RESEARCH PAPER

Antibiotic Resistance in *Klebsiella pneumoniae*: Biofilm Formation, Fimbriae, and Virulence Factors – A Comprehensive Review"

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How To Cite This Article: Joshi F, Shrivastava P, Gwamaka P, Rohinkar Ad. Antibiotic Resistance In Klebsiella Pneumoniae: Biofilm Formation, Fimbriae, And Virulence Factors – A Comprehensive Review. Int J Drug Deliv Technol. 2026;16(27s):630-644. Doi: 10.25258/ijddt.16.27s.73

INTRODUCTION

1.1. BACKGROUND OF THE STUDY

Klebsiella spp. are ubiquitous in nature. ***Klebsiella*** probably have two common habitats, one being the environment, where they are found in surface water, sewage, and soil and on plants and the other being the mucosal surfaces of mammals such as humans, horses, or swine, which they colonize. In this respect, the genus ***Klebsiella*** is like *Enterobacter* and *Citrobacter* but unlike ***Shigella spp***. or ***E. coli***, which are common in humans but not in the environment (**Podschum, R; Ullmann et al., 1998**).

Bacteria belonging to the genus **Klebsiella** frequently cause human nosocomial infections. In particular, the medically most important **Klebsiella** species, **Klebsiella pneumonia**, accounts for a significant proportion of hospital-acquired urinary tract infections, pneumonia, septicemias, and soft tissue infections. The principal pathogenic reservoirs for transmission of **Klebsiella** are the gastrointestinal tract and the hands of hospital personnel. Because of their ability to spread rapidly in the hospital environment, these bacteria tend to cause nosocomial outbreaks. New types of strains, known as extended-spectrum-β-lactamase (ESBL) producers, often cause hospital outbreaks of multidrug-resistant Klebsiella spp., especially in neonatal wards. The incidence of ESBL-producing strains among clinical **Klebsiella** isolates has been steadily increasing over the past years. The resulting limitations on the therapeutic options demand new measures for the management of **Klebsiella** hospital infections. While the different typing methods are useful epidemiological tools for infection control, recent findings about **Klebsiella** virulence factors have revealed novel insights into the pathogenic strategies of these bacteria. **Klebsiella** pathogenicity factors such as capsules or lipopolysaccharides are presently considered to be promising candidates for vaccination efforts that may serve as immunological infection control measures (**Janda, J. M; Abbott, S. L et al., 2006**).

Several studies indicate a significant relationship between biofilm formation and antimicrobial resistance in **Klebsiella pneumoniae**. To overcome the problematic biofilms and factories, a comprehension of the interaction of the materials is necessary.

K. pneumoniae is able to form a dense film of extracellular biofilm to safeguard and minimize the impact of antibiotics by promoting bacterial attachment to living or non-living surfaces (Shadkam, S., et al., 2021). Capsule polysaccharides and fimbriae types 1 and 3 are crucial virulence factors contributing to biofilm formation in **K. pneumoniae**.

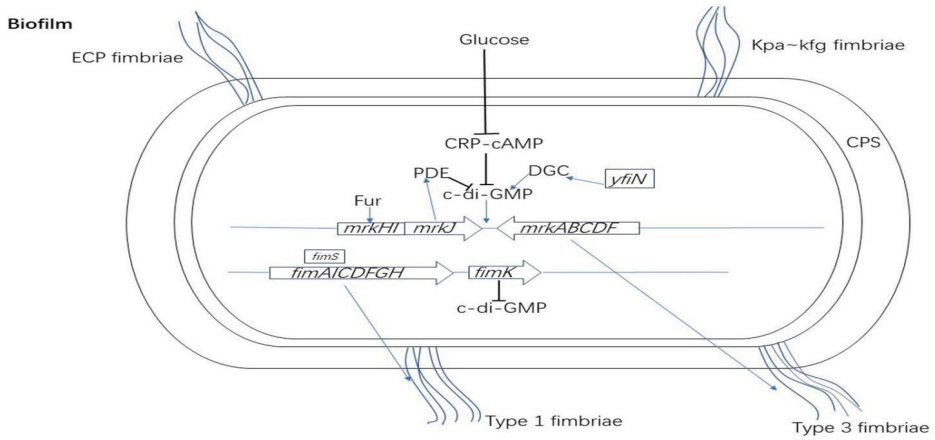

Figure 1: shows the systemic mechanisms stages of biofilm formation (Li, Y. & Ni, M. (2023)).

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1.2. EPIDEMIOLOGY IN INDIA: CASE STUDY

The epidemiology of **Klebsiella** infections in India and other countries is closely linked to virulence, antibiotic resistance, and diagnostic reporting. In addition to highlighting the changing reporting mechanisms that influence our comprehension of burden and spread, the body of work presented here charts a progression from the emergence of virulent and resistant Klebsiella strains in India to more extensive regional and global patterns of hospital-associated disease.

The rise of hypervirulent strains that acquire virulence plasmids and carbapenemase-producing capacities is highlighted by **Shankar et al. (2022),** which highlights the convergence of antimicrobial resistance and virulence within ***Klebsiella pneumoniae*** populations in India. The argument presented in this paper states that this collection of multidrug-resistant hyper-virulent organisms crosses the clonal borders as the resistance genes are transferred into the chromosomal background which increases the minimal resistance. The virulence and resistance that has been disseminating across various clones as the scientists witnessed is a worldwide one, and containment measures to the problem are required, which is specific to the various microbial settings of India.

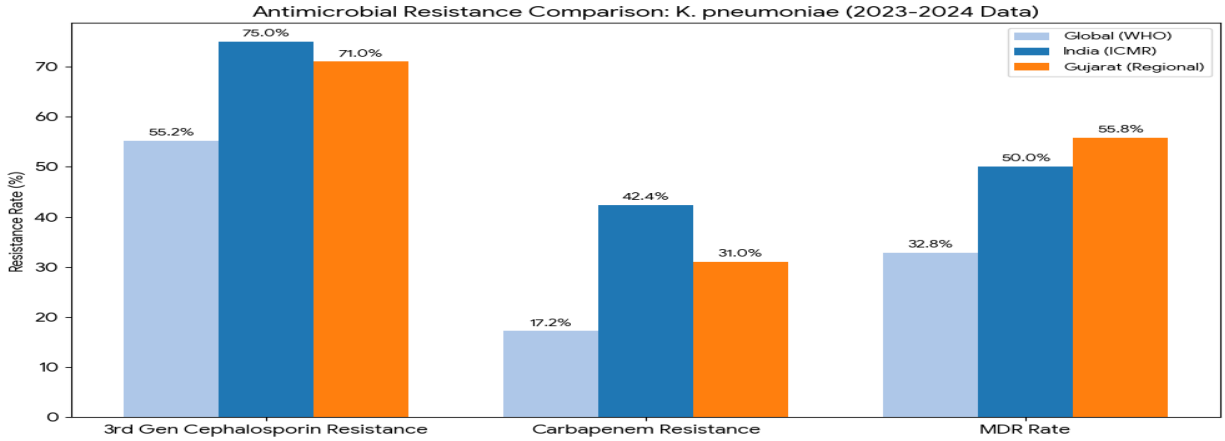
According to local epidemiology, (**Lin et al., 2023**) provide both international and national surveys of hospital-acquired carbapenem-resistant Klebsiella pneumoniae (CRKP). It is clear in their meta-analysis that utilized a vast amount of nosocomial data, that CRKP is the cause of most of the hospital related Klebsiella infections across the globe, with India showing that such infections are prevalent. According to them, in part, they describe this indicator by the differences in ecological niches and mosaic genomic architecture existing in Indian healthcare structures. They also illustrate high provincial heterogeneity in other states and this can be used as an indication that the burden and transmission of CRKP are determined by the issues that are particular to both states and regions.

Moving further to more general African and Ethiopian contexts, (**Gebremeskel et al., 2023) and (Kijineh et al., 2024**) describe the issue of the enduring resistance in Ethiopia and incessant percentages of plasmid-mediated resistance in Klebsiella species, which are the primary cause of Gram-negative infections. Determine the current occurrence of phenotypic multidrug-resistant Klebsiella in hospital-related samples, take into account regional and specimen-type variations and re-examine the general increase in ESBL-producing Klebsiella in the majority of infections and circumstances. Both of the studies relate resistance to the healthcare exposure and a high use of antibiotics but are limited due to the limitations of methods used that cannot be further generalized.

In addition, the analysis touches upon the meeting of the Klebsiella with new clinical conditions like co-infection with SARS-CoV-2. (**de Lima das Chagas et al., 2024**) discusses the prevalence and the resolution of co-infection of the Klebsiella and the COVID-19 patients admitted to the hospital. Work suggests that diagnostic and treatment interventions need to be put on watch in the state of high burden, such as in the case of India, and helps to comprehend the role of the Klebsiella in the evolving epidemiological situation of the interaction of viruses and bacteria, although it is dedicated to the COVID-19 era.

The literature has generally presented some areas of concern to the epidemiological situation in India. Firstly, virulence and antibiotic resistance can coexist and be transferred among different lineages of Klebsiella that makes it more difficult to treat and contain. Second, hospital-based transmission is a major contributor to CRKP and India has demonstrated a remarkable prevalence and geographical dispersion of such transmission and this necessitates certain surveillance and control. Third, reported prevalence and resistance patterns are determined by only methodological differences and resource constraints, but in Ethiopian and African data, which, despite these constraints, show global trends, reporting procedures and diagnostic skills affect our perception of burden. The necessity to introduce the infection control and antimicrobial stewardship in India, as well as in other similar cases, is further explained by the coinfection setting, i.e. with SARS-CoV-2, which introduces the dynamic clinical setting in which the Klebsiella infections and the evolving management of respiratory diseases interact.

By synthesis, the review places India-Gujarat into the global epidemiological context of acknowledging the importance of strong reporting and surveillance systems, clonal diversities and resistance and virulence phenotypes, and CRKP overload. In Gujarat and others, integrated learning can be adopted to carry out directed research on the molecular epidemiology of infections and reporting and regional patterns of transmission that may be utilized in informing effective infection control, antimicrobial stewardship, and policy interventions.



1.3. GLOBAL SPREAD

The emergence of resistance in Enterobacteriaceae is a big threat in the 21st century. In most countries the resistance in Enterobacteriaceae is rising rapidly against third-generation antibiotics. There has been a 5-fold increase in CRE infections in USA between 2008 and 2012; similarly, carbapenem-resistant K. pneumoniae has increased by 30% in Europe. More countries affected are Saudi Arabia, Egypt, Iraq, South East Asia, Europe, and North America. The threats posed in the above-mentioned countries are carbapenem-resistant K. pneumoniae, MDR- E. coli, and Proteus spp. In Greece and Italy, CRE infections are endemic. (Mohammad, K. S et al., 2005)

Epidemiological studies of genome analysis reveal quick genome changes. Though it might sometimes lead to misinterpretations, it is a generally very effective way of studying quick genome changes and point mutations to pinpoint gene changes. (Moser, A. I., et al. (2022).

East and Southeast Asia, particularly Taiwan and China, predominantly exhibited the most common strains of hypervirulent Klebsiella pneumoniae. However, it is now emerging globally. (Russo, T. A., & Marr, C. M. (2019).

These resistant strains in livestock mirror human infections. The most common reservoirs include water and farm waste and even cattle waste. Such behaviour leads to easy spread of resistance across borders. (Mohammad, K. S et al., 2005)

Figure 2: the global spread of Klebsiella pneumonia

2.1. CLASSIFICATION OF KLEBSIELLA PNEUMONIAE BASED ON STRAIN

Due to ongoing research into genetic diversity and the pathogenic potential of the different **Klebsiella pneumoniae** strains, this bacterium has become very difficult to classify. As such, Wesley Long et al. conducted a comprehensive whole-genome sequencing analysis on **Klebsiella pneumoniae** and showed the genetic diversity present among **Klebsiella** bacteria groups. The analysis proved that the genetic diversity is present mainly among **K. pneumoniae**, **K. variicola**, and **K. quasipneumoniae**.

Klebsiella quasipneumoniae strains, whether they produce ESBL or not, share a common genetic ancestor. This fact indicates a process of intermingling and, hence, a difficulty in classification.

Based on this premise, L. **Ferreira et al. (2019)** sought to explore the molecular epidemiology of K. pneumoniae in the Brazilian intensive care unit, emphasizing the dominance of the multidrug-resistant strains, particularly sequence type 437. The work also pointed out the increasing occurrence of hypervirulent **K. pneumoniae** (hvKP), which evokes clinical challenges due to resistance mechanisms and virulence

factors, as well as the relationship between virulence gene factors and clinical outcomes, highlighting the importance of precise virulence gene categorization for the purpose of treatment strategies.

In a similar context, **Nirwati et al. (2019)** studied the biofilm formation and antibiotic resistance in **K. pneumoniae** isolated from clinical samples, which depicted that biofilm assists **K. pneumoniae** in developing resistance, making it hard to deal with disease infection outcomes, hence presenting a key relevance for understanding its relevance and importance while categorizing its characteristics based on its genetic and phenotypic properties.

The relevance and implications of **K. pneumoniae** as an opportunistic pathogen, especially with nosocomial infections, were analysed and expanded on by **P. Herridge et al. (2020),** focusing on the implications and importance of resistance genes within **K. pneumoniae**, which have a major bearing on its pathogenicity and, therefore, its categorization and relevance as a microorganism.

The identification of risk factors, such as the previous use of antibiotics and invasive procedures, linked with **K. pneumoniae** infections also indicates the influence of the environmental/clinical context in determining the virulence/resistance of the different strains of the pathogen. (**Choi et al., 2020**), which was devoted to the understanding of the pathogen, also aimed to enhance the knowledge about the different aspects of **K. pneumoniae**, addressing the diversity of lipopolysaccharide and capsular polysaccharide antigens in invasive strains, thereby giving insight into the vaccination strategies against **K. pneumoniae**, indicating the "immunological classification" of **K. pneumoniae** infection, where antigenic variation was found to be a "critical factor" in the virulence of the different **K. pneumoniae** isolates.

The most recent is that of (**J. Kochan et al., 2023**), who offered interested parties a detailed nuance in the classification of **K. pneumoniae** as classical **K. pneumoniae** and hypervirulent **K. pneumoniae**. In their work, they realized that convergent **K. pneumoniae** strains exist, which have the characteristics of the classifications. Thus, they provided additional information about the **K. pneumoniae** classification which was believed to remain the same. **K. pneumoniae** is categorized as classical **K. pneumoniae** that causes nosocomial infections, and is concurrently resistant to antibiotics and hypervirulent **K. pneumonia**e, which is virulent and sensitive to antibiotics.

Overall, these studies indicate some alterations of the existing concepts on **K. pneumoniae** classifications that revolve around the pattern of genetic variation, virulence determinants, and antimicrobial resistance determinants among others. Therefore, in the farther step, the literature review will focus on investigating the clinical implications of such studies.

2.2. LIFE CYCLE OF Klebsiella pneumoniae, BIOFILM FORMATION AND HOST EFFECT

We will follow the path in this chapter. Investigating the life cycle of **Klebsiella pneumoniae**: transmission, environmental factors, and developmental stages in the host body.

As we proceed to research the topic of **Klebsiella pneumoniae, a** multifaceted issue to human health, we shall delve further and learn more about a myriad of circumstances such as community and environmental reservoirs and medical environments. Altogether, the existing literature emphasizes the virulence, resistance, and persistence of the organism, which have a certain impact on the developmental patterns within the host body, host-tissue relationships, and dynamics of transmission.

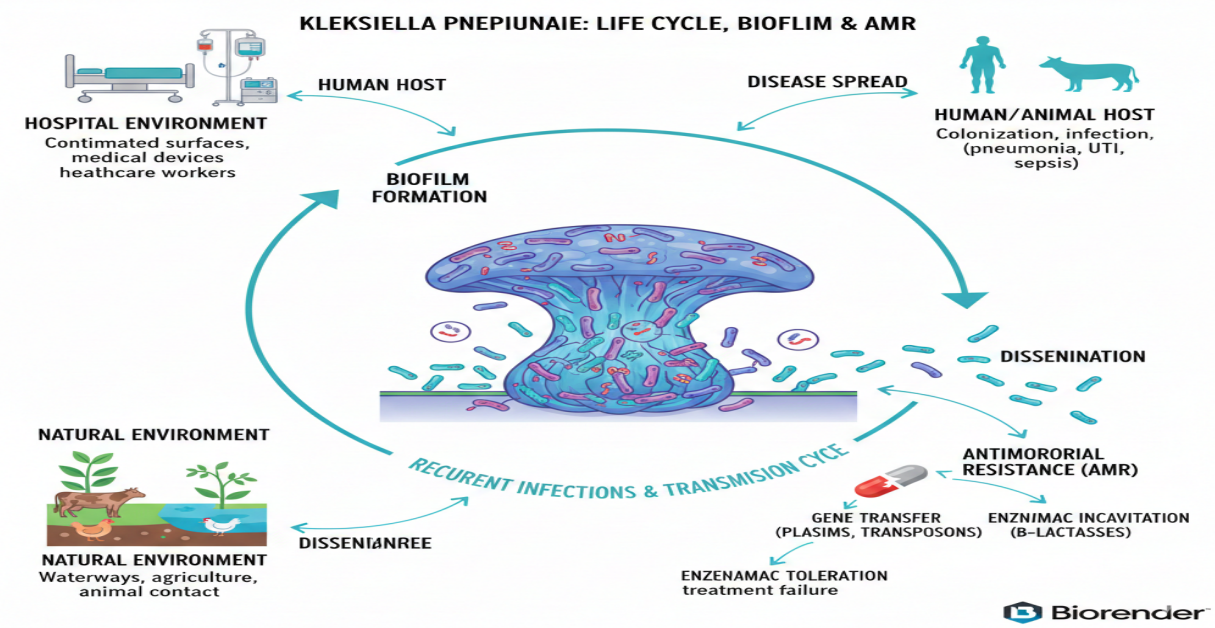


Figure 3: Life circle of *Klebsiella pneumoniae* and biofilm formation and antimicrobial resistance mechanism

Klebsiella pneumoniae is pathogenic, and its pathogenesis and transmission cycle is shown in the figure above. It focuses on the route through which the pathogen is transmitted to infect human hosts by moving between the clinical (surface of the hospitals and medical devices) and environmental reservoirs (soil, water, and animals). It primarily focuses on the biofilm life cycle, which illustrates how bacteria come together in protective communities that drive antimicrobial resistance (AMR). The primary causes of this resistance are horizontal gene transfer (through plasmids) and enzymatic inactivation (such as β-lactamases) that ultimately lead to a failure in treatment and chronic infection.

As per early clinical and public health perspectives, **K. pneumoniae** is a non-motile, Gram-negative bacterium, which can be found in human mucosa and in soil, water, and sewage. This basic view stresses the fact that there may be benign colonization and invasive diseases, including endocarditis, bacteremia, liver abscesses, respiratory and urinary infections, and endophthalmitis. The strains that are hypervirulent and carbapenem-resistant are particularly important in newborn environments, as the disease can most probably be related to localized infections, which disseminate into the blood. The anti-phagocytic properties of the capsule, adhesion through pili, and synthesis of siderophores, especially aerobactin, offer a molecular basis of initial colonization and subsequent systemic distribution (**Mukherjee et al., 2021**).

Strain classification methods, including MLST and capsular serotyping, represent an ongoing effort to monitor the changes and epidemiology of clinical populations of virulence.

The formation of biofilms becomes a major virulence factor when the infection progresses to an extent that it becomes more resistant to host defenses and antimicrobial therapy. The biofilms have been reported in the literature as being important in catheter-associated infections and in the creation of persistent reservoirs of abiotic surfaces and medical devices. The treatment of the issues related to device-associated and nosocomial infections necessitates the knowledge of the regulatory networks that regulate biofilm formation, the existing models that were applied to study the processes, and the implications of such studies on treatment strategies (**Eduarda Souza Guerra et al., 2022**).

The contact between the bacterium and the host intracellular pathways also renders contacting the host and pathogen more complicated. To survive within the host cells and modify cytokine responses, **K. pneumoniae** is able to intracellularly infiltrate macrophages and regulate the process of phagosome maturation, autophagy, and cell death. This intra-cellular niche provides a therapeutic feature because the drugs can be improved by immunomodulatory strategies and host signaling pathway inhibition. Hypervirulence and carbapenem resistance are greatly associated with epidemic strains, which may influence clinical risk, which is why an understanding of how host cell signaling can affect disease development and maintenance is essential (**Wei et al., 2022**).

The clinical history is influenced by ecological and environmental factors of the resistance. The effluents of the hospital and **K. pneumoniae** contain antibiotic resistance determinants, indicating the transfer of the resistance genes in the waterways outside the patient care environment.

Monitoring the resistance phenotypes and gene structures of the environmental isolates can aid in better understanding the resistance distribution and the possibility of spreading new infection through the environmental reservoirs with the help of the environmental perspective (**U. Okafor & U. Nwodo, 2023**).

Lastly, the threat of the biocide resistance and the biocide resistance in the practical applications of the infection management is also warned. The accessory genome is also strong and **K. pneumoniae** this is also able to form biofilms, thereby allowing this bacteria to endure in healthcare facilities despite cleaning. The necessity to use effective disinfection practices and constant resistance surveillance in a clinical and environmental setting is underscored by the fact that sub-lethal exposure to biocides and organic load may support the emergence of resistant subpopulations (**Ntshonga et al., 2024**).When taken as a whole, these studies show how **K. pneumoniae** uses hospital ecosystems, host niches, and environmental reservoirs to maintain transmission, build resistance, and modify virulence tactics. The changing picture highlights how the organism's life cycle within the host and larger transmission networks is shaped by the interaction of capsule-mediated immune evasion, biofilm-associated survival, intracellular persistence, and environmental dissemination.

Below is the diagram that generise, show How the disease spread form the original place the human, what order, family of the species which are likely to have more infection for the diseases, and also explain what environment or time that the species are more likely to be active, hence to the host body from the the initial process of development in the host body and how it can be transmitted from one host to another.

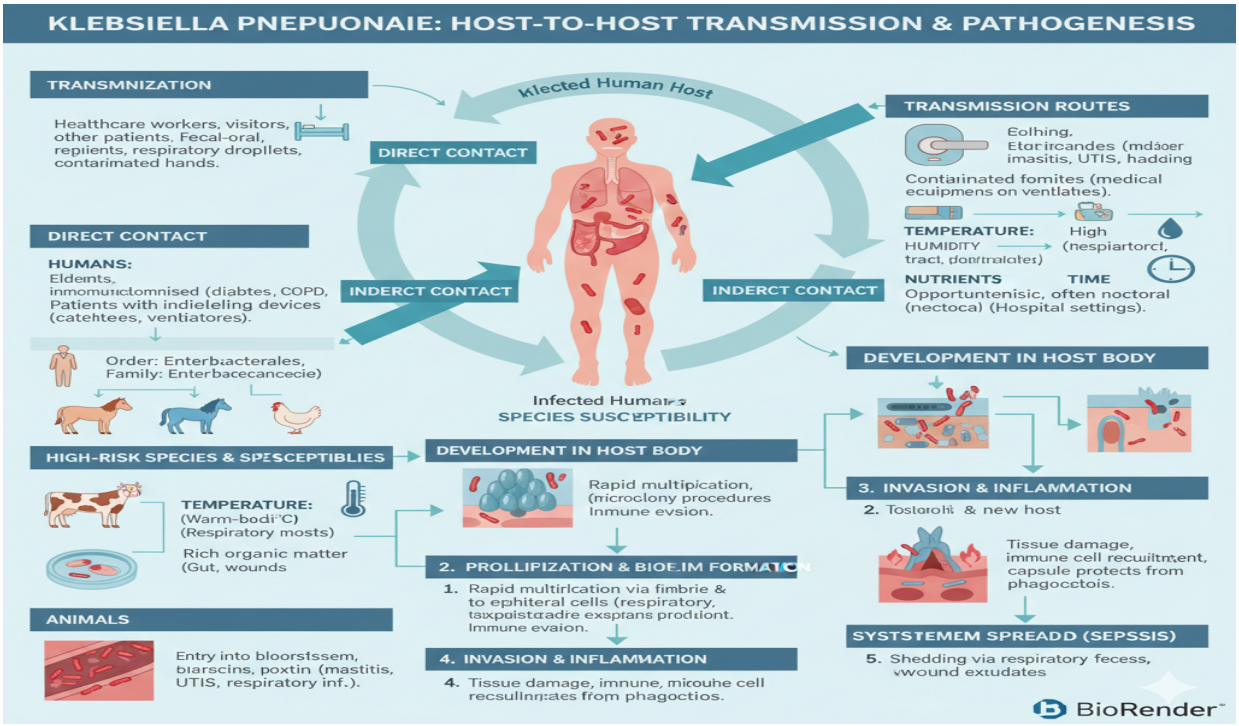


Figure 4: Pneumonia, U.T.I diseases can be transformed form the host to host

2.4. TYPES OF FIMBRIAE IN *Klebsiella pneumoniae*

Fimbriae, often called pili, look like tiny hairs sticking out from the surfaces of many Gram-negative bacteria, including Klebsiella species. Smaller and far more numerous than flagella, these appendages help the microbes cling to solid surfaces and to the cells of their hosts. **Duguid's classic 1959 paper** still stands as one of the first broad studies of Klebsiella fimbriae, describing the main types he saw and showing how each type stuck to things.

He worked with 154 non-motile **Klebsiella** strains from capsule serotypes 1 through 72. He looked at 125 of them under electron microscopes and simple blood-clumping tests and saw fimbriae. More to the point, he noted that the hairs could not resemble the thick capsule and it can even exist on mutants who lost the capsule. These findings indicated fimbriae to be their adhesion organs and not simply shreds or extensions of capsular material.

And also The literature on the fimbriae types of **Klebsiella pneumoniae** is suggestive of a complex interaction between bacterial adhesion mechanisms and biofilm formation that are key to the pathogenesis of Klebsiella pneumoniae-caused infections.

The former study by (**Vuotto et al., 2014**) brought out the significance of type 3 pili that initiates adherence to abiotic surfaces resulting to a biofilm formation caused by type 3 fimbrial shaft (MrkA).

The paper has highlighted the significant roles played by type 1 and type 3 fimbriae in biofilm development and how type 3 fimbrial expression by MrkJ a phosphodiesterase regulates the expression of type 3 fimbriae.

On this basis, (**Arato et al., 2021**) further described the fimbrial structures that were expressed in clinical isolates of K. pneumoniae in more detail. In their opinion, the majority of Gram-negative enterobacteria contain both type 1 and type 3 fimbriae including type 1 fimbriae that are linked specifically to urinary infections. The type 1 fimbriae structural characteristics including the size and genetic expression as well as evidence of upregulation of one of the key adhesins, FimH, in urinary infections were defined by the authors. The significance of type 1 fimbriae in the invasion procedures and biofilm formation of mice with urinary tract infection was demonstrated in this study.

The regulatory processes governing the expression of fimbials, in particular how the environmental factors and the sites governing the colonization of the type 1 fimbriae are important in the regulation of the fimbial expression of the type 1 fimbriae were further explored as (**Eduarda Souza Guerra et al., 2022**) discovered. The researchers found these repressive factors that prevent formation of biofilms as FimK and KpfR. Based on their results the expression of fimbriae is not merely a predetermined characteristic, but an act which reacts based on the bacterial surroundings, and may play a vital role in determining the ability of **K. pneumoniae** to adhere and establish biofilms in the various habitats of the host.

In another study, (**Venkitapathi et al., 2022**) evaluated the expression of **K. pneumoniae** and **K. quasipneumoniae** type 1 and type 3 fimbriae. They described the regulatory circumstances that control fimbrial expression particularly the phase shift of the fimS regulatory sequence of the type 1 fimbriae and mrkHIJ gene cluster of type 3 fimbriae. It has also been

identified that the fimK allele of **K. quasipneumoniae** was truncated in a peculiar manner which resulted in the pattern of the altered expression of type 3 fimbriae which demonstrates the sensitive regulation environment in which the fimbial expression is regulated and the effect of such a regulation on bacterial attachment to host tissues.

Together, these results result to an integrated understanding of the type of fimbriae in **K. pneumoniae**, their structure and mechanism, and ways of regulating these to alter their expression in the circumstances of their hosts. The literature published demonstrates the topicality of fimbrial adhesins in the pathology of **K. pneumoniae** and their potential application as the object of therapeutic activity.

2.5. STRAINS -HYPOMUCOVIRULENT(HPV) TYPE OF Klebsiella

Literature on the topic of **Klebsiella pneumoniae** has been more concerned lately with the spread of hypervirulent forms of the organism, especially in the realms of such public health concerns as antibiotic resistance and virulence factors.

The first exploration by (**Shi et al., 2018**) brings out the evidence of hypervirulent **Klebsiella pneumoniae** (hvKP) in the Asian-Pacific region which is causing the alarming increase of the epidemic. A major finding of theirs highlights the high prevalence of multidrug-resistant hypervirulent strains, in particular, the K1 ST23 clone that is a serious threat to both healthy and immunocompromised people. The paper singles out key genomic virulence factors, including iroBCD, rmpA/A2, which play a role in the pathogenicity of hvKP.

Using this background (**Mai et al., 2023**) further expound on the identification and clinical implications of hvKP and explain how it has demonstrated an upward trend in the healthcare facility and the subsequent emergence of hypervirulent hvKP resistant to the carbapenem (CR-hvKP). Their study brings out the importance of a standardized method of identification, the accuracy of the string test and the developments in molecular methods such as PCR and multilocus sequence typing. The hvKP strains also have large virulence plasmids which complicate the treatment process, because they carry genes that are associated with the pathogenicity and resistance to antibiotics.

In an opposing point of view, (**J. Kochan et al., 2023**) report the results of an investigation that imply that there are subgroups of **K. pneumoniae** that are multidrug-resistant and hypervirulent but show unexpectedly low virulence. They screened 2,608 isolates on their genome, which showed convergent strains that have a combination of characteristics of classical and hypervirulent strains, bringing up the concern of the virulence potential of these hybrids. The research recommends conducting additional research regarding the pathogenic organisms of convergent strains since the initial findings reveal that not all of them carry the virulence capacity characteristic of hvKP in trials.

(**Teo et al., 2024**) are an addition to the discussion as they study the mucosal tropism and patterns of distribution of both classical and hypervirulent strains. Their study indicates that there exists a great gap in the knowledge about pathogenesis of **K. pneumoniae**, especially the role of mucosal surfaces in the development of the disease. According to them, the interaction of virulence and the transfer of antibiotic resistance genes increases the threat of an outbreak, and a deeper insight into the pathogenic mechanisms of **K. pneumoniae** will be needed.

Continuing on the clinical implications, (**Dingiswayo et al., 2024**) examine the hypermucoviscous phenotype of hvKP strains in South African situation. Studies show that hypervirulent **Klebsiella pneumoniae** (hvKP) strains tend to be more aggressive than E. coli in experimental models. Nevertheless, hypervirulent **Klebsiella pneumoniae** (hvKP) strains do not act in a consistent manner - there is a high degree of variability in the degree of virulence. The virulence of an hvKP is polyfactorial, and it is closely linked with the expression of virulence genes, especially those of the iron acquiring systems, as well as, with the presence of essential metabolic pathways. The existing scientific sources indicate the constantly developing idea of Klebsiella pneumoniae, typically hypermucoviscous and hypervirulent types. Current studies have reconsidered the interplay between hypervirulence and growing resistance to antimicrobials, and therapeutic dilemmas, since this is a highly difficult clinical management scenario.

2.6. KLEBSIELLA PNEUMONIAE INFECTIONS MATRIX METALLOPROTEINASES (MMPS).

The study of matrix metalloproteinases (MMPs) in the infection of **Klebsiella pneumoniae** shows that there is a complicated interaction between bacterial virulence, host immune systems, and extracellular matrix (ECM) remodelling. MMPs are enzymes that break down the ECM components and are necessary in normal tissue repair and remodelling. Nevertheless, in case their activity is dysregulated, they may be involved in pathology of the disease.

Schaaf et al. (2008) also found high levels of pulmonary MMP activity in high-risk infection by high-risk pathogens including **Klebsiella pneumoniae**. Their results show that neutrophil-released MMPs, which are major immune effector cells, contribute to the fight against infection, but in the same way, they contribute to lung tissue damage in cases of hospital-acquired pneumonia. This shows how, MMPs act as defensive and, at the same time, deleterious agents.

In line with this idea, **Cathcart et al. (2015)** explained that the maladaptation of MMPs is related to various disease conditions, cancer included. They highlighted that although MMPs play a crucial role in physiological ECM turnover, their disequilibrium in their control may contribute greatly to the development of disease. MMPs

are therefore useful mediators of recovery in controlled conditions but they may cause pathological effects in case regulatory mechanisms are dysfunctional.

Ferreira et al. (2019) also put **Klebsiella pneumoniae** in clinical disease, especially multidrug-resistant ones, into perspective. Their work describes a number of virulence factors, such as, those that facilitate the formation of biofilms and degradation of ECM. They further point out the role of MMP activity in tissue remodeling and biofilm persistence, mechanisms that increase bacterial survival in the face of immune and antimicrobial challenge.

Cabral-Pacheco et al. (2020) wrote an article that gives an extensive background of MMPs and their endogenous regulators i.e., the tissue inhibitors of metal proteinases (TIMPs). They emphasized that the correct MMPTIMP balance is paramount in respect to the integrity of the ECM and normal tissue functioning. Disturbance of this balance is associated with the chronic inflammatory process and tissue destruction, which explains why the control of MMP activity may be a possible treatment approach in Klebsiella pneumoniae infections with excessive inflammation and remodelling.

Ahmed Rashid and **Bardaweel (2023)** narrowed down to MMP-9, which is also one of the most widely investigated MMPs. MMP-9 has also attracted interest because of its multifaceted and context-specific involvement in ECM remodeling and pathophysiology including cancer. Their article emphasizes MMP-9 as a major cause of pathological tissue remodelling and as a potential biomarker and therapeutic target especially in disorders that involve inflammation and structural tissue remodelling. The issues they highlight about the development of effective MMP-9 inhibitors may mean a lot in the fight against infections involving MMP-9 in the pathogenesis of tissue damage and inflammatory reactions.

Highlighting the importance of biofilms in the pathogenesis of **K. pneumoniae**, (**Eduarda Souza Guerra et al., 2022**) pointed out the fact that virulence factors related to biofilms contribute to survival and resistance. In this study, the idea that, besides facilitating persistence, biofilms play a role in spreading resistance genes is strengthened.

Reviewing different methods to fight biofilm formation, (**Tasmia Asma et al., 2022**) states that the use of antibiotics can reduce the growth of biofilm, but in many cases, it does not eliminate the existence of biofilms. This explains why new methods of intervention that are more effective than conventional antimicrobial treatments are of utmost importance.

(**Sabenca et al., 2023**) paid attention to the abilities of **K. pneumoniae** strains to form biofilms, especially strains containing ESBL and KPC genes. They stated that the production of biofilms was high in these strains, which casts doubt on the implication of the same regarding infection management and treatment effectiveness.

Finally,(**Dell'Annunziata et al., 2024**) discuss the dynamics between Klebsiella pneumoniae and host cells, especially the release of the outer membrane vesicles (OMVs). Such OMVs have been demonstrated to trigger death-signaling pathways in human bronchial epithelial cells, which is another indication of complexity to the connection between MMPs, bacterial virulence factors, and host immune responses. It is proposed in the paper that Klebsiella pneumoniae is dangerous not only due to its own virulence factors, but also due to its impact on the host cell pathways. In this respect, matrix metalloproteinases (MMPs) seem to be involved in this process because the bacterium could indirectly modulate MMP activity to aid infection, persistence, and tissue infiltration.

Combined, the existing literature provides a very comprehensive and subtle perspective of the role of MMPs in the case of infection with **Klebsiella pneumoniae**. MMPs have dual roles in the host defense and in the tissue damage and due to this duality, they are currently being considered as potential therapeutic targets in the management of infection-related complications. The interaction of the MMP activity, bacterial virulence and host immune responses is a significant and increasingly popular research area, with implications of value to treatment regimens against this opportunistic pathogen.

The genes that cause biofilm formation in **Klebsiella pneumoniae** also have a considerable amount of research done on them. This literature demonstrates that the formation of biofilms is regulated by a complicated system of genetic factor which improves attachment, colonization and survival of bacteria in a diversity of environments. These genetic processes contribute to the understanding of the effectiveness of the organism to endure both in the clinical and natural environment. The original contribution by **Eko Niba et al**.

The article (**Tabe Eko Niba et al., 2007**) emphasizes the role of certain genes in change of the planktonic to the biofilm forms of bacterial life. They find that genes that control surface structures, like flagella and fimbriae are essential in the formation of biofilms and propose that similar processes are in effect in **Klebsiella pneumoniae**.

Based on this, (**Nucleo et al., 2009**) directly distinguishes curli genes and cellulose biosynthesis genes as central actors in biofilm formation in **Klebsiella pneumoniae**. This observation highlights the role of the process of matrix production and adhesion control in the formation of biofilms.

(**MS Lery et al., 2014**) further explain the contribution of the type 3 pili to the adherence to surfaces, which provides biofilm formation, and discusses the connection between quorum sensing and biofilm formation pointing to the complexity of the regulatory network.

Laverty et al.(**Laverty et al., 2014**) describe the biosynthesis of pili and exopolysaccharide generation highlighting the significance of these elements in

different Gram-negative pathogens, such as **Klebsiella pneumoniae**. The review depicts the relevance of certain genes that participate in the production of polysaccharides, which are extremely vital in the maturation of biofilms as well as the development of capability of the **Klebsiella pneumoniae** to resist environmental impacts. These polysaccharides are significant constituents of the biofilm matrix giving it structural integrity and protection to the bacterial community.

Vuotto et al. (2014) extended this knowledge to MrkA, the type 3 fimbriae structural shaft protein, without which the formation of biofilms in **Klebsiella pneumoniae** is impossible. Their article also analyzed other types of fimbials and the regulatory enzyme MrkJ phosphodiesterase to show that biofilm formation is not regulated by one specific genetic and regulatory factor. Such a multilayered regulation is useful in explaining the strength and diversity of biofilm formation.

Wang et al. (2016) found that the production of cellulose grow higher when bacteria are exposed to simulated microgravity conditions, which leads to a higher growth of biofilms. The observation is how the environmental factors can directly affect the expression of genes and the biofilm behavior. In favor of the idea of multifaceted control, mrkABCDF operon, coding type 3 fimbriae, was demonstrated to be highly regulated by various regulatory cues.

Palacios et al. (2018) found other types of fimbials which also play a role in virulence as well as biofilm formation. Their findings demonstrate that type 1 and type 3 fimbriae play different and complementary roles of successful surface attachments and colonization of the environment. They also noted that the genetic determinants are highly conserved in the various **Klebsiella pneumonia** strains suggesting the biological and clinical significance of these genetic determinants.

The article by **Kumar Singh et al. (2019)** has shown that biofilm architecture is dependent on the environmental conditions, where external stressors have a substantial impact on the structure of the biofilm matrix. Their results also indicate that the genetic and biochemical pathways that determine the biofilm structure in Klebsiella pneumoniae should be investigated further. Genetic mobility is another important factor of biofilm capacity. The type 3 fimbriae encoded in the plasmid were also mentioned by **Element et al. (2023)**, which implies that horizontal gene transfer can positively influence the capacity of the bacterium to create biofilms and adapt to infection. This gives its survival strategy an added flexibility.

Li and Ni (2023) presented a general review of the multifactorial regulation of biofilm formation in **Klebsiella pneumoniae** synthesizing the important works on fimbrial determinants, and their regulation systems. The resulting analysis of their review is that biofilm formation is regulated by a multifaceted genetic network and that further studies are required to be able to unravel all these processes.

On the whole, as demonstrated in the literature, biofilm formation in **Klebsiella pneumoniae** is mediated by a set of coordinated mechanisms - such as fimbriae, curli fibers, cellulose production, and quorum sensing - all of which are involved in the formation and maintenance of stable biofilm communities.

Table: Antibiotic resistance and involved genes

Antibiotic class	Example of antibiotic(s)	Resistance genes	Mechanism of resistance
β-lactams	Ampicillin, Cefotaxime	TX-Mbla_TEM, bla_SHV,bla_C	β-lactamase enzymesthat hydrolyzeβ-lactam ring
	Imipenem, Meropenem	bla_KPC, bla_NDM, bla_OXA-48	Carbapenemases degrading carbapenems
Aminoglycosides	Gentamicin, Amikacin	aac(6')-Ib, aph(3')-Ia, armA	Enzymatic modification or methylation of 16S rRNA
Fluoroquinolones	Ciprofloxacin, Levofloxacin	qnrA, qnrB, qnrS,gyrA, parC	Target site mutations or protection of DNA Gyrase
Tetracyclines	Tetracycline, Tigecycline	tet(A), tet(B), tet(X)	Efflux pump activation or enzymatic inactivation
Polymyxins	Colistin	mcr-1, mgrB mutations	Lipid A modification reduces colistin binding
Sulfonamides	Sulfamethoxazole	sul1, sul2	Altered dihydropteroate synthase
Chloramphenicol	Chloramphenicol	catA1, cmlA	Acetylation-mediated Inactivation
Macrolides	Azithromycin (rare use)	mph(A), erm(B)	Methylation of 23S rRNA or drug inactivation

2.7. CORRELATION IN ANTIBIOTIC RESISTANCE AND BIOFILM FORMATION

Research work conducted nowadays also suggests that there is a close and complicated connection between inertness to antibiotics and biofilm development in **Klebsiella pneumoniae**. Biofilms do not only shield the bacteria of environmental strains and immune response, but also adversely affect the efficacy of antibiotics to an extent of prolonged infection and compromised clinical outcome.

The contribution of biofilms to the pathogenesis of chronic infections was initially noted by (**J Sanchez et al., 2013**), and it is important to point out that multidrug-resistant (MDR) organisms such as **K. pneumoniae** prefer to live in the biofilm, which provides them with increased resistance to antimicrobial therapy along with host immunity. Their results established the scene of the clarification of how the formation of biofilms is not just a strategy to survive but the key in the maintenance of the infections.

The genetic basis of biofilm formation in MDR pathogens was also investigated by (**Thummeepak et al., 2016**), but the study was on Acinetobacter baumannii. The paper has an indirect informational impact on the K. pneumoniae context by implying that virulence genes related to biofilm formation can also be involved in resistant mechanisms, hence showing that the Gram-negative bacteria share a common evolutionary approach.

Specifically, (**Anes et al., 2017**) studied **K. pneumoniae** and attributed hypermucoid phenotypes to a higher virulence and biofilm formation. Their study highlighted the ability of the bacterium to acquire resistance not only genetically but also in terms of structural modification that strengthens biofilm. This flexibility complicates the treatment methods because biofilms may drastically decrease the effects of antibiotics. Drugs have a shorter penetration and less activity in their protective matrix and changed state of metabolism, which frequently results in persistent or recurrent infections.

Anti-biofilm strategies on quorum-sensing interference were also addressed in 2018 as a promising therapeutic option, which is an indication of the increasing pressure to seek alternative ways of treating biofilm-related infections. This is consistent with the growing appreciation of biofilms as reservoirs of antibiotic resistance and thus making infection control more difficult and necessitating new methods of treatment. The study by Lenchenko et al. (2020) supported this point of view by examining the structural and adhesive characteristics of Klebsiella pneumoniae biofilms. They discovered that the thick biofilm structure limits penetration of antibiotics thus it becomes hard to eliminate and necessitates the consideration of specific anti- biofilm therapy in addition to traditional antimicrobial therapy.

Alamri et al. (2020) studied the association between the biofilm formation and the antibiotic resistance in the case of Acinetobacter baumannii and discovered that the linkage between them could not be always predicted. This is an essential warning that the connection between biofilm generation and resistance differs in different bacterial species, and insists on species-specific research, such as in **Klebsiella pneumoniae**.

The significance of biofilms in clinical chronic infections and antimicrobial resistance, **Luo et al. (2020)** emphasized that the behavior and dynamics of biofilms must be thoroughly studied to facilitate the creation of more efficient measures to prevent and cure them. This is especially true in the case of Klebsiella pneumoniae that is still evolving in the wake of the rising antimicrobial pressure.

Shadkam et al. (2021) examined the isolates of **K. pneumoniae** in hospitals directly using hospitalized patients, and the authors identified that biofilm-forming strains were very widespread among the multidrug-resistant (MDR) ones. Their conclusions provide a clear evidence of the high clinical significance of biofilm formation in nosocomial infections. The same way, **Haghighifar et al. (2021)** studied molecular resistance mechanisms in particular, extended-spectrum b-lactamases (ESBLs) and their relationship with biofilm formation. They demonstrated that biofilm-associated virulence factors are responsible in the treatment failure and suggested more specific interventions against biofilm-based infections.

Sahoo et al. (2021) conducted a review of the patterns of antibiotic resistance among biofilm forming bacterial isolates like **K. pneumoniae** and indicated the necessity of alternative or combination treatments to deal with these infections that persist. They also observed the increasing rate of **K. pneumoniae** infections in the health care facilities, particularly in the immunocompromised patients.

Oleksy-Wawrzyniak et al. (2021) assessed the in vitro biofilm-forming capacity of **K. pneumoniae** and discovered major virulence elements that promote the pathogenicity of the bacteria. Their article revealed the specific challenge of managing biofilm-related urinary tract infections, one of the frequent clinical manifestations of this organism.

Gavino Donadu et al. (2022) were interested in adaptive resistance of biofilm embedded bacteria (such as **K. pneumoniae**). They found that the dynamics and context-dependent nature of resistance in biofilms provide a more refined view of the interaction between biofilm formation and antibiotic resistance, and this field is currently under investigation.

The study by **Shebl et al. (2023**) examined the effects of antibiotic combinations on the biofilm formation of **K. pneumoniae** and an idea of using combination therapy could help overcome certain types of resistance. This

justifies the fact that more effective treatment measures are still in need of development to address the biofilm-related infections in clinical practice.

Bai and Guo (2024) conducted a review of the interactions of **Klebsiella pneumoniae** with the human immune system, with biofilm forming as one of the main ways of immune resistance. Their discussion highlights the necessity of alternative and adjunctive treatment to raise the urgency of the necessity to treat the increasing threat of multidrug-resistant strains.

El Naggar et al. (2024) examined the interconnection between biofilm formation and carbapenemase production and the overall interaction of the virulence and antimicrobial resistance systems of **Klebsiella pneumoniae**. Their results offer valuable information regarding the interrelation between these factors and their role in the clinical outcome of the pathogen. It is important to know these links in order to make sound decisions about treatment especially when there is a serious infection.

Lastly, **Bereanu et al. (2024)** wrote about the significance of **K. pneumoniae** biofilm formation in medical devices-related infections. The urgency of their work is based on the fact that control and prevention methods are inadequate, and the clinical risk caused by this pathogen is high when using devices.

Altogether, the literature is a compelling account of the complex interrelation between the problem of antibiotic resistance and biofilm formation in the **Klebsiella pneumoniae** that requires additional research and innovative approaches to address the crucial issue of the contemporary social problem.

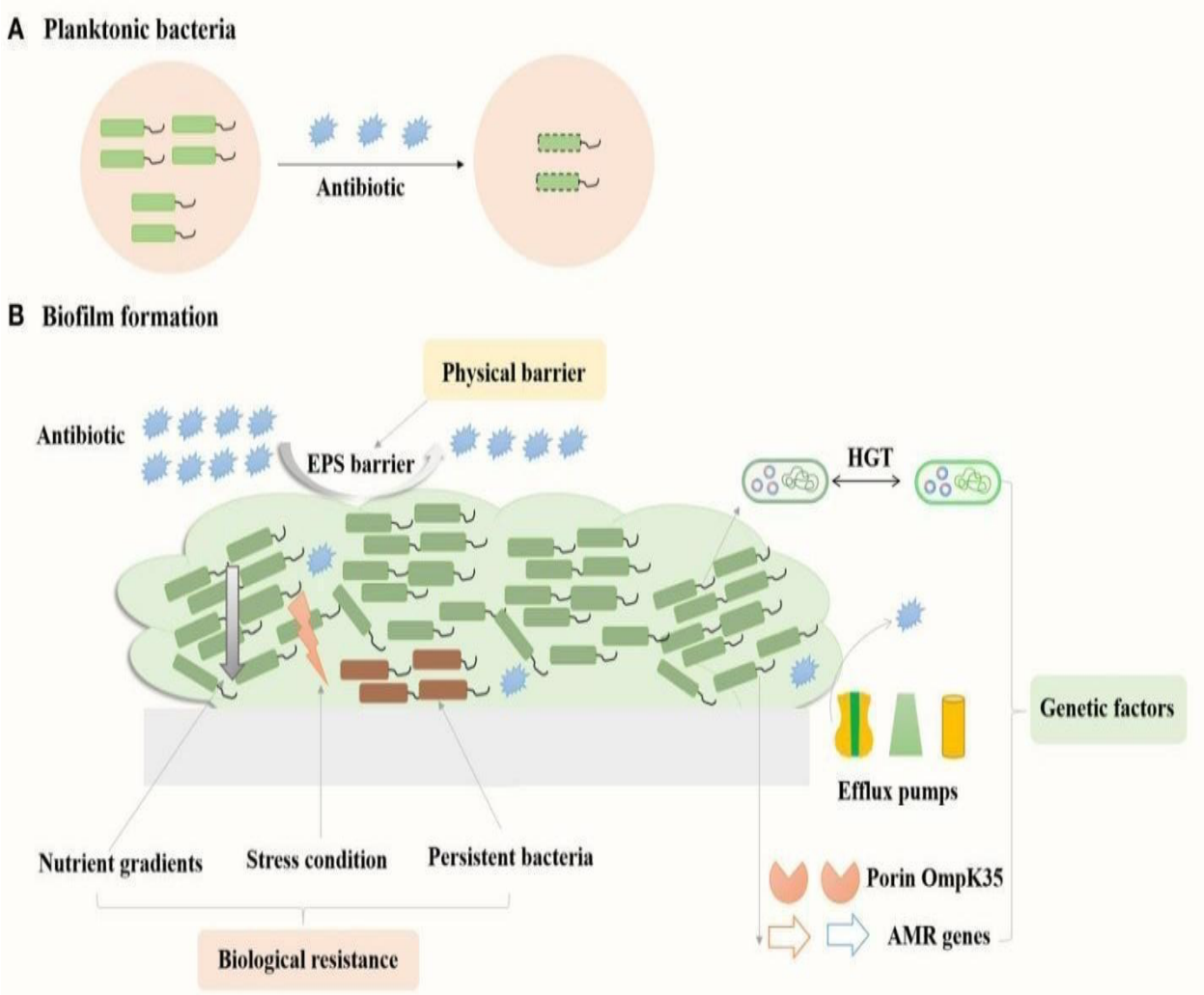
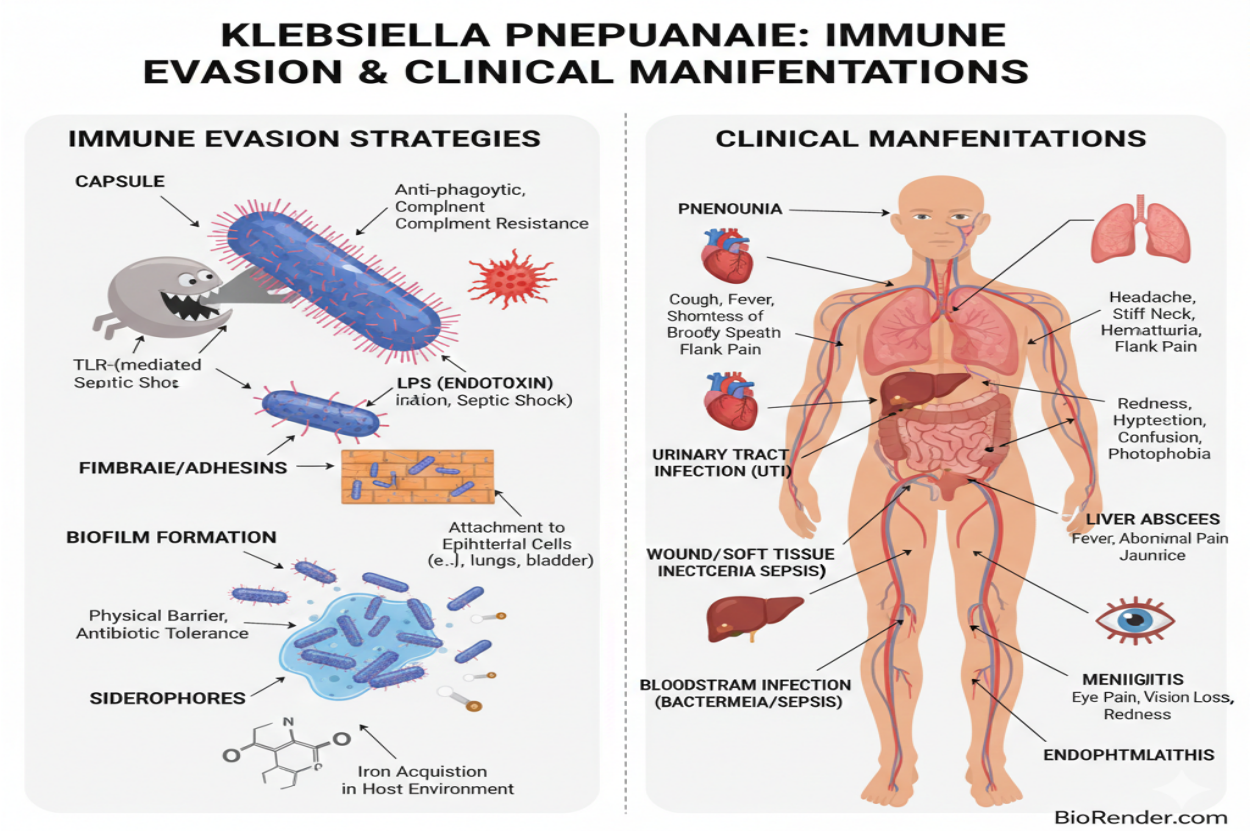


Figure: A.Bacteria with non-biofilm state B. Bacteria with biofilm its resistance following application of the antibiotic (Li, L., Gao.,et al(2024)).

CLINICAL FUTURE AND CONTROL MEASURE

Further it is discussed how the *Klebsiella* species infection characteristics interact with the host immune system, the symptoms of this bacterial infection can take many different form. As shown in the diagram below.



Infections with *Klebsiella* species have complex interactions with the host immune system that influence the course of the illness as well as the range of clinical symptoms. The given articles, arranged chronologically, show how host cell death pathways, intracellular trafficking, bacterial surface features, and biofilm biology interact to influence immune activation, pathogen removal, and clinical consequences.

(**Sá-Pessoa et al., 2020**) show that **Klebsiella pneumoniae** can weaken host defenses by altering inflammatory signals in a way that is dependent on NOD1. The article demonstrates that the reception of mutant polysaccharides in the human airway epithelial cells results in inflammatory responses, indicating a complex relationship between immunological sensing and the capsule structure. That alveolar macrophages play a pivotal role in the protective pulmonary responses; in their absence, they increase neutrophil recruitment and neutrophil survival at the expense of bacterial clearance and survival. The paper also presents the cooperation between innate lymphocytes and monocytes that ensure clearance and the importance of the bacterial surface attributes to the interactions with phagocytes and further immune reactions.
This body of findings introduces the concept of early infection as a battlefield in which the coordination of leukocytes, the macrophage activity, and the alterations of the capsules make the host defenses either enhanced or halted.

Adhering to the route between colonization and dissemination in mouse models, (**Joseph et al., 2021**) broaden the discussion to living systems. They emphasize that, in immunocompromised hosts, in particular, the mucosa of the respiratory and gut can be colonized *by* ***Klebsiella pneumoniae***, which raises the chance of invasive disease. As an example of the ways in which accessory genomes contribute to pathogenic capability, virulence plasmid-carrying hypervirulent strains have contributed to making colonization a deadly disease causing bacteremia, meningitis, pneumonia, and liver abscesses.
The application of mammalian models to study the host-pathogen interactions identifies possibilities to improve therapeutic actions that enhance host defenses and underscores the importance of immunity in determining clinical outcome. In addition, biofilm development on medical devices and the ability of the organism to adapt to various niches increases the risk of diseases. Facets of epithelial cells Clinical Epidemiology, Risk Factors, and Control Strategies (**Chang et al., 2021**) note the presence of colonization in the respiratory and gastrointestinal tract and the fact that the transmission often occurs through self-colonizing strains.

The findings indicate a high proportion of hospital-acquired cases are due to latent carriage and consider the outcome of colonization into the nosocomial infections. Besides clinical, laboratory, and imaging details, proper assessment of the presence of Klebsiella in the blood should be conducted to differentiate colonization and

active infection. Therefore, this paper would place immune-mediated disease development within the broader clinical context, in which host variables and colonization condition combine to provoke symptoms that require specific treatment.

It identifies the possible vaccine and therapeutic targets in the biofilm machinery is also prominent in the review, but also identifies the necessity of combining in vitro and in vivo models to represent the complexity of biofilm-related infections. Biofilm biology and host defense interactions underscore the mechanisms that explain the inconsistency in symptoms and disease severity and why conventional therapies frequently fail to eliminate deep-rooted infections. **Guerra et al. (2022)** pay attention to biofilms as a key factor in the pathogenesis of the disease.

It focuses on the decisions of host cell fate and has an intra-cellular survival strategy (**Wei et al., 2022**). The article discusses the immunotherapy coverage of the therapeutic horizon by capsule- or LPS-specific antibodies despite serotype variation and how multidrug-resistant and hypervirulent strains are problematic in terms of treatment. Among them, one should mention that infections employ endocytosis and phagosomes maturation to escape clearance and alter cell death pathways and signals of danger to sustain intracellular presence. Gaining an insight into such molecular pathways can improve the general ability to gain an understanding of the mechanisms which take place when the host immune system recognizes and reacts to infection and why specific clinical phenotypes are suggesting an abnormal inflammatory response and cell death. The perspective correlates clinical symptoms seen, potential immunomodulatory treatment, and intracellular survival mechanisms.

When taken as a whole, these articles chart a path from molecular interactions at the interfaces between macrophages and airway epithelium to colonization and dissemination processes, population-level epidemiology, biofilm-mediated disease, immunomodulatory approaches, and treatment considerations. Host immunity is frequently positioned in the literature as a factor influencing symptomatology as well as a possible target for treatments meant to stop the development of invasive disease from colonization. awareness the relationship between Klebsiella species infections and the host immune system requires an awareness of the roles played by bacterial surface features, intracellular trafficking, biofilm biology, and cytokine signalling.

IMPLICATION AND CHALLENGES

Research from, **Mirzaie, A., & Ranjbar, R. (2021)** Reveal the high percentage of MDR **K. pneumoniae** is supported by some previous studies. This MDR pattern exhibited by microbes is causing a great challenge in managing infections, and consequently, it is very important to monitor and optimize antibiotic use through antibiotic stewardship programs. Several studies have shown that treatment with a combination of antibiotics can help to prevent the emergence of new resistant strains, as therapy failures are commonly found in individuals who only receive single antibiotic therapy. It is also important for clinicians and microbiologists to collaborate to warrant effective management of infections as promoted in the Rational Use of Medicine (RUM) Program. Managing infections with currently accessible antibiotics and evaluating the outcomes have become important and urgent protocols for the successful treatment of biofilm-associated infections.

2.3. ALTERNATIVE TREATMENT BY USING PLANT EXTRACT (OIL)

Several methods provide a thorough blueprint of the gene when processed. Common technologies used are: Illumina, PacBio, Oxford Nanopore. Repeat regions of the genome make it difficult to read SNVs and might give out false positives but on contrast it is one of the effective ways to determine genetic mutations. (Moser, A. I., et al., 2022).

Medicinal extracts from plants that are commonly available but are not predominant in this field are used, such as, cranberry, capers, garlic, peppermint, fennel, thyme etc. There are approximately 20,000 identified medicinal plants out of which around 1340 have been known for their antimicrobial properties. These extracts are responsible for inhibition of microbial growth and enhancement of antibiotic activity. They show incredible synergy when mixed with antibiotics. (Vaou, N., et al., (2021.; Moser, A. I., et al., 2022.; Silva, S. L., Araújo, F. S. M., Silva, P. O. A., Silva, E. V. A., et al., 2023.)

These extracts are used in a 1:1 ratio with antibiotics such as ampicillin, nalidixic acid, erythromycin, amikacin. Proven interaction in the studies show higher efficacy of nalidixic acid when mixed with green tea extract against salmonella. Similarly, rosmarinic acid when mixed with erythromycin enhances efflux pump inhibition. Several isolates of Klebsiella pneumoniaeare tested against essential oils using agar well diffusion and disc diffusion methods. After these diffusion methods are performed, MIC and MBC are performed to ensure formulation of perfect dosage for humans. We use essential oils that are tested on 100 samples, (50 human and 50 bovine) 10 oils were tested including thyme, sage, garlic, nigella, moringa, marjoram and tea tree. The main component is carvacrol and other components include cymene, gamma terpene, alpha pinene and myrcene. Oregano oil is one of the unconventional oils used in the synergistic approach. (El-Demerdash, et al., (2024)

Confirmed virulence/resistance genes of virulence by PCR uge, wabG, fimH, blaNDM-1 by PCR for isolates and reference strain. Tested 15 EOCs (including thymol, carvacrol, geraniol, linalool, eugenol, 1,8- cineole, menthol isomers, etc.). Results show most active antibacterial EOCs: thymol showed the strongest inhibitory activity (MIC ≈ 0.78 mg/mL; MBC ≈ 1.56

mg/mL, bactericidal), while carvacrol and geraniol also exhibited strong bactericidal or low- MIC activity across isolates. Some compounds (e.g., menthol) had very weak activity (high MICs, no MBC within tested range). Gentamicin control showed variable MICs and, in some strains, very high values indicating resistance. (Kwiatkowski, P., et al., 2022).

FUTURE PERSPECTIVE AND DIRECTION

The therapeutic strategies on this due to limitation of new antibiotic, many researches has focus on drugs combinations to combat ***Klebsiella pneumoniae*** biofilm, combining traditional antimicrobials with antibiotics that have diverse action mechanisms or incorporating other anti-biofilm agents may result in unforeseen effectiveness against biofilm caused by ***Klebsiella pneumoniae***. antimicrobial peptides (AMPs) are also being explored as potential therapeutic agents due to their broadspectrum antibiotic activities and biofilm inhibition capabilities. Nanoparticles (NPs) are also attracting increasing interest as effective antimicrobial agents due to their large surface area and ease for surface modification. Natural products and their derivatives are also being investigated for their antibiofilm activity against ***Klebsiella pneumoniae.***

REFERENCES

1. Anes, J., Hurley, D., Martins, M., & Fanning, S. (2017). Exploring the genome and phenotype of multidrug-resistant Klebsiella pneumoniae of clinical origin. Frontiers in Microbiology.
2. Arato, V., Raso, M. M., Gasperini, G., Berlanda Scorza, F., & Micoli, F. (2021). Prophylaxis and treatment against Klebsiella pneumoniae: Current insights on this emerging antimicrobial-resistant global threat. Frontiers in Cellular and Infection Microbiology.
3. Bai, R., & Guo, J. (2024). Interactions and implications of Klebsiella pneumoniae with human immune responses and metabolic pathways: A comprehensive review. Frontiers in Immunology.
4. Bereanu, A. S., Vintilă, B. I., Bereanu, R., Codru, I. R., Hașegan, A., Olteanu, C., Săceleanu, V., & Sava, M. (2024). TiO_2 nanocomposite coatings and inactivation of carbapenemase-producing Klebsiella pneumoniae biofilm—Opportunities and challenges. Nanomaterials.
5. Cabral-Pacheco, G. A., Garza-Veloz, I., Castruita-De la Rosa, C., Ramirez-Acuña, J. M., Perez-Romero, B. A., Guerrero-Rodriguez, J. F., Martinez-Avila, N., & Martinez-Fierro, M. L. (2020). The roles of matrix metalloproteinases and their inhibitors in human diseases. International Journal of Molecular Sciences.
6. Cathcart, J., Pulkoski-Gross, A., & Cao, J. (2015). Targeting matrix metalloproteinases in cancer: Bringing new life to old ideas. Genes & Diseases.
7. Chang, D., Sharma, L., Dela Cruz, C. S., & Zhang, D. (2021). Clinical epidemiology, risk factors, and control strategies of Klebsiella pneumoniae infection. Infection and Drug Resistance.
8. Choi, M., Hegerle, N., Nkeze, J., Sen, S., Jamindar, S., Nasrin, S., et al. (2020). The diversity of lipopolysaccharide (O) and capsular polysaccharide (K) antigens of invasive Klebsiella pneumoniae in a multi-country collection. Frontiers in Microbiology.
9. Dell'Annunziata, F., Ciaglia, E., Folliero, V., Lopardo, V., Maciag, A., Galdiero, M., Puca, A. A., & Franci, G. (2024). Klebsiella pneumoniae OMVs activate death-signaling pathways in human bronchial epithelial host cells (BEAS-2B). Microorganisms.
10. Dingiswayo, L., Adelabu, O. A., Arko-Cobbah, E., Pohl, C., Mokoena, Z., Du Plessis, M., & Musoke, J. (2024). Hypervirulent Klebsiella pneumoniae in a South African tertiary hospital—Clinical profile, genetic determinants, and virulence in Caenorhabditis elegans. Microbiology Spectrum.
11. Donadu, M. G., Ferrari, M., Mazzarello, V., Zanetti, S., Kushkevych, I., Rittmann, K. M. R., et al. (2022). No correlation between biofilm-forming capacity and antibiotic resistance in environmental Staphylococcus spp.: In vitro results. Pathogens.
12. Eduarda Souza Guerra, M., Destro, G., Vieira, B., Lima, A. S., Ferraz, L. F. C., Hakansson, A. P., Darrieux, M., & Converso, T. R. (2022). Klebsiella pneumoniae biofilms and their role in disease pathogenesis. Pathogens.
13. El-Demerdash, A. S., Alfaraj, R., Farid, F. A., Yassin, M. H., Saleh, A. M., &Dawwam, G. E. (2024). Essential oils as capsule disruptors: enhancing antibiotic efficacy against multidrug-resistant Klebsiella pneumoniae. Frontiers in Microbiology, 15, 1467460.
14. Element, S., Moran, R. A., Beattie, E., Hall, R. J., van Schaik, W., & Buckner, M. M. C. (2023). Growth in a biofilm promotes conjugation of a blaNDM-1-bearing plasmid between Klebsiella pneumoniae strains. Microbial Genomics.
15. Ferreira, R. L., da Silva, B. C. M., Rezende, G. S., Nakamura-Silva, R., Pitondo-Silva, A., Campanini, E. B., et al. (2019). High prevalence of multidrug-resistant Klebsiella pneumoniae harboring several virulence and β-lactamase encoding genes in a Brazilian intensive care unit. Frontiers in Microbiology.
16. Haghighifar, E., Norouzi, F., & Kamali Dolatabadi, R. (2021). Molecular detection of ESBLs and biofilm formation in uropathogenic Klebsiella pneumoniae in Iran. Infection and Drug Resistance.
17. Herridge, W. P., Shibu, P., O'Shea, J., Brook, T. C., & Hoyles, L. (2020). Bacteriophages of Klebsiella spp., their diversity, and potential therapeutic uses. Journal of Medical Microbiology.

18. Janda, J. M., & Abbott, S. L. (2006). The genera Klebsiella and Raoultella. In The Enterobacteria (2nd ed., pp. 115–129). ASM Press.
19. Jochan, T. J., Nozick, S. H., Valdes, A., Mitra, S. D., Cheung, B. H., Lebrun-Corbin, M., et al. (2023). Klebsiella pneumoniae clinical isolates with features of both multidrug resistance and hypervirulence have unexpectedly low virulence. mBio.
20. Kwiatkowski, P., Sienkiewicz, M., Pruss, A., Łopusiewicz, Ł., Arszyńska, N., Wojciechowska-Koszko, I., ... &Dołęgowska, B. (2022). Antibacterial and anti-biofilm activities of essential oil compounds against New Delhi Metallo-β-lactamase-1-producing uropathogenic Klebsiella pneumoniae strains. Antibiotics, 11(2), 147.
21. Li, L., Gao, X., Li, M., Liu, Y., Ma, J., Wang, X., et al. (2024). Relationship between biofilm formation and antibiotic resistance of Klebsiella pneumoniae and updates on antibiofilm therapeutic strategies. Frontiers in Cellular and Infection Microbiology, 14, Article 1324895. https://doi.org/10.3389/fcimb.2024.1324895
22. Li, Y., & Ni, M. (2023). Regulation of biofilm formation in Klebsiella pneumoniae. Frontiers in Microbiology.
23. Luo, Y., Yang, Q., Zhang, D., & Yan, W. (2020). Mechanisms and control strategies of antibiotic resistance in pathological biofilms. Frontiers in Microbiology.
24. Mai, D., Wu, A., Li, R., Cai, D., Tong, H., Wang, N., & Tan, J. (2023). Identification of hypervirulent Klebsiella pneumoniae based on biomarkers and Galleria mellonella infection model. Microbiology Spectrum.
25. Mirzaie, A., & Ranjbar, R. (2021). Antibiotic resistance, virulence-associated genes analysis and molecular typing of Klebsiella pneumoniae strains recovered from clinical samples. AMB Express. https://doi.org/10.1186/s13568-021-01282-w
26. Mohammad, K. S., & Abada, K. Z. (2005). The Antibiotic-Resistance Era in Enterobacteriaceae: Mechanisms, Epidemiology, and Emerging Countermeasures. Prevalence, 10.
27. Moser, A. I., Campos-Madueno, E. I., Perreten, V., &Endimiani, A. (2022). Genome stability during serial subculturing in hyperepidemic multidrug-resistant Klebsiella pneumoniae and Escherichia coli. Journal of Global Antimicrobial Resistance, 31, 152-161.
28. Mukherjee, S., Mitra, S., Dutta, S., & Basu, S. (2021). Neonatal sepsis: The impact of carbapenem-resistant and hypervirulent Klebsiella pneumoniae. Frontiers in Pediatrics.
29. Nirwati, H., Sinanjung, K., Fahrunnisa, F., Wijaya, F., Napitupulu, S., Hati, V. P., et al. (2019). Biofilm formation and antibiotic resistance of Klebsiella pneumoniae isolated from clinical samples. BMC Proceedings, 13(Suppl 11), 1–8. https://doi.org/10.1186/s12919-019-0176-7
30. Oleksy-Wawrzyniak, M., Junka, A., Brożyna, M., Paweł, M., Kwiek, B., Nowak, M., et al. (2021). The in vitro ability of Klebsiella pneumoniae to form biofilm and the potential of various compounds to eradicate it from urinary catheters. Pathogens.
31. Palacios, M., Miner, T. A., Frederick, D. R., Sepulveda, V. E., Quinn, J. D., Walker, K. A., & Miller, V. L. (2018). Identification of two regulators of virulence that are conserved in classical and hypervirulent Klebsiella pneumoniae. Infection and Immunity.
32. Podschun, R., & Ullmann, U. (1998). Klebsiella spp. as nosocomial pathogens: Epidemiology, taxonomy, typing methods, and pathogenicity factors. Clinical Microbiology Reviews, 11(4), 589–603. https://doi.org/10.1128/CMR.11.4.589
33. Russo, T. A., & Marr, C. M. (2019). Hypervirulent Klebsiella pneumoniae. Clinical microbiology reviews, 32(3), 10-1128.
34. Sá-Pessoa, J., Przybyszewska, K., Vasconcelos, F. N., Dumigan, A., Frank, C. G., Hobley, L., & Bengoechea, J. A. (2020). Klebsiella pneumoniae reduces SUMOylation to limit host defense responses. mBio.
35. Sahoo, A., Swain, S. S., Behera, A., Sahoo, G., Mahapatra, P. K., & Panda, S. K. (2021). Antimicrobial peptides derived from insects offer a novel therapeutic option to combat biofilm. Frontiers in Microbiology.
36. Shadkam, S., Goli, H. R., Mirzaei, B., Gholami, M., & Ahanjan, M. (2021). Correlation between antimicrobial resistance and biofilm formation capability among Klebsiella pneumoniae strains. Annals of Clinical Microbiology and Antimicrobials. https://doi.org/10.1186/s12941-021-00418-x
37. Silva, S. L., Araújo, F. S. M., Silva, P. O. A., Silva, E. V. A., Bezerra, M. M. S. L., Diniz, A. F., ... & Oliveira Filho, A. A. (2023). Evaluation of the antimicrobial effect of the Origanum vulgare L essential oil on strains of Klebsiella pneumoniae. Brazilian Journal of Biology, 83, e269317
38. Tasmia Asma, S., Imre, K., Morar, A., Herman, V., Acaroz, U., Mukhtar, H., et al. (2022). An overview of biofilm formation—Combating strategies and mechanisms of action of antibiofilm agents. Frontiers in Microbiology.
39. Teo, T. H., Ayuni, N., Yin, M., Liew, J. H., Chen, J. Q., Kurepina, N., et al. (2024). Differential mucosal tropism and dissemination of classical and hypervirulent Klebsiella pneumoniae infection. Nature Communications.
40. U. Nwodo, U., Green, E., & Okoh, A. I. (2012). Bacterial exopolysaccharides: Functionality and

prospects. International Journal of Molecular Sciences.

41. Vaou, N., Stavropoulou, E., Voidarou, C., Tsigalou, C., &Bezirtzoglou, E. (2021). Towards advances in medicinal plant antimicrobial activity: A review study on challenges and future perspectives. Microorganisms, 9(10), 2041.
42. Vuotto, C., Longo, F., Pascolini, C., Donelli, G., Balice, M. P., Libori, M. F., Tiracchia, V., Salvia, A., & Varaldo, P. E. (2017). Biofilm formation and antibiotic resistance in Klebsiella pneumoniae urinary strains. Journal of Applied Microbiology, 123(4), 1003–1018. https://doi.org/10.1111/jam.13533
43. Wei, S., Xu, T., Chen, Y., & Zhou, K. (2022). Autophagy, cell death, and cytokines in Klebsiella pneumoniae infection: Therapeutic perspectives. Frontiers in Immunology.