



Universiteit  
Leiden  
The Netherlands

## **Klebsiella pneumoniae: a growing threat in the era of antimicrobial resistance**

Asokan, S.; Jacob, T.; Jacob, J.; AlSosowaa, A.A.; Cherian, T.; Peijnenburg, W.J.G.M.; Vijayan, S.

### **Citation**

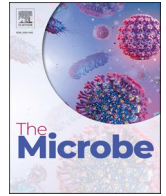
Asokan, S., Jacob, T., Jacob, J., AlSosowaa, A. A., Cherian, T., Peijnenburg, W. J. G. M., & Vijayan, S. (2025). *Klebsiella pneumoniae: a growing threat in the era of antimicrobial resistance*. *The Microbe*, 7. doi:10.1016/j.microb.2025.100333

Version: Publisher's Version

License: [Creative Commons CC BY-NC-ND 4.0 license](#)

Downloaded from: <https://hdl.handle.net/1887/4246614>

**Note:** To cite this publication please use the final published version (if applicable).



# *Klebsiella pneumoniae*: A growing threat in the era of antimicrobial resistance

Sijo Asokan<sup>a</sup>, Teena Jacob<sup>b</sup>, Jenny Jacob<sup>a</sup>, Afaf A. AlSosowaa<sup>a</sup>, Tijo Cherian<sup>a</sup>,  
Willie J.G.M. Peijnenburg<sup>c</sup>, Smitha Vijayan<sup>a,\*</sup><sup>1</sup> 

<sup>a</sup> School of Biosciences, Mar Athanasios College for Advanced Studies Tiruvalla (MACFAST), Pathanamthitta, Kerala, India

<sup>b</sup> Department of Microbiology, Pushpagiri Institute of Medical Sciences and Research Centre, Tiruvalla, Kerala, India

<sup>c</sup> Institute of Environmental Sciences (CML), Leiden University, Leiden, the Netherlands

## ARTICLE INFO

### Keywords:

*Klebsiella pneumoniae*  
Antibiotic resistance  
Virulence factors  
ESBL  
COVID-19  
Risk factors

## ABSTRACT

*Klebsiella pneumoniae* is an opportunistic bacterial pathogen that causes a variety of infections, particularly in hospitals. The growing prevalence of antibiotic resistance in *K. pneumoniae* strains has heightened its clinical significance and threatens patient outcomes. Furthermore, the COVID-19 pandemic has exacerbated the problem by disrupting healthcare systems and influencing antibiotic use practices. The purpose of this review article is to examine the evolving threat of *K. pneumoniae* in the context of antibiotic resistance. Beyond antibiotic resistance, the review also explores epidemiology, virulence factors, patient risk factors, and treatment strategies for *Klebsiella* infections, offering a comprehensive perspective on the multifaceted challenges posed by this opportunistic pathogen in the healthcare settings.

## 1. Introduction

The *Enterobacteriaceae* family includes *Klebsiella pneumoniae*, which is found in the gastrointestinal tract microbiome of healthy people and animals. It is an opportunistic pathogen that is frequently found in hospitals and is responsible for about one-third of all Gram-negative infections. Despite this, it can also cause a number of extra-intestinal infections, such as septicemia and endocarditis, as well as life-threatening infections like pneumoniae and septic shock. Furthermore, it contributes significantly to the development of severe community-acquired infections such as necrotizing pneumonia, pyogenic liver abscesses, and endogenous endophthalmitis (Podschn and Ullmann, 1998).

Particularly in the current era of rising antibiotic resistance, *K. pneumoniae* is regarded as one of the most concerning pathogens involved in antibiotic resistance. Along with other highly significant multi-drug resistant (MDR) pathogens, it has been categorized as an ESKAPE organism (Boucher et al., 2009). The growing prevalence of *K. pneumoniae* strains that produce extended spectrum  $\beta$ -lactamase (ESBL) and carbapenemase in healthcare facilities is a matter of worldwide apprehension. In this review, we focus on the main features

of *K. pneumoniae* that shape it as a highly important worldwide antibiotic resistant pathogen which includes epidemiology, virulence factors, ESBL types, patient risk factors and treatment.

## 2. Taxonomy and diversity of *Klebsiella* species

*Klebsiella* bacteria are Gram-negative, encapsulated, non-motile, rod-shaped, and lack oxidase activity. Trevisan (1885) named ten strains in this genus after German microbiologist Edwin Klebs (1834–1913 Podschn and Ullmann, 1998). *Klebsiella* belongs to the *Enterobacteriaceae* family, which also includes *Salmonella*, *Enterobacter*, *Citrobacter*, *Cluyvera*, *Leclercia*, *Raoultella*, and *Cronobacter*, among other well-known bacteria. The *Klebsiella* genus (Fig. 1) encompasses the *K. pneumoniae* species complex (KpSC) along with other *Klebsiella* species such as *K. indica*, *K. terrigena*, *K. spallanzanii*, *K. huaxiensis*, *K. oxytoca*, *K. grimontii*, *K. pasteurii*, and *K. michiganensis*, which share a 90 % nucleotide identity with KpSC. The term "KpSC" denotes closely related species with a 95 %-96 % nucleotide identity to *K. pneumoniae sensu stricto*. Within KpSC, there are seven phylogroups: *K. pneumoniae* (Kp1), *K. quasipneumoniae subsp. quasipneumoniae* (Kp2), *K. variicola subsp. variicola* (Kp3), *K. quasipneumoniae subsp. similipneumoniae* (Kp4), *K.*

\* Corresponding author.

E-mail address: [smitha@macfast.org](mailto:smitha@macfast.org) (S. Vijayan).

<sup>1</sup> <https://orcid.org/0000-0002-6376-306X>

variicola subsp. tropica (Kp5), *K. quasivariicola* (Kp6), and *K. africana* (Kp7).

Biochemical and proteomics assays previously misidentified all KpSC taxa as *K. pneumoniae* due to overlapping features. Modern species identification techniques use sequence-based classifiers or MALDI-TOF platforms, which analyze strains or reference genomes and require up-to-date databases for accurate taxonomy. For *K. pneumoniae* isolates, the multilocus sequence typing (MLST) strategy used is applicable to the whole species complex. Moreover, based on their rpoB sequences, *K. terrigena*, *K. planticola*, and *K. ornithinolytica* have been added to the *Raoultella* genus (Dong et al., 2022).

### 3. *Klebsiella pneumoniae*

In 1882, while investigating lung samples from pneumonia patients, Friedlander made a significant discovery of a bacterium that was later identified as *K. pneumoniae*. Originally referred to as Friedlander's bacillus, it was later renamed *Klebsiella* in 1886. Additional research revealed that *Klebsiella* is not only present in the human gastrointestinal tract but also colonizes the skin and nasopharynx as a saprophytic microorganism (Rasmussen and Bush, 1997 Feb).

*K. pneumoniae* is found in the respiratory tracts and faeces of approximately 5 % of healthy individuals, and it accounts for only about 1 % of bacterial pneumonia cases. Nevertheless, *K. pneumoniae* can result in severe lung consolidation due to hemorrhagic necrotizing. In immunocompromised patients, it may lead to urinary tract infections and bacteremia, causing focal lesions. Some enteric bacteria can also lead to pneumonia. *K. pneumoniae* and *K. oxytoca* are accountable for hospital-acquired infections (Brooks et al., 2007).

*K. pneumoniae* is responsible for the highest rates of morbidity and mortality in developed nations. The most frequent underlying conditions that result in *K. pneumoniae* infections are diabetic mellitus, neoplastic diseases, and hepatobiliary diseases. In patients with community-acquired *K. pneumoniae* bacteremia, diabetes mellitus was frequently observed as an associated condition. On the other hand, neoplastic diseases are often linked with nosocomial *K. pneumoniae* bacteremia due to factors such as prolonged hospitalization, invasive

procedures, chemotherapy, and antibiotic use (Tsai et al., 2010).

### 4. Epidemiology

*K. pneumoniae* primarily resides in humans, with 5–38 % of individuals in the general community carrying it in their stool and 1–6 % in their nasopharynx. The gastrointestinal tract and the hands of hospital workers are the main sources of infection, which can result in hospital-acquired outbreaks. Chinese individuals and those with chronic alcoholism have higher rates of colonization. *K. pneumoniae* is much more prevalent among hospitalized patients than in the general population, with carrier levels in the stool of up to 75 % observed in some cases, likely due to the administration of antibiotics (Esposito et al., 2018; Walter et al., 2018).

### 5. Virulence factors of *K. pneumoniae*

Several factors contribute to *K. pneumoniae*'s increased survival and virulence, including capsule polysaccharides (CPS), lipopolysaccharides (LPS), siderophores (e.g., aerobactin), and fimbriae (Fig. 2).

#### 5.1. Capsule polysaccharide (CPS)

The *Klebsiella* strains are surrounded by a thick hydrophilic polysaccharide capsule, which is commonly found and gives them a shiny, mucoid appearance on agar colonies. This capsule is resistant to various host defense mechanisms (Ahmadi et al. 2022a). The loss of this characteristic has been associated with reduced virulence in subcultures (Brisse et al., 2006). *In vitro*, the presence of the capsule significantly hinders the deposition of bacterial complement components and leads to a noticeable decrease in bacterial phagocytosis by macrophages (Cortés et al., 2002). The formation of capsules also interferes with the proper formation of Type 1 fimbriae on the bacterial surface, which may obstruct the transcription of other adhesive molecules (Matatov et al., 1999). As a result, isogenic capsule-negative variants show increased adherence to and invasion of different cultured cells compared to wild-type strains (Sahly et al., 2008).

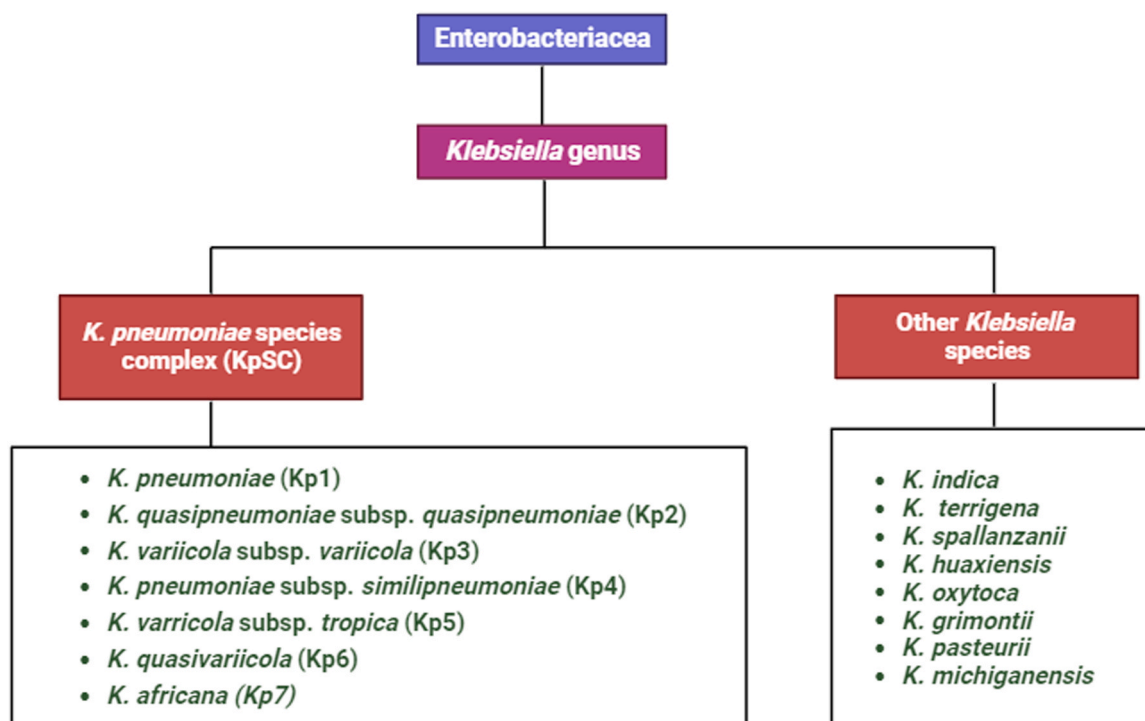


Fig. 1. Species composition of the *Klebsiella* genus.

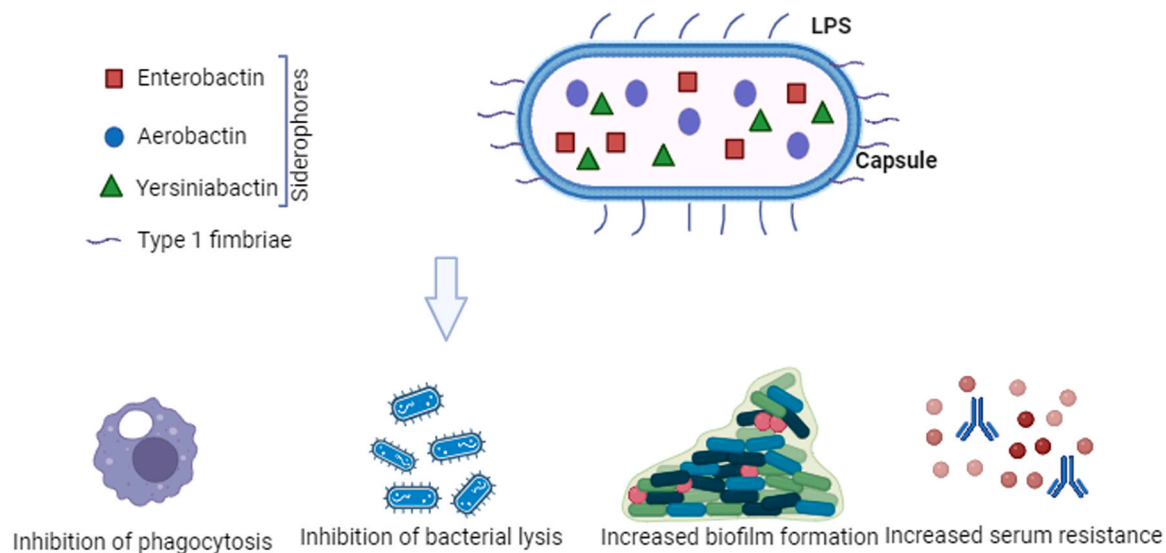


Fig. 2. Virulence factors of *Klebsiella pneumoniae*.

Numerous capsule types (K), particularly K1, K2, K54, K57, K20, and K5, are commonly linked to pneumonia, septicemia, and community-acquired invasive pyogenic liver abscess. Furthermore, K1, K2, K20, K54, and K57 are frequently associated with severe infections in humans and cause significant harm in experimental infections in mice (Wei et al., 2016).

### 5.2. Lipopolysaccharides

*K. pneumoniae* has lipopolysaccharides (LPS) integrated into its membrane. LPS consists of three structural components: a lipid A component that secures the entire structure in the bacterial membrane, an oligosaccharide core, and a terminal side chain known as the O antigen. The lipid A is a hydrophobic moiety that is located in the outer leaflet of the outer membrane. Lipid A is critical in host recognition of LPS because it binds strongly to Toll-like receptor 4 (TLR4), triggering the immune response (Opoku-Temeng et al., 2019).

The O antigen is a polymer composed of repeating oligosaccharide units located on the LPS structure's outermost region. *K. pneumoniae* has nine identified O antigens, with diversity attributed primarily to differences in the structure and arrangement of the sugar monomers. Bacteria that express full length O antigens, also known as the "smooth" LPS phenotype, are less susceptible to complement-mediated killing than strains that lack or express truncated O antigens, also known as the "rough" LPS phenotype. Therefore, the length of O antigens is also crucial for bacterial survival. *K. pneumoniae* resistance to complement-mediated death is associated with LPS's ability to bind and sequester complement system components, particularly the O antigens, or effectively inhibit their attachment to the bacterial surface (Opoku-Temeng et al., 2019).

### 5.3. Siderophores

*K. pneumoniae* requires iron, a critical nutrient derived entirely from the environment, to thrive during infection. This metal is usually not readily available in the host during an infection. Host plasma typically has low levels of free iron because iron transport molecules such as transferrin bind to it. In response to bacterial infection, mammalian hosts can further diminish iron levels by redirecting iron binding to lactoferrin, an innate defense protein in body fluids. To thrive during mammalian infection, *K. pneumoniae*, like many other bacterial pathogens, must use iron acquisition strategies (Cortés et al., 2002).

Many pathogens, including *K. pneumoniae*, use siderophore secretion

as their primary mechanism for iron acquisition. When it comes to binding iron, these molecules have a greater affinity than host transport proteins. Siderophores function by either scavenging iron from the surrounding environment or sequestering it from host iron-chelating proteins. Some strains of *K. pneumoniae* produce enterobactin, salmochelin, yersiniabactin, and aerobactin, among other siderophores (Fig. 3). The main siderophore of *K. pneumoniae* is enterobactin; however, lipocalin-2, the host molecule, inhibits enterobactin's ability to function. Lipocalin-2 no longer inhibits salmochelin, which is a c-glucosylated form of enterobactin. Yersiniabactin and aerobactin have structural differences from enterobactin and salmochelin. Lipocalin-2 does not inhibit either siderophore, but the efficacy of yersiniabactin is diminished when the host molecule transferrin is present. *K. pneumoniae*'s ability to produce multiple siderophores may allow it to colonize and spread throughout the host, with each siderophore playing a distinct role in different niches. FepA, IroN, YbtQ, and IutA are transporters for enterobactin, salmochelin, yersiniabactin, and aerobactin, respectively (Cortés et al., 2002).

### 5.4. Type 1 and 3 fimbriae

The main adhesive structures identified as pathogenicity factors are type 1 and 3 fimbriae. Type 1 fimbriae are slender, thread-like projections on the surface of bacterial cells that are found in 90 % of clinical and environmental *K. pneumoniae* isolates, as well as nearly all Enterobacteriaceae family (Stahlhut et al., 2009). Type 1 fimbriae of *K. pneumoniae* bind to d-mannosylated glycoproteins, indicating that they are mannose sensitive. The genes that control Type 1 fimbriae expression are active in the urinary tract and have been linked to UTIs. Type 3 fimbriae are helical filaments. Unlike type 1 fimbriae, type 3 fimbriae are "mannose insensitive," which means they do not bind to mannose. Furthermore, type 3 fimbriae aren't required for gastrointestinal tract colonization or lung virulence (Di Martino et al., 1996). They are associated with biofilm formation on catheters (Behzadi et al., 2023) and endotracheal tubes. Type 3 fimbriae, and possibly Type 1 fimbriae, may facilitate the entry, persistence, and delivery of *K. pneumoniae* in ventilator-associated pneumonias (Paczosa and Mecsas, 2016).

### 5.5. Biofilm formation by *K. pneumoniae*

A group of microorganisms that adhere to a surface form a biofilm. Extracellular polymer (EPS), a slimy substance, surrounds and holds the cells in the biofilm together. Proteins, carbohydrates, and elements like

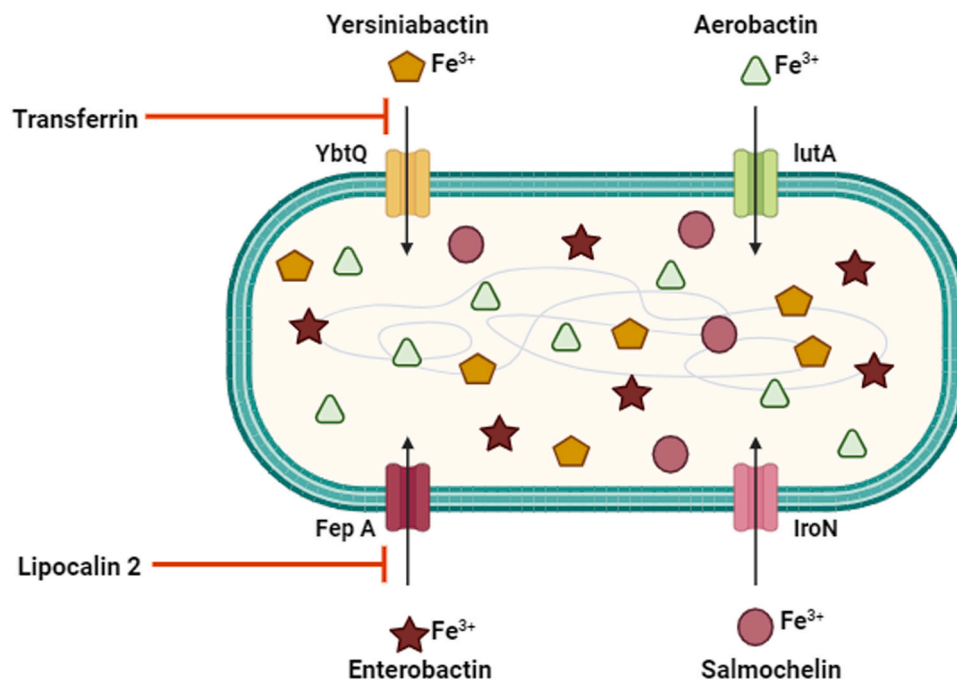


Fig. 3. Siderophore production in *K. pneumoniae*.

DNA make up the EPS (Ahmadi et al. 2022b). Biofilms can develop on both living and non-living surfaces and are found in a variety of settings, including hospitals, industry, and natural environments. Despite the fact that they resemble one another in some aspects, slime and a biofilm are distinct from one another (Lear and Lewis, 2012).

Certain types of bacteria can bind to the slimy substance or directly to other bacteria that are already there but cannot connect to their own surface. These bacteria may use acylated homoserine lactone (AHL), a chemical used in quorum sensing, to interact with one another as they colonise. However, some bacteria are less efficient for developing biofilms because of their restricted range of motion. In comparison to bacteria that can move, those that cannot move easily have a harder time gathering and growing on surfaces (Li et al., 2001).

Biofilm formation (Fig. 4) involves the following key stages:

#### 5.5.1. Reversible attachment

A number of physical, chemical, and biological processes occur on a surface when bacteria come into contact with it. In most cases, general

interactions like electrical forces, hydrophobic forces, or van der Waals forces are involved when bacteria adhere to non-living surfaces, such as abiotic surfaces. On the other hand, more complicated molecular mechanisms, such as lectin or adhesive interactions, are involved when bacteria attach to living surfaces, such as tissues (Dunne, 2002). According to some studies, bacteria that can move, known as planktonic cells, may use this ability to move around to make initial contact with inanimate objects (O'Toole and Kolter, 1998).

#### 5.5.2. Irreversible attachment

The next step involves the irreversible attachment, growth, and layering of bacterial cells after the exopolymer provides a surface for bacteria to adhere to. Extracellular matrices, which include polysaccharides, proteins, DNA, and other components, hold these layers together. According to Davies and Geesey (1995) (Davies and Geesey, 1995), these matrices are crucial for maintaining the biofilm's structure, obtaining and holding onto nutrients needed for biofilm production, and protecting the cells from drying out and the negative effects of

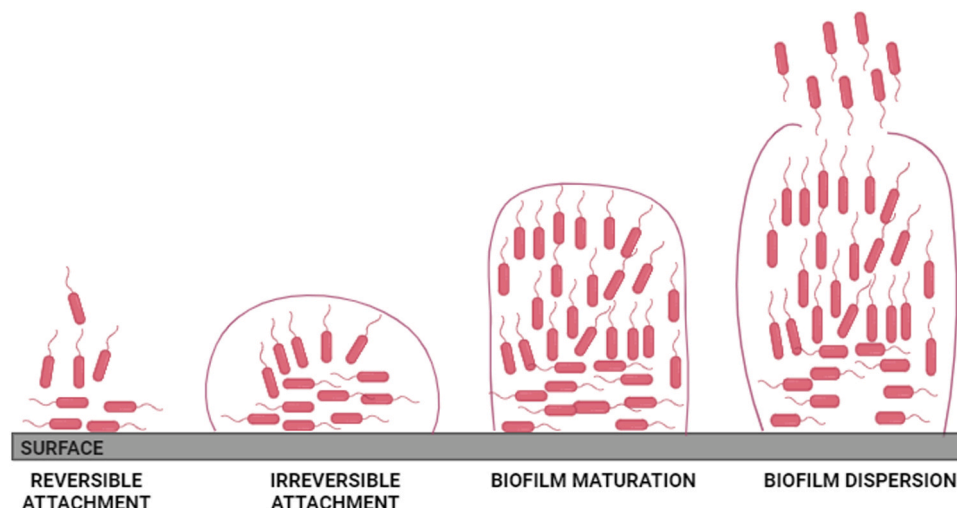


Fig. 4. Process of biofilm formation in *K. pneumoniae*.

antimicrobial agents.

### 5.5.3. Maturation of biofilm formation

After the bacterial cells have committed themselves to a surface irrevocably, they go through phenotypic changes, and the biofilm maturation process begins. In order to start establishing micro-colonies, bacteria either gather already secured cells, clonally proliferate, or recruit planktonic or bulk fluid cells. Numerous extracellular components produced by the connected cells interact with other organic and inorganic molecules to form glycocalyx (Lawrence et al., 1991).

It was suggested that the microcolony, similar to the tissues that make up more complex species, acts as the main basis for the development of biofilms. Similar to this, the water channels in the biofilm constitute an early circulatory system that resembles that of higher organisms. There is a secure time and space structure in microbial biofilms. Nutrients are delivered to bacteria at a low water flow rate through water channels, making the basic "style" of mushroom-like microcolonies with overriding water canals appropriate for nutrient admittance (Stoodley et al., 2002).

Since the late 1980s, *K. pneumoniae* has been observed to develop a biofilm *in vitro*. However, only Reid and other members studied some bladder epithelial cells of a person with an asymptomatic urinary tract infection rise by Electron Microscope *K. pneumoniae* in 1992 and presented clear evidence for *in vivo* biofilm (Reid et al., 1992).

Subsequent laboratory tests show that approximately 45 % of *K. pneumoniae* was found not only in urine but also in sputum, blood, and tumour swabs. Furthermore, in laboratory conditions, approximately 63 % of *K. pneumoniae* samples taken from catheterized urinary tract infections (UTIs) produced biofilm (Niveditha et al., 2012; Behzadi et al., 2023).

Furthermore, *K. pneumoniae* strains isolated from the endotracheal tube (ETT) in patients with ventilator-associated pneumonia (VAP) have the ability to form biofilms in laboratory settings (Singhai et al., 2012).

## 6. Clinical manifestations

The clinical manifestations include pneumonia, bacteremia, wound infection, pyogenic liver abscess, meningitis and urinary tract infections (Fig. 5).

### 6.1. Pneumonia

Friedlander described the first case of lobar pneumonia caused by *K. pneumoniae* in 1882. The infection typically manifests as a consolidation in a specific region of the lung and causes varying degrees of tissue damage, including abscesses, cavities, bronchiectasis, empyema, and pleural adhesions. Because of the presence of the *Klebsiella* bacteria's mucoid coating, the affected areas may appear slimy. This type of pneumonia can look like tuberculosis in chronic cases, with bronchiectasis and scarring in the lung tissue (Hansen et al., 1998).

The two main types of pneumonias caused by *K. pneumoniae* are hospital-acquired pneumonias (HAPs) and community-acquired pneumonias (CAPs). *K. pneumoniae* HAPs are much more common than *K. pneumoniae* CAPs. Hospital-acquired pneumonia (HAP) is defined as pneumonia that develops at least 48 hours after a person's admission to a hospital, in the absence of any pneumonia symptoms before admission. *K. pneumoniae* is the cause of approximately 11.8 % of HAPs. Like other nosocomial pneumonias, *K. pneumoniae* HAP causes systemic symptoms like fever and leukocytosis in addition to respiratory symptoms like cough and unilateral pulmonary infiltrates. Both ventilated and non-ventilated patients experience these HAPs. CAP refers to potentially serious infections that are associated with hospitalization, ICU admissions, and increased morbidity and mortality rates. *K. pneumoniae* CAPs produce acute pneumonia-like symptoms such as cough, fever, leukocytosis, and chest pain. A distinguishing feature of these infections is the production of "currant jelly sputum," which is thick, blood-tinged mucus caused by increased inflammation and necrosis in the lungs and is a sign of *K. pneumoniae* infection (Paczosa and Meccas, 2016).

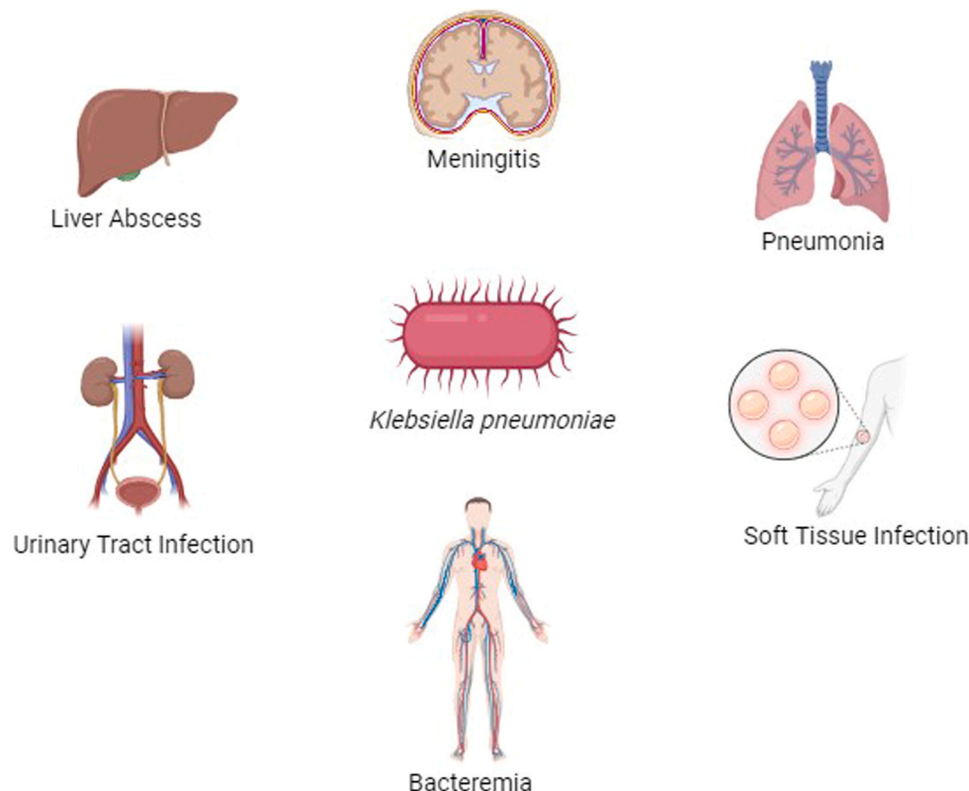


Fig. 5. Clinical manifestations of *K. pneumoniae*.

## 6.2. Bacteremia and septicemia

Bacteremia is the presence of bacteria in the bloodstream, whereas septicemia is a condition in which bacteria actively multiply in the blood, causing toxic substances to be produced. Gram-negative bacilli commonly associated with bacteremia include *Klebsiella pneumoniae*, *E. coli*, *P. aeruginosa*, *Proteus* species, *Salmonella* species, and *Acinetobacter* species. The invasion of these organisms is considered a severe condition in infectious diseases. Infections that begin outside of blood vessels are more common than infections that begin within them, and bacteria enter the bloodstream via the lymphatic system. These infections often stem from local sites of infection, such as the genitourinary tract, respiratory tract, or surgical sites. Infective endocarditis and bacteremia associated with intravenous lines and catheters are examples of intravascular infections (Banerjee et al., 1993).

*K. pneumoniae* is the second most common Gram-negative bacteria that cause nosocomial and community-associated bacteremias, after *E. coli*. 50 % of nosocomial *K. pneumoniae* bacteremia develops by primary lung infections. *Klebsiella pneumoniae* bacteremias are a serious threat, with a high death rate. Several patient factors contribute to the higher risk of mortality associated with *K. pneumoniae* bacteremia. These include ICU admission, age over 65, an underlying malignancy, pneumonia, the need for mechanical ventilation or urinary catheters, and alcoholism (Paczosa and Meccas, 2016; Behzadi et al., 2023).

## 6.3. Wound infection and surgical site infection (SSI)

Wound infections can develop as a result of surgical procedures, trauma, burns, or any condition that compromises the integrity of the mucosal, cutaneous, or tissue barriers. Burns and surgical site infections (SSIs) are especially dangerous. An infection that occurs within 30 days of a procedure and affects the skin, subcutaneous tissue, and deeper soft tissues of the incisions is referred to as SSI. *Klebsiella pneumoniae* is commonly found in the gastrointestinal tract and is a Gram-negative microorganism. The majority of these infections occur after laparotomies, and the majority of wounds were either clean-contaminated, contaminated, or dirty, with GI spillage contributing to SSIs. Low serum albumin levels, anaemia, and the type of suture used have all been linked to the development of SSI (Kamal et al., 2017).

## 6.4. Pyogenic liver abscess

Hypervirulent *K. pneumoniae* is a gram-negative bacterium that can cause pyogenic liver abscess (PLA) even when there is no hepatobiliary disease. Diabetes patients are at a higher risk of contracting this infection. Endophthalmitis (inflammation of the inner eye), meningitis (inflammation of the membranes surrounding the brain), brain abscess, septic pulmonary emboli (infected blood clots in the lungs), lung abscess, splenic abscess, and osteomyelitis (bone infection) can occur in patients with *Klebsiella* liver abscess (Tang and Chen, 1994). Because of the hematogenous spread from the liver, liver abscesses can cause a variety of various secondary infections. Although *K. pneumoniae* meningitis is uncommon in many parts of the world, it has been reported in Taiwan as a complication of a community-acquired liver abscess (Paczosa and Meccas, 2016).

## 6.5. Meningitis

*Klebsiella* meningitis is more common in the elderly and has been linked to alcoholic liver disease, diabetes, and transfusion-dependent thalassemia major. Co-infection with other pathogens, such as *Enterobacter*, is possible. *K. pneumoniae* was responsible for 13 % of culture-positive meningitis cases, while *K. oxytoca* was responsible for 2.3 percent, according to a series of cases reported in Taiwan. It is worth noting that *Klebsiella* meningitis can occur in Asian countries as a secondary metastatic infection caused by a liver abscess (Sathyavathy and

Madhusudhan, 2020).

## 6.6. Urinary tract infection

*Klebsiella* is responsible for approximately 6–17 % of nosocomial urinary tract infections (UTIs) acquired in healthcare settings (Fig. 6). *Klebsiella*-related UTIs are more common in certain patient populations at risk, such as those with neuropathic bladders or those with diabetes mellitus (Sathyavathy and Madhusudhan, 2020; Behzadi and Behzadi, 2008). These infections frequently produce symptoms such as painful urination, frequent and urgent urination, and blood in the urine, which are similar to those seen with other bacterial infections (Paczosa and Meccas, 2016).

## 7. Patient risk factors

Compared to the general population, those with underlying immunodeficiency are far more vulnerable to infections with classical *K. pneumoniae* strains. Dialysis, solid-organ transplantation, diabetes, malignancy, and chronic liver disease are risk factors for contracting nosocomial bacteremia caused by *K. pneumoniae* (Fig. 7). Given the alarming rise in the overall number of diabetics in the general population, it is reasonable to expect an increase in the number of CAPs caused by *K. pneumoniae* strains. The suppression of the innate immune system is common among diabetics, cancer patients, and alcoholics. Cytotoxic treatments that target rapidly dividing cells, such as immune and cancerous cells, frequently cause decreased levels of innate immune cells in cancer patients, resulting in conditions such as neutropenia. Furthermore, people with diabetes have weakened bacterial defenses due to changes in chemokine and cytokine production, reduced neutrophil responses, and impaired phagocytic functions. These deficiencies are most likely associated with altered glucose metabolism and oxidative stress, as well as other immune system changes (Paczosa and Meccas, 2016).

Neonates and elderly people are also at risk of contracting *K. pneumoniae* infections. Neonates, particularly those born prematurely or in intensive care units, are vulnerable due to their underdeveloped immune systems, lack of established microbiota, and relatively high mucosal permeability in their gastrointestinal tracts. *K. pneumoniae* is frequently the causative agent of sepsis in neonates, and it is the leading cause of neonatal sepsis in some developing countries. The elderly people are more prone to infections because of alterations in their immune responses over time that reduce their ability to fight off infections (Paczosa and Meccas, 2016).



Fig. 6. Colonies of *Klebsiella pneumoniae* on MacConkey Agar isolated from urine sample.

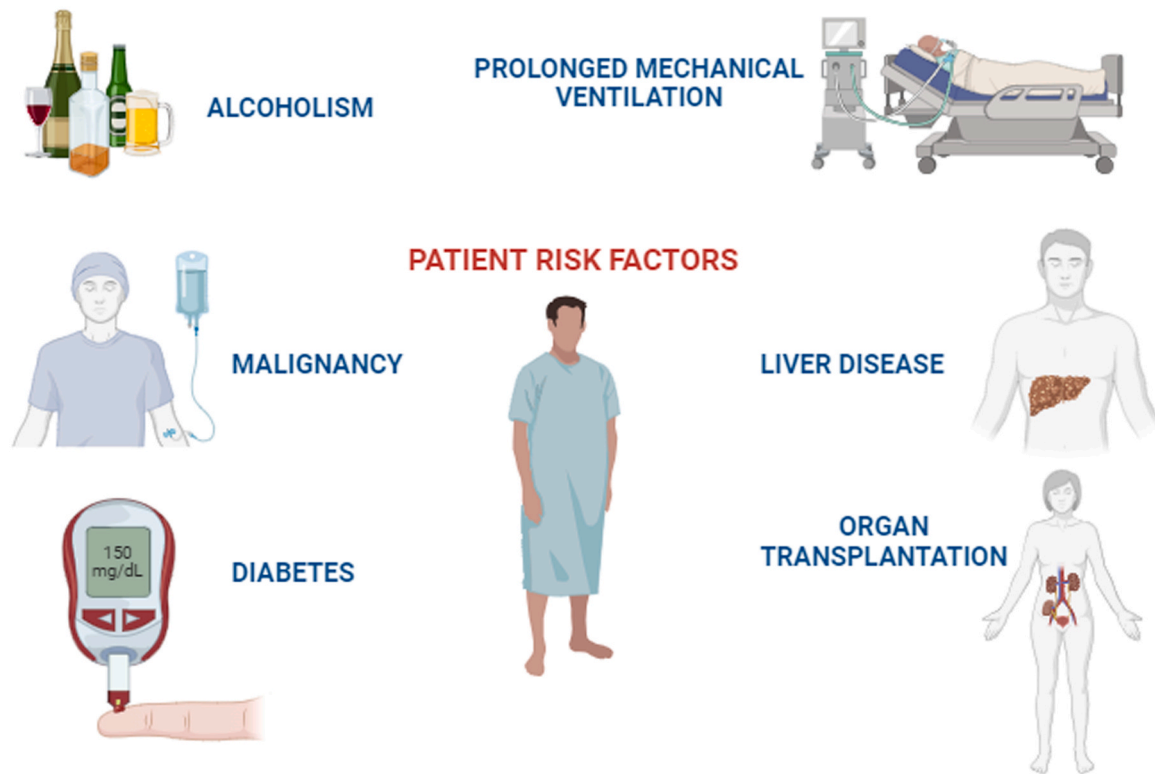


Fig. 7. Risk factors related to *K. pneumoniae* infection.

Patients who undergo procedures involving medical equipment or implants provide an open pathway for *K. pneumoniae* entry. Endotracheal intubation is a common way to acquire *K. pneumoniae* pneumonias, which increases the risk of developing ventilator-associated pneumonia (VAP), a type of nosocomial pneumonia that appears at least 48 hours after intubation. This procedure has the potential to increase the risk of pneumonia through multiple mechanisms. These include disrupting the mechanisms that clear the respiratory tract, creating an environment that encourages the formation of bacterial biofilms, and possibly accumulating oropharyngeal secretions that carry bacteria around the endotracheal tube's cuff. Another way for *K. pneumoniae* to infect someone is through the insertion of a catheter, since the implant acts as a substrate for *K. pneumoniae* to grow a biofilm and as an entrance point into the urinary tract (Paczosa and Mecsas, 2016).

Most *K. pneumoniae* infections are caused by bacteria found in a person's microbiota. For example, when *K. pneumoniae* colonizes the gastrointestinal (GI) tract, people are more likely to develop infections from both classical strains commonly found in hospitals and abscesses caused by hypervirulent *K. pneumoniae* strains. Similarly, *K. pneumoniae* colonization in the oropharynx increases the risk of *K. pneumoniae* ventilator-associated pneumonia (VAP). Extended hospitalization is associated with a higher prevalence of *K. pneumoniae* carriage. *K. pneumoniae* is frequently transmitted through the hands of healthcare personnel or contaminated medical devices in healthcare settings. Furthermore, antibiotic therapy removes antibiotic-sensitive microorganisms from the gut microbiota, allowing a previously small population of *K. pneumoniae* to grow rapidly, increasing carriage rates (Paczosa and Mecsas, 2016).

## 8. Mechanism of antibiotic resistance

*K. pneumoniae* is resistant to key antibiotic classes such as carbapenems, cephalosporins, aminoglycosides, and fosfomycin, making these agents ineffective and causing therapeutic failures (Karampatakis et al., 2023). The spread of antibiotic resistance in *K. pneumoniae* has reduced

the efficacy of conventional treatments against this pathogen. Resistance can occur as a result of increased efflux, drug inactivation, or altered target site binding. Resistance is further exacerbated by the production of ESBL or biofilms by numerous *K. pneumoniae* strains. The five main mechanisms by which *K. pneumoniae* develops antibiotic resistance are as follows: (1) enzymatic antibiotic inactivation and modification; (2) antibiotic target alteration; (3) porin loss and mutation; (4) increased efflux pump expression of the antibiotic; and (5) biofilm formation (Li et al., 2023; Ahmadi et al. 2022c). Fig. 8 and Table 1 illustrate the five mechanisms that give *K. pneumoniae* antibiotic resistance.

### 8.1. Genetic exchanges, pan-genome, and antibiotic resistance in *K. pneumoniae*

Antibiotic resistance in *K. pneumoniae* is primarily mediated by horizontal gene transfer (HGT), which facilitates the rapid dissemination of antimicrobial resistance genes (ARGs) across bacterial populations (Karampatakis et al., 2024). This process occurs through conjugation, transformation, and transduction, with conjugation playing the most significant role. Conjugative plasmids, which frequently carry multiple ARGs, enable the spread of resistance determinants such as *bla*CTX-M, *bla*SHV, *bla*TEM (conferring ESBL production), and *bla*KPC, *bla*NDM, *bla*OXA-48 (associated with carbapenem resistance) (Behzadi et al., 2020). Class 1 integrons within these plasmids further promote the acquisition and dissemination of diverse resistance gene cassettes, exacerbating multidrug resistance. Transformation contributes to ARG acquisition, particularly in biofilm-associated infections, where extracellular DNA from lysed bacterial cells is readily integrated into the genome. Transduction, mediated by bacteriophages, facilitates additional gene exchange among *K. pneumoniae* strains. Recently, outer membrane vesicles (OMVs) have emerged as a novel HGT mechanism, encapsulating and delivering genetic material that enhances both resistance and virulence (Algammal et al., 2023).

The *K. pneumoniae* pan-genome, comprising core, accessory, and unique genes, underscores its genetic diversity and adaptability. The

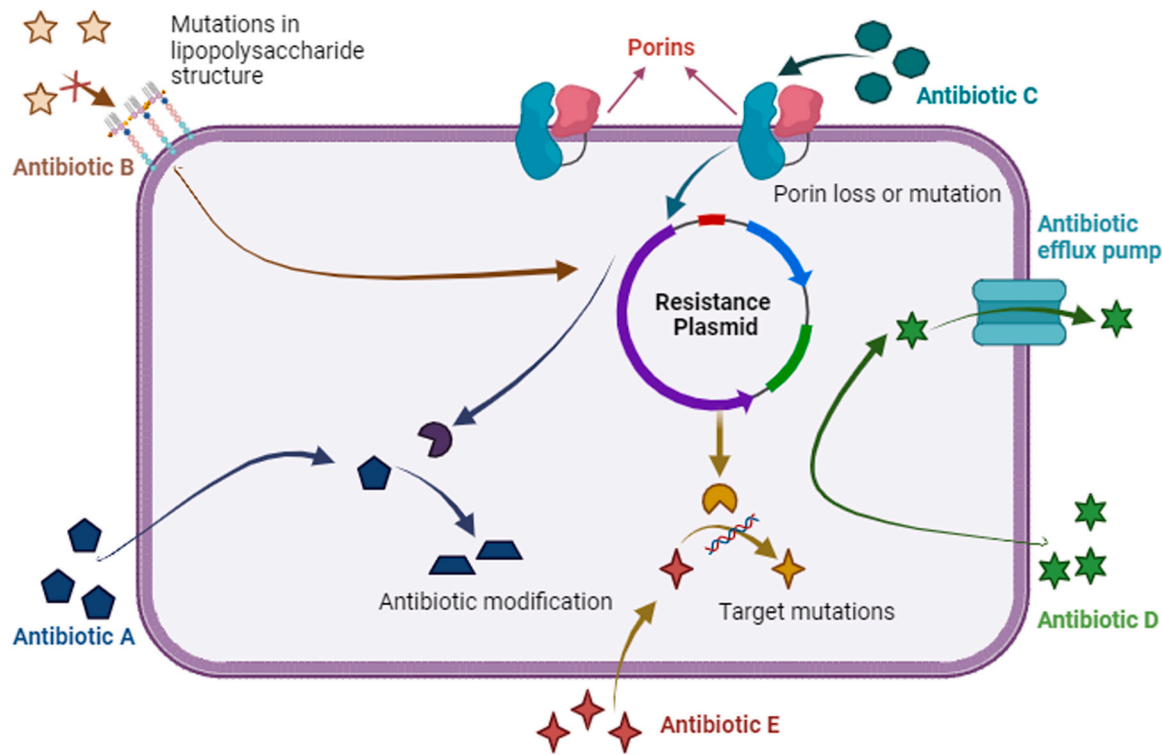


Fig. 8. Antibiotic Resistance Mechanism in *K. pneumoniae*.

**Table 1**  
Mechanism of Drug Resistance in *K. pneumoniae*.

| Mechanism of Resistance                            | Key findings                                                                                                                                           |
|----------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------|
| Enzymatic antibiotic inactivation and modification | $\beta$ -Lactamase is a key resistance mechanism, classified as ESBLs, AmpC, and carbapenemases.                                                       |
| Antibiotic target alteration                       | <i>K. pneumoniae</i> modifies the target gene or methylates certain bases to produce drug resistance.                                                  |
| Porin loss and mutation                            | By decreasing the outer membrane pore protein, <i>K. pneumoniae</i> becomes resistant to antibiotics by limiting their ability to enter the bacterium. |
| Increased efflux pump expression of the antibiotic | Efflux pumps lower intracellular drug concentrations by releasing antimicrobial cells outside the cell, which reduces resistance to many antibiotics.  |
| Biofilm formation                                  | Biofilms are resistant to antibacterial treatments and possess osmotic barrier characteristics.                                                        |

core genome includes conserved genes essential for fundamental cellular functions, ensuring bacterial survival. The accessory genome, present in only some strains, encodes traits such as antimicrobial resistance and virulence, enabling adaptation to diverse environments, particularly in hospital settings under antibiotic pressure. Unique or singleton genes, often acquired via HGT, confer specific survival advantages in distinct ecological niches. Unlike bacteria with a closed pan-genome, *K. pneumoniae* exhibits an open pan-genome, continuously incorporating new genetic material through plasmids, transposons, and integrons. This genetic plasticity accelerates its evolution, particularly in clinical environments, fostering resistance development. The bacterium's ability to acquire genes encoding capsular polysaccharides and iron acquisition systems further enhances its virulence, aiding immune evasion and infection establishment. Consequently, *K. pneumoniae* is a major pathogen responsible for severe healthcare-associated infections, including pneumonia, bloodstream infections, and urinary tract infections (Karampatakis et al., 2024; Issakhanian and Behzadi, 2019). The ongoing evolution of *K. pneumoniae* presents significant challenges

for public health, necessitating continuous genomic surveillance and targeted therapeutic interventions to mitigate its spread.

Beyond HGT, antibiotic resistance in *K. pneumoniae* arises from genetic mutations affecting drug targets, porins, and efflux pumps. Fluoroquinolone resistance is predominantly driven by mutations in *gyrA* and *parC*, encoding DNA gyrase and topoisomerase IV, respectively. Mutations within quinolone resistance-determining regions (QRDRs) alter drug-binding affinity, diminishing fluoroquinolone efficacy and leading to high-level resistance. Carbapenem resistance frequently results from mutations in outer membrane porins, particularly OmpK35 and OmpK36. Structural modifications, downregulation, or loss of these porins reduce membrane permeability, restricting antibiotic influx (Behzadi et al., 2020). When coupled with carbapenemase production, porin loss significantly compromises carbapenem effectiveness, posing a major clinical challenge. Mutations in *rpoB*, encoding the RNA polymerase  $\beta$ -subunit, confer rifampin resistance by altering the drug-binding site, reducing its inhibitory effect. Additionally, efflux pump mutations enhance resistance by actively expelling antibiotics. Overexpression or structural changes in the AcrAB-TolC efflux system reduce intracellular concentrations of  $\beta$ -lactams, aminoglycosides, tetracyclines, and fluoroquinolones, further limiting therapeutic efficacy (Issakhanian and Behzadi, 2019). Understanding the genetic mechanisms underlying *K. pneumoniae* resistance is essential for developing effective antimicrobial strategies. Continuous genomic surveillance and targeted therapeutic interventions remain crucial in managing the growing threat of multidrug-resistant *K. pneumoniae* infections.

### 9. Extended spectrum $\beta$ -lactamase (ESBL) producing *K. pneumoniae*

The global prevalence of antibiotic-resistant  $\beta$ -lactam bacteria, including cephalosporin-resistant Enterobacteriaceae, has risen dramatically in recent years. Resistance has increased in humans, various animal species, and the environment (Huijbers et al., 2015). The production of ESBL and AmpC  $\beta$ -lactamase enzymes are the primary causes of cephalosporin resistance (Bevan et al., 2017; Jacoby, 2009).

Since the early 1980s, the emergence of drug-resistant microorganisms, particularly Enterobacteriaceae producing ESBL, has been documented globally (World Health Organization, 2015). Bacterial resistance is being attributed not only to natural evolution of microorganisms, but also to the overuse of antimicrobial agents, which has accelerated this process. Antibiotic resistance genes have been discovered to originate from environmental bacteria, affecting the environment's microbiota. Antibiotic overuse, both preventive and therapeutic, as well as the release of human and animal microbiota containing resistance genes, all contribute to the worsening of the situation (Martinez, 2009; Ahmadi et al. 2022a).

ESBLs aid in the breakdown of penicillins and cephalosporins. Gram-negative enteric bacteria from the Enterobacteriaceae family have developed resistance to these beta-lactam drugs by acquiring the ESBL gene and producing similar enzymes. ESBL-producing Enterobacteriaceae infections affect over 1.5 billion people worldwide (Woerther et al., 2013). *Klebsiella pneumoniae* is resistant to a wide variety of antibiotics, including  $\beta$ -lactams, fluoroquinolones, and aminoglycosides (Fair and Tor, 2014; Dsouza et al., 2017). This bacterium can spread through a variety of routes, including bacterial transfer between hosts, clonal

transfer, and resistance gene transfer. These resistance genes are typically found in mobile genetic material and can be horizontally transferred between bacterial species. Both pathogenic and non-pathogenic strains are involved in this transfer process. Antibiotic use in both human and veterinary medicine has a significant impact on these processes (Smet et al., 2009).

*Klebsiella pneumoniae* also has an impact on the environment, including soil, wastewater, animals, and food products. Humans can become infected by coming into close contact with the blood, saliva, faeces, and urine of animals carrying ESBLs, or by consuming contaminated water or food products (Founou et al., 2016). ESBL-producing enterobacteriaceae have been found in patients (Leverstein-van Hall et al., 2011 Jun; Overdevest et al., 2011) as well as in healthy community members (Vinué et al., 2009; Geser et al., 2012). ESBL infections have also been found in meat (Overdevest et al., 2011; EFSA Panel on Biological Hazards BIOHAZ, 2011) livestock (Dierikx et al., 2010; Hartmann et al., 2012), and companion animals (Dierikx et al., 2010).

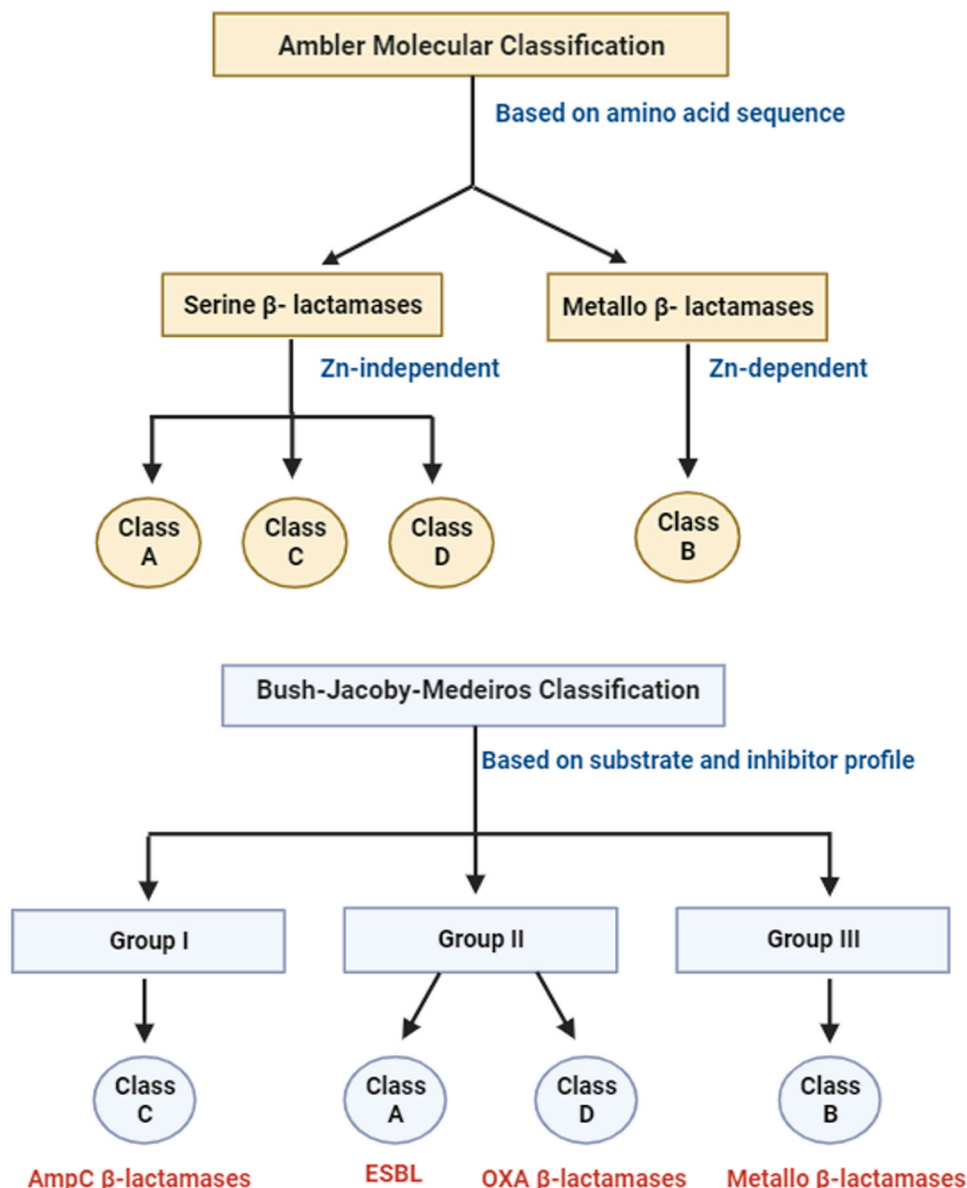


Fig. 9. Classification of Beta Lactamases.

### 9.1. ESBL types

The overuse of newer generation antibiotics such as cephalosporins, carbapenems, and monobactams in clinical settings (Liakopoulos et al., 2016) has resulted in the emergence of various types of  $\beta$ -lactamases. The two primary systems for classifying  $\beta$ -lactamases are the Bush–Jacoby–Medeiros functional classification and the Ambler molecular classification (Fig. 9). Based on amino acid sequence, the Ambler scheme is categorized (Ambler et al., 1991; Pitout and Laupland, 2008). Serine  $\beta$ -lactamases belong to classes A, C, and D, while metallo- $\beta$ -lactamases belong to class B enzymes. Based on the substrate and inhibitor profiles of enzymes, the Bush–Jacoby–Medeiros functional classification is further subdivided into three subgroups:

**Group I:** Enterobacteriales' genetic material contains the genetic code for cephalosporinases, which include enzymes like AmpC, CMY, ACT, FOX, and MIR. Plasmids contain numerous variants of these enzymes.

**Group II:** The largest group of  $\beta$ -lactamase is a serine-based enzyme with broad activity against penicillins, cephalosporins, and carbapenems. Enzymes in this class include TEM, SHV, CTX, OXA, and KPC. These enzyme genes are found in plasmids, which can be horizontally transmitted to other bacterial genera.

**Group III:** This category includes metal-based  $\beta$ -lactamases (MBLs), which rely on the presence of zinc. MBLs are represented by enzymes such as NDM, IMP, VIM, and SPM.

The most common and clinically significant ESBL genes are those from the CTX-M, TEM, and SHV families. The CTX-M enzyme is the most prevalent among them. CTX-M enzymes are divided into five groups based on their amino acid sequences: CTX-M-1, CTX-M-2, CTX-M-8, CTX-M-9, and CTX-M-25am (Perovic et al., 2014). Although *Klebsiella pneumoniae* can produce enzymes from all of these groups, the emergence of carbapenemase, which is colistin resistant (Perovic et al., 2014; Hudson et al., 2014) is cause for concern. Mobile genetic elements (MGEs) transport these resistant genes and facilitate their transfer between bacteria (Founou et al., 2016). MGEs increase the likelihood of *K. pneumoniae* infections, which are difficult to treat clinically. ESBL enzymes encoded by different gene groups can hydrolyze a wide range of penicillins and cephalosporins. CTX-M is the most important enzyme in this class. In recent years, the most common groups are ESBLs and ESVs (Cantón et al., 2012).

ESBL have been found in a variety of ecological niches around the world over the last decade, including environmental contaminants and commensal organisms in humans and animals. These ecological niches act as reservoirs and transmission routes for ESBL. Because production animals have a direct connection to the food chain, they are the primary source of ESBL spread (Madec et al., 2017). Infection occurs when hosts become infected with antibiotic-resistant bacteria, resulting in host-microbial interactions that can harm the host and disrupt its homeostasis (Pirofski and Casadevall, 2002).

Enterobacteriaceae-produced  $\beta$ -lactamase is widely transmitted among animal and human populations. Several risk factors are linked to the spread of these infections. Understanding the dynamics of transmission within animals and between animals and humans is critical for effective interventions.

Transmission is confirmed when two isolates from the same person have identical bacterial species, plasmid types, and ESBL genes. This suggests that Enterobacteriaceae are transmitted between humans in hospital and household settings (Harris et al., 2007; Valverde et al., 2008; Hilty et al., 2012).

### 10. Carbapenem resistance in *K. pneumoniae*

Carbapenems are the recommended treatment for severe infections caused by *K. pneumoniae* strains that produce extended-spectrum  $\beta$ -lactamases (ESBLs). These drugs are highly resistant to  $\beta$ -lactamase enzyme breakdown and continue to be effective against ESBL-producing bacteria

(Colodner et al., 2004). However, the emergence of *K. pneumoniae* carbapenemase (KPC) has led to an increase in carbapenem resistance among *K. pneumoniae* strains, posing significant clinical challenges and complicating treatment options (Nordmann et al., 2009). *K. pneumoniae* strains carrying KPC-1 have moderate to high carbapenem resistance, whereas strains carrying KPC-2 and KPC-3 only have high resistance when certain outer membrane porins are lost (Yigit et al., 2001; Smith Moland, 2003; Woodford et al., 2004). KPC-producing bacteria are not restricted to *K. pneumoniae*; they have spread globally and have been found in other Gram-negative bacteria such as *Pseudomonas aeruginosa* and *Escherichia coli* (Kitchel et al., 2009 Aug). A strain of *K. pneumoniae* known as clonal complex (CC) 258, which produces either KPC-2 or KPC-3, has been found all over the world (Brisse et al., 2009). This strain frequently carries the KPC gene, as well as a number of other acquired antimicrobial resistance genes (Chen et al., 2014).

*K. pneumoniae* isolates also contain metallo-lactamases (IMP, VIM, and NDM), plasmid-mediated clavulanic acid-inhibited  $\beta$ -lactamases (NmCA, IMI, SME, and GES), and expanded-spectrum oxacillinase (OXA-48). The Indian subcontinent is where New Delhi metallo-lactamase-1 (NDM-1) is most frequently found in *K. pneumoniae* (Yong et al., 2009; Dortet et al., 2014). However, *K. pneumoniae* with the NDM-1 gene has been found in patients who had previously been hospitalized in the Indian subcontinent, with cases reported in Europe, the Middle East, North Africa, Asia, and North America (Wailan and Paterson, 2014; Zhu et al., 2016). OXA-48, discovered in *K. pneumoniae* in Turkey in 2004, is now endemic in Turkey and neighbouring Mediterranean countries, and is rapidly spreading to other European countries (Voulgari et al., 2013). KPC-producing strains are more common in nosocomial (hospital-acquired) isolates than NDM and OXA-48, which appear in both nosocomial and community-acquired strains. Genetic studies show that the OXA-48 gene is more likely to spread among enterobacterial species than the KPC and NDM genes (Nordmann and Poirel, 2014).

### 11. Hypervirulent *K. pneumoniae*

Hypervirulent *K. pneumoniae* (hvKp) has emerged as a potentially dangerous global pathogen. hvKp is more virulent than classical *K. pneumoniae* (cKp) and has the potential to cause community-acquired infections, which are frequently seen in people who have no underlying health conditions. hvKp is prevalent in the gastrointestinal tract, which contributes to its spread in both community and clinical settings. Infection rates are higher in the Asia-Pacific Rim region. The most common cause of pyogenic liver abscesses has been identified as hvKp. One feature that distinguishes hvKp from cKp is that it has a tendency to spread to distant anatomical locations, such as the lung, the central nervous system (CNS), and the eye (Fig. 10). Moreover, hvKp has been linked to primary extrahepatic infections, which include soft tissue infections, pneumonia, and bacteremia. The genetic components linked to increased virulence are typically found on chromosomal mobile genetic elements and large virulence plasmids. These components function as recognizable indicators for separating clinical isolates of hvKp from cKp. Important virulence traits unique to hvKp include the expression of the colibactin toxin, the existence of K1 and K2 capsule types, increased production of capsules, and the presence of up to four siderophore systems intended for iron acquisition. Furthermore, hypermucoviscosity is a phenotypic trait shared by hvKp strains in laboratory settings, and it has evolved into a distinguishing feature of many hypervirulent isolates. A common marker for hvKp is the string test, which evaluates the hypermucoviscous phenotype (string  $\geq 5$  mm). Its prediction accuracy for clinical hvKp strains is estimated to be 90 %. Alarming, the emergence of multidrug-resistant hypervirulent strains creates a new and difficult challenge in combating this already formidable pathogen (Zhu et al., 2021). Table 2 lists the key differences between cKp and hvKp.

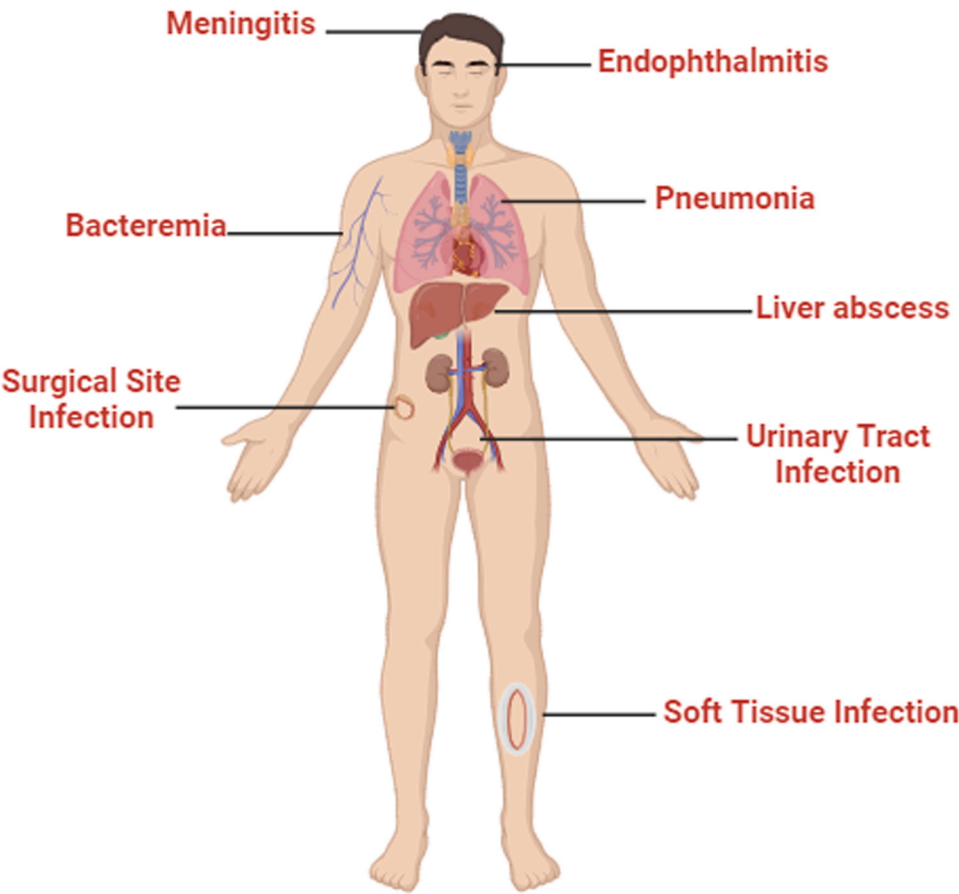


Fig. 10. Anatomical sites of Hypervirulent *K. pneumoniae*.

**Table 2**  
Primary difference between cKP and hvKP.

| Characteristics                      | Classical <i>K. pneumoniae</i> (cKp)            | Hypervirulent <i>K. pneumoniae</i> (hvKp)                                  |
|--------------------------------------|-------------------------------------------------|----------------------------------------------------------------------------|
| Acquisition Host                     | Nosocomial<br>Immunocompromised patients        | Community<br>Healthy adults                                                |
| Geographic region                    | The whole world                                 | Asia Pacific Rim Region                                                    |
| Sites of infection                   | Pneumonia, Urinary Tract Infections, Bacteremia | Pyogenic liver abscess, meningitis, endophthalmitis, necrotizing fasciitis |
| Copathogens at the site of infection | Polymicrobial at the site of infection          | Monomicrobial at the site of infection                                     |
| Metastasis                           | Uncommon                                        | Common                                                                     |
| Phenotypes                           | Non-hypermucoviscosity and string < 5 mm        | Hypermucoviscosity and string ≥ 5 mm                                       |
| Common serotypes                     | K1-K79                                          | K1, K2, K5, K16, K20, K54, K57, KN1                                        |
| Siderophores                         | Enterobactin, yersiniabactin                    | Enterobactin, yersiniabactin, Salmochelin, and aerobactin                  |

12. *K. pneumoniae* infections in COVID-19 patients

The global emergence of COVID-19 drew widespread attention, resulting in a focus on studying the virus and preventing its transmission while ignoring concurrent infections. Initially, studies on co-infections among COVID-19 patients were limited or ignored, particularly during the early stages of the SARS-CoV-2 outbreak. Because of varying opinions regarding the mortality rate and severity of COVID-19, researchers began looking into co-infections. Several studies have found that the occurrence of co-infections varies among SARS-CoV-2 patients. According to Lai et al., (Lai et al., 2020) there was a wide range of

co-infection rates among SARS-CoV-2 patients, ranging from 0.6 % to 50 %. Furthermore, an extensive review conducted across multiple medical centres revealed that 50 % of deceased COVID-19 patients had secondary bacterial co-infections acquired during treatment (Zhou et al., 2020). These investigations have shown that the mortality rate associated with viral-bacterial co-infections is higher than the mortality rate associated with bacterial or viral infections alone. This discovery is consistent with previous findings by Brundage and Shanks in 2008, (Brundage and Shanks, 2008) who discovered that viral infections enhance bacterial development and increase the likelihood of infection. For example, influenza virus replication can injure the lungs by producing entry sites for bacterial invasion and delaying bacterial clearance from the respiratory system.

The underlying issue in viral infections is immune system weakness, which contributes to the occurrence of subsequent bacterial and fungal infections. As previously stated in study, complications from bacterial infections are recognised as one of the leading causes of influenza fatalities. A wide range of bacteria and fungi have been identified as co-pathogens in COVID-19 patients. Among them are *Candida species*, *Aspergillus flavus*, *K. pneumoniae*, *Chlamydia pneumoniae*, *S. pneumoniae*, *Mycoplasma pneumoniae*, *A. baumannii*, *S. aureus*, and *L. pneumophila*. Furthermore, a number of co-pathogens have been identified, including enterovirus, coronavirus, parainfluenza, influenza B virus, human immunodeficiency virus, and metapneumovirus (Lai et al., 2020).. During therapy, seven COVID-19 patients in the intensive care unit (ICU) were co-infected with carbapenemase-producing *K. pneumoniae* (CP-Kp) (Montrucchio et al., 2020). In Taiwan, Wei-Cheng et al. in 2020 (Chen et al., 2020) reported the first COVID-19-related fatality due to severe community-acquired pneumonia (CAP) caused by *K. pneumoniae* in a case study. Within the first four days after contracting COVID-19, co-infections are common.

In 2020, Massey et al. (Massey et al., 2020). discovered that *K. pneumoniae* and *Moraxella catarrhalis* were the most common severe co-pathogens found in SARS-CoV-2 patients in the United States, causing severe respiratory infections. Similarly, a study in China (Zhu et al., 2020), found that *K. pneumoniae* was the second most prevalent respiratory pathogen among COVID-19 patients, following closely behind *S. pneumoniae*. The COVID-19 pandemic has had a significant impact on Italy, and Arcari et al. in 2021 (Arcari et al., 2021) discovered carbapenemase-producing *K. pneumoniae* in 34 % of Italian COVID-19 patients. Among 108 COVID-19 patients in Saudi Arabia, *K. pneumoniae* and *A. baumannii* were the most common bacterial species, with complete resistance to all antibiotics except colistin (Bazaid et al., 2022)..

Said et al. in 2022 (Said et al., 2022) investigated the presence of microbial co-infection in 301 people infected with SARS-CoV-2. The researchers discovered numerous important microbes in these individuals, the most common of which was multidrug-resistant *K. pneumoniae*, which was present in 37 % of cases. Furthermore, 26 % of the patients were infected with the multidrug-resistant *A. baumannii*, while 18.6 % were infected with the very drug-resistant *A. baumannii*. The infection was caused by very drug-resistant *P. aeruginosa* in 8.5 % of cases, while other bacteria were responsible for the remaining 9.3 % of cases. Furthermore, the study found a link between bacterial illness and an increased risk of death, especially when *K. pneumoniae* and *A. baumannii* were present.

García-Menio et al. in 2021 (García-Meniño et al., 2021) investigated the impact of multidrug-resistant *K. pneumoniae* infections on COVID-19 patients and concluded that strict infection control measures are critical for preventing the spread of *K. pneumoniae* and other multidrug-resistant bacteria among COVID-infected patients. To effectively combat these forms of illnesses, they emphasised the significance of maximising infection control strategies.

Overuse of antibiotics during COVID-19 treatment is problematic because it promotes the development of bacteria that are resistant to antibiotics. *K. pneumoniae* strains that exhibit multidrug resistance not only show resistance to multiple types of antibiotics but also resistance to specific types of antibiotics. Because of the increased prevalence of antibiotic resistance, especially colistin resistance, seen in *K. pneumoniae* isolates, especially among patients co-infected with COVID-19, ongoing surveillance and evaluation of antibiotic usage are essential (Yahya, 2022).

## 13. Treatment of *K. pneumoniae* infection

### 13.1. Antibiotic therapy

Given the rarity of *K. pneumoniae* in the general population, it is best to follow established antibiotic treatment guidelines when dealing with pneumonia cases. Once a *K. pneumoniae* infection is suspected or confirmed, the antibiotics used should be tailored to the specific sensitivities observed in the local area. Currently, the recommended treatment plans for community-acquired *K. pneumoniae* infection include a 14-day regimen of either a third or fourth-generation cephalosporin as a monotherapy or a respiratory quinolone as a monotherapy. A combination of either of these regimens with an aminoglycoside can also be considered. If the patient is allergic to penicillin, a course of aztreonam or a respiratory quinolone is an ideal choice (Liu and Guo, 2018; Mitharwal et al., 2016; Thakuria et al., 2013). Because of its widespread effectiveness, carbapenem therapy is recommended following the diagnosis of ESBL. In the case of CRE (carbapenem-resistant Enterobacteriaceae), infectious disease specialists should be consulted to guide the treatment process. Antibiotics from the polymyxin class, tigecycline, fosfomycin, aminoglycosides, and dual therapy with carbapenems are among the options for treating CRE. Combining two or more of these agents in a treatment regimen may result in lower mortality rates than using a monotherapy alone.

### 13.2. Phage therapy

Bacteriophages could be used as an alternative method of treating bacterial infections, including those resistant to antibiotics. A single administration of phage  $\Phi$ NK5 effectively suppressed *K. pneumoniae*-induced liver damage, bacteremia, and cytokine production, according to (Hung et al., 2011). This suggests that phages may hold promise as a potential therapeutic agent for treating *K. pneumoniae*-caused liver infections. Furthermore, the use of bacteriophages in conjunction with iron-chelating agents against *K. pneumoniae* has yielded promising results. Chhibber, Nag, and Bansal (2013) (Chhibber et al., 2013) discovered that this approach resulted in thinner biofilms and a greater number of inactive bacterial cells. Genetically engineered phages containing depolymerase were found to reduce the extracellular polymeric substances (EPS) of the biofilm (Lu and Collins, 2007). These findings highlight the potential of phage-based strategies in mitigating the impact of *K. pneumoniae* biofilms.

### 13.3. Photodynamic therapy

Photodynamic therapy (PDT) has emerged as a promising treatment option for infections caused by  $\beta$ -lactamase-producing *K. pneumoniae*. PDT involves exposing photosensitizers (PS) to appropriate wavelength visible light. The photosensitizer's excitation generates reactive oxygen species (ROS), particularly singlet oxygen. Bacterial resistance to PDT is unlikely due to the non-specific mechanism of action of ROS (Wood et al., 2006). 5-aminolevulinic acid (5-ALA) and its derivative 5-ALA methyl ester (MAL), precursor compounds of photosensitizers, were found to have significant effects on *K. pneumoniae* cells in a recent study by Liu et al. (2016) (Liu et al., 2016). When exposed to white light, these compounds caused cell envelope damage, intracellular biopolymer leakage, cytoplasmic denaturation, and disruption of extracellular polymeric substances (EPS) in *K. pneumoniae* planktonic cells and biofilms. This study demonstrates the efficacy of PDT based on 5-ALA and MAL in combating *K. pneumoniae* infections, particularly those associated with  $\beta$ -lactamase production.

### 13.4. Vaccination

Vaccination is thought to be the most effective and cost-effective way to prevent infectious diseases. Several attempts have been made over the last 40 years to develop effective vaccines against *K. pneumoniae*. Among these efforts, capsular polysaccharide was identified as a vaccine antigen, but its variable serotypes limited its use in vaccine production. The approach of focusing on non-capsular protein antigens revealed that outer membrane proteins protect the immune response induced by *K. pneumoniae*. Extracellular vesicles (EVs) produced by bacteria are spherical, nanoscale proteolipids that are enhanced with outer membrane proteins. Because of the abundance of outer membrane proteins and the presence of immune-stimulating pathogen-associated molecular patterns such as lipopolysaccharide, *K. pneumoniae* extracellular vesicles (EVs) may be a promising vaccine candidate. The specific mechanism by which EV vaccines confer protection has yet to be fully understood. It sheds new light on a novel vaccine development strategy that uses EVs derived from Gram-negative bacteria to protect against bacterial infections, particularly those caused by multidrug-resistant bacteria (Lee et al., 2015).

## 14. Conclusion

The emergence of strains of *K. pneumoniae* with increased virulence and drug resistance has made the organism a serious concern in hospital settings. *K. pneumoniae* isolates have undergone genetic analyses that have uncovered a wide variety of genetic variations, including both multidrug resistance and hypervirulence. The prevalence and global spread of these high-risk isolates have drastically reduced the treatment

options available to clinicians. Several reports have shown that *K. pneumoniae* co-infections occur and spread among critically ill COVID-19 patients, particularly during their hospitalization. The widespread use of antibiotics in the early stages of COVID-19 diagnosis raises alarm because while it may reduce bacterial co-infections, it also encourages antibiotic resistance in bacteria like *K. pneumoniae* strains. The accurate identification of multidrug-resistant *K. pneumoniae* is critical for guiding the diagnosis and treatment of COVID-19 patients. To reduce the risk associated with COVID-19, additional efforts are needed to control and prevent *K. pneumoniae* infections. Despite these strengths, some limitations should be acknowledged. A more in-depth discussion on host immune responses and environmental reservoirs could enhance our understanding of *K. pneumoniae* persistence and transmission. Moreover, given the rapid evolution of resistance mechanisms, continuous genomic surveillance is essential to track emerging resistance patterns. While alternative therapies show promise, their clinical efficacy, safety, and large-scale applicability require further investigation.

### CRedit authorship contribution statement

**Vijayan Smitha:** Writing – review & editing, Validation, Supervision, Project administration, Methodology, Conceptualization. **Asokan Sijo:** Writing – original draft, Investigation. **Jacob Teena:** Writing – review & editing, Supervision, Conceptualization. **Cherian Tijo:** Writing – review & editing. **Peijnenburg Willie JGM:** Validation, Formal analysis. **Jacob Jenny:** Writing – review & editing. **AlSosowaa Afaf A:** Investigation.

### Funding

The research did not receive any specific grant from funding agencies in the public, commercial or nonprofit sectors.

### Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

### Acknowledgements

The authors are thankful to the authorities of the MACFAST College and Pushpagiri Institute of Medical Sciences and Research Centre, Tiruvalla for providing the necessary support and facilities throughout the study.

### Data availability

No data was used for the research described in the article.

### References

- Ahmadi, Z., Noormohammadi, Z., Behzadi, P., Ranjbar, R., 2022a. Molecular detection of *gyrA* mutation in clinical strains of *Klebsiella pneumoniae*. *Iran. J. Public Health* 51 (10), 2334.
- Ahmadi, Z., Noormohammadi, Z., Ranjbar, R., Behzadi, P., 2022b. Prevalence of tetracycline resistance genes *tet* (A, B, C, 39) in *Klebsiella pneumoniae* isolated from Tehran, Iran. *Iran. J. Med. Microbiol.* 16 (2), 141–147.
- Ahmadi, M., Ranjbar, R., Behzadi, P., Mohammadian, T., 2022c. Virulence factors, antibiotic resistance patterns, and molecular types of clinical isolates of *Klebsiella pneumoniae*. *Expert Rev. anti-Infect. Ther.* 20 (3), 463–472.
- Algammal, A., Hetta, H.F., Mabrok, M., Behzadi, P., 2023. Emerging multidrug-resistant bacterial pathogens “superbugs”: A rising public health threat. *Front. Microbiol.* 14, 1135614.
- Ambler, R.P., Coulson, A.F.W., Frère, J.M., Ghuyssen, J.M., Joris, B., Forsman, M., et al., 1991. A standard numbering scheme for the class A  $\beta$ -lactamases. *Biochem. J.* 276 (1), 269–270. <https://doi.org/10.1042/bj2760269>.
- Arcari, G., Raponi, G., Sacco, F., Bibbolino, G., Di Lella, F.M., Alessandri, F., Coletti, M., Trancassini, M., Deales, A., Pugliese, F., Antonelli, G., 2021. *Klebsiella pneumoniae* infections in COVID-19 patients: a 2-month retrospective analysis in an Italian hospital. *Int. J. Antimicrob. Agents* 57 (1), 106245. <https://doi.org/10.1016/j.ijantimicag.2020.106245>.
- Banerjee, M., Sahu, K., Bhattacharya, S., Adhya, S., Bhowmick, P., Chakraborty, P., 1993. Outbreak of neonatal septicemia with multidrug resistant *Klebsiella pneumoniae*. *Indian J. Pediatr.* 60, 25–27. <https://doi.org/10.1007/bf02860501>.
- Bazaid, A.S., Barnawi, H., Qanash, H., Alsaif, G., Aldarhami, A., Gattan, H., Alharbi, B., Alrashidi, A., Al-Soud, W.A., Moussa, S., Alfouzan, F., 2022. Bacterial coinfection and antibiotic resistance profiles among hospitalised COVID-19 patients. *Microorganisms* 10 (3), 495. <https://doi.org/10.3390/microorganisms10030495>.
- Behzadi, P., Behzadi, E., 2008. The microbial agents of urinary tract infections at central laboratory of Dr. Shariati Hospital, Tehran, Iran. *Turk. Klin. Tip. Bilim* 28 (4), 445.
- Behzadi, P., García-Perdomo, H.A., Autrán Gómez, A.M., Pinheiro, M., Sarshar, M., 2023. Uropathogens, urinary tract infections, the host-pathogen interactions and treatment. *Front. Microbiol.* 14, 1183236.
- Behzadi, P., García-Perdomo, H.A., Karpiński, T.M., Issakhanian, L., 2020. Metallo- $\beta$ -lactamases: a review. *Mol. Biol. Rep.* 47 (8), 6281–6294.
- Bevan, E.R., Jones, A.M., Hawkey, P.M., 2017. Global epidemiology of CTX-M  $\beta$ -lactamases: temporal and geographical shifts in genotype. *J. Antimicrob. Chemother.* 72 (8), 2145–2155. <https://doi.org/10.1093/jac/dkx146>.
- Boucher, H.W., Talbot, G.H., Bradley, J.S., Edwards, J.E., Gilbert, D., Rice, L.B., et al., 2009. Bad bugs, no drugs: no ESCAPE! an update from the infectious diseases society of America. *Clin. Infect. Dis.* 48 (1), 1–12. <https://doi.org/10.1086/595011>.
- Brisse, S., Fevre, C., Passet, V., Issenuth-Jeanjean, S., Tournibize, R., Diancourt, L., Grimont, P., 2009. Virulent clones of *Klebsiella pneumoniae*: identification and evolutionary scenario based on genomic and phenotypic characterization. *PloS One* 4 (3), e4982. <https://doi.org/10.1371/journal.pone.0004982>.
- Brisse, S., Grimont, F., Grimont, P.A., 2006. The genus *klebsiella*. *prokaryotes* 159–196. <https://doi.org/10.1007/0-387-30746-x.8>.
- Brooks, G.F., Butel, J.S., Carroll, K.C., Morse, S.A., 2007. Jawetz, Melnick, and Medical microbiology, 24th ed. McGraw-Hill, New York, pp. 254–255.
- Brundage, J.F., Shanks, G.D., 2008. Deaths from bacterial pneumonia during 1918–19 influenza pandemic. *Emerg. Infect. Dis.* 14 (8), 1193. <https://doi.org/10.3201/eid1408.071313>.
- Cantón, R., González-Alba, J.M., Galán, J.C., 2012. CTX-M enzymes: origin and diffusion. *Front. Microbiol.* 3, 110. <https://doi.org/10.3389/fmicb.2012.00110>.
- Chen, W.C., Lai, Y.C., Lin, C.H., Zheng, J.F., Hung, W.C., Wang, Y.J., Kuo, T.H., Wu, L.H., Tong, Y.S., Lu, M.C., Liu, K.S., 2020. First COVID-19 mortality case in Taiwan with bacterial co-infection by national surveillance of critically ill patients with influenza-negative pneumonia. *J. Microbiol., Immunol. Infect.* 53 (4), 652–656. <https://doi.org/10.1016/j.jmii.2020.05.005>.
- Chen, L., Mathema, B., Chavda, K.D., DeLeo, F.R., Bonomo, R.A., Kreiswirth, B.N., 2014. Carbapenemase-producing *Klebsiella pneumoniae*: molecular and genetic decoding. *Trends Microbiol.* 22 (12), 686–696. <https://doi.org/10.1016/j.tim.2014.09.003>.
- Chhibber, S., Nag, D., Bansal, S., 2013. Inhibiting biofilm formation by *Klebsiella pneumoniae* B5055 using an iron antagonizing molecule and a bacteriophage. *BMC Microbiol.* 13 (1), 1–8. <https://doi.org/10.1186/1471-2180-13-174>.
- Colodner, R., Raz, R., Chazan, B., Sakran, W., 2004. Susceptibility pattern of extended-spectrum  $\beta$ -lactamase producing bacteria isolated from inpatients to five antimicrobial drugs in a community hospital in Northern Israel. *Int. J. Antimicrob. Agents* 24 (4), 409–410. <https://doi.org/10.1016/j.ijantimicag.2004.06.001>.
- Cortés, G., Borrell, N., de Astorza, B., Gómez, C., Saulea, J., Alberti, S., 2002. Molecular analysis of the contribution of the capsular polysaccharide and the lipopolysaccharide O side chain to the virulence of *klebsiella pneumoniae* in a murine model of pneumonia. *Infect. Immun.* 70 (5), 2583–2590. <https://doi.org/10.1128/iai.70.5.2583-2590.2002>.
- Davies, D.G., Geesey, G.G., 1995. Regulation of the alginate biosynthesis gene *algC* in *Pseudomonas aeruginosa* during biofilm development in continuous culture. *Appl. Environ. Microbiol.* 61 (3), 860–867. <https://doi.org/10.1128/aem.61.3.860-867.1995>.
- Dierikx, C., van Essen-Zandbergen, A., Veldman, K., Smith, H., Mevius, D., 2010. Increased detection of extended spectrum beta-lactamase producing *Salmonella enterica* and *Escherichia coli* isolates from poultry. *Vet. Microbiol.* 145 (3–4), 273–278. <https://doi.org/10.1016/j.vetmic.2010.03.019>.
- Dong, N., Yang, X., Chan, E.W., Zhang, R., Chen, S., 2022. *Klebsiella* species: taxonomy, hypervirulence and multidrug resistance. *EBioMedicine* 79. <https://doi.org/10.1016/j.ebiom.2022.103998>.
- Dortet, L., Poiriel, L., Nordmann, P., 2014. Worldwide dissemination of the NDM-type carbapenemases in Gram-negative bacteria. *BioMed. Res. Int.* 2014. <https://doi.org/10.1155/2014/249856>.
- Dsouza, R., Pinto, N.A., Hwang, I., Cho, Y., Yong, D., Choi, J., Lee, K., Chong, Y., 2017. Panel strain of *Klebsiella pneumoniae* for beta-lactam antibiotic evaluation: their phenotypic and genotypic characterization. *PeerJ* 5, e2896. <https://doi.org/10.7717/peerj.2896>.
- Dunne, W.M., 2002. Bacterial adhesion: seen any good biofilms lately? *Clin. Microbiol. Rev.* 15 (2), 155–166. <https://doi.org/10.1128/cmr.15.2.155-166.2002>.
- EFSA Panel on Biological Hazards (BIOHAZ), 2011. Scientific Opinion on the public health risks of bacterial strains producing extended-spectrum  $\beta$ -lactamases and/or AmpC  $\beta$ -lactamases in food and food-producing animals. *EFSA J.* 9 (8), 2322. <https://doi.org/10.2903/j.efsa.2011.2322>.
- Esposito, E.P., Cervoni, M., Bernardo, M., Crivaro, V., Cuccurullo, S., Imperi, F., et al., 2018. Molecular Epidemiology and virulence profiles of colistin-resistant *klebsiella pneumoniae* blood isolates from the hospital agency “ospedale dei colli,” Naples, Italy. *Front. Microbiol.* 9. <https://doi.org/10.3389/fmicb.2018.01463>.
- Fair, R.J., Tor, Y., 2014. Antibiotics and bacterial resistance in the 21st century. *Perspect. Med. Chem.* PMC-S14459. <https://doi.org/10.4137/pmc.s14459>.

- Founou, L.L., Founou, R.C., Essack, S.Y., 2016. Antibiotic resistance in the food chain: a developing country-perspective. *Front. Microbiol.* 7, 1881. <https://doi.org/10.3389/fmicb.2016.0188>.
- García-Meniño, