilm-associated infections are particularly difficult to treat and account for an estimated 65–80% of bacterial infections.
6. **Hypermucosal viscosity phenotype:** Commonly associated with hypervirulent strains, this phenotype increases bacterial resistance to phagocytosis and antibiotics, contributing to severe infection

Multidrug Resistance and Its Implications

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 **(Daef EA,2012)**

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.

Genomic Insights into *Klebsiella* spp.

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 **(Li W, Sun G,2014)**

Phenotypic Analysis of Virulence Traits

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**. (Zhan L, Wang S,2017)**

Hypermucoviscosity, another phenotypic characteristic, is frequently associated with infectious disease. Plants exhibiting this phenotype are active colonies and enhance immune resistance. The hypermucosadhesion phenotype is usually determined by the cord test, where the colonies form a cord-like structure when stretched with a loop

Pathogenicity in In Vivo Models

The Galleria mellonella larval model has emerged as a valuable system for testing Klebsiella spp. This model offers several advantages, including cost-effectiveness, ease of implementation, and relevance to human diseases. Infection with highly pathogenic Klebsiella isolates results in high tick mortality, making this model a reliable tool for pathogen studies **(Lee C-R,2017).**

In this study, the pathogenicity of Klebsiella isolates was evaluated using the G. mellonella model. Trees with specific serotypes and hypermucoviscosity phenotypes showed increased virulence, highlighting the role of these traits in disease severity Such findings highlight the importance of virulent virus detection for prevention oral and emphasizes infection prevention and management.

Continuous Molecular Surveillance

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 makes a difference recognize rising clones with expanded resistance but too gives unused restorative techniques **(Shah RK, Ni ZH, Sun XY, Wang GQ, Li F,2017)**

Material 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 **(Rastegar S, Moradi M,2019).** 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 **(Zhang Y, Zhao C,2016).** The bacteria were specifically selected on the basis of 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 **(Soltani E,2020)**

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**(Luo Y, Wang Y, Ye L, Yang J,2014)**

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 **(Luo Y, Wang Y, Ye L, Yang J,2014)**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 **(Luo Y, Wang Y, Ye L, Yang J,2014)**

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 and discussion

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 research also linked specific genetic features to factors that increase disease-causing ability. For example, some aggressive strains had genes that help them gather iron, form protective capsules, and stick to surfaces. This interaction is especially clear in ST15 and ST454, where infection-related genes and strong immune responses are present. Overall, this study emphasizes the importance of detailed genetic analysis in understanding how drug-resistant Klebsiella pneumoniae can cause disease. The data also suggest that information from these genetic analyses can help predict how well the bacteria can resist treatments and help in choosing better therapies.

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

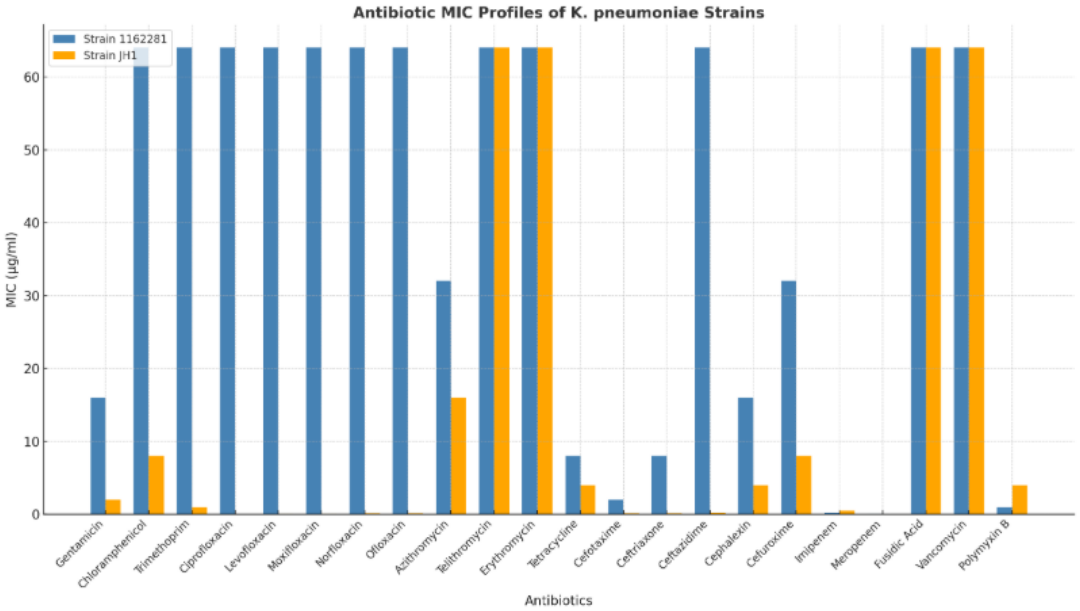

Fig1. Antibiotic MIC profiles of k.pneumonae strain

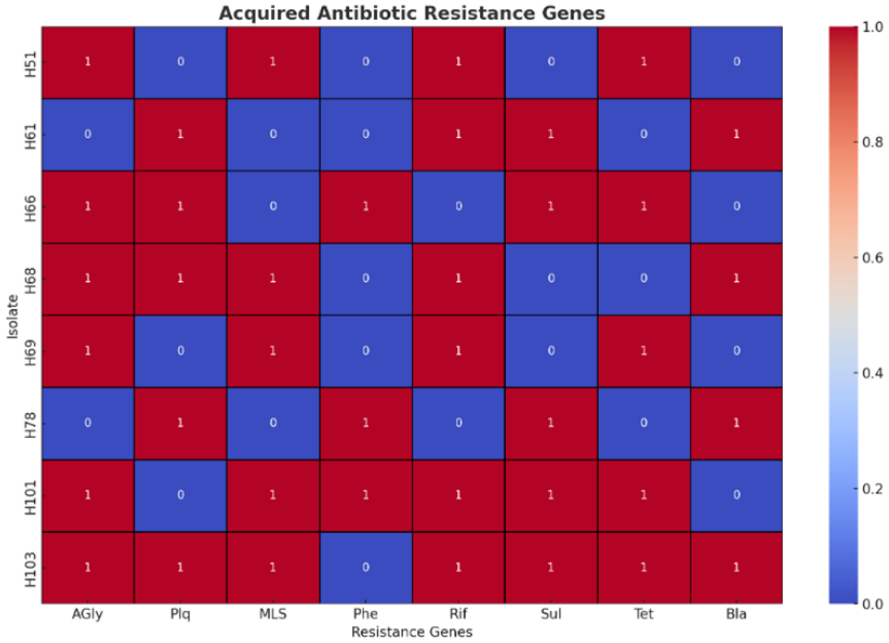

Fig2: heatmap Acquired Antibiotic resistance genes

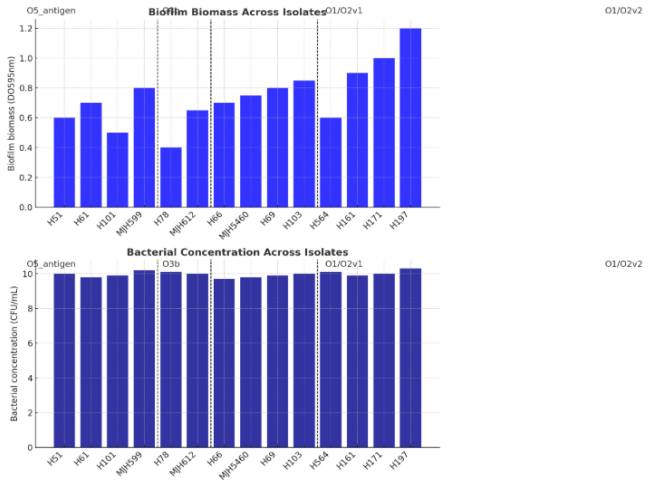

Fig 3 anova analysis

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**(Wu H, Li D, Zhou H, Sun Y, Shen D.,2014)**

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

The Role of Media and Technology

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 important that these professionals are aware of the latest diagnoses, treatments and preventive measures so they can help effectively. We need an ongoing training program to keep healthcare professionals aware of new risk factors and best practices. Training may include topics such as the recognition and treatment of Klebsiella infection, judicious use of antibiotics, and clinical infection control. By sharing this knowledge with healthcare providers, we can improve screening, improve patient care, and reduce the spread of antibiotic-resistant .

Conclusion:-

Analysis of bacterial serotypes and their associated characteristics provided valuable insights into virulence, virulence, and antimicrobial resistance profiles This study found that O1/O2 serotypes were prevalent among isolates tested in 2010, and were 67% of all cases (14 of 21 extracted) This chart shows that so these serotypes are common in the community and hospital. Such differences reinforce the notion that O1/O2 serotypes are an important focus of research and investigation in understanding Klebsiella infections.

In addition to the number of O1/O2 serotypes, the study found remarkable variation in K locus (KL) serotypes, a key component of capsular polysaccharide synthesis genes This variation reflects the genetic and phenotypic diversity of the Klebsiella genus which is emphasized. Among the identified K locus serotypes, K19 emerged as the most prevalent (4 of 21 isolates), most closely related to the O1/O2v2 serotype. The inclusion of the K19 repeat in O1/O2v2 highlights a potential link that deserves further investigation, particularly in terms of its importance for toxicity and immune evasion The capsule polymer encoded by locus K plays an important role in protecting viruses from the host immune system, facilitating their persistence in the host and clearing infectious agents high This study found that K19-related isolates exhibit unique phenotypic characteristics, particularly in terms of the ability to avoid the host defenses and by further investigation that may contribute to the severity of the pathogen is increased It could be clarified whether this dominance is due to selective pressure in specific regions or reflects a broad 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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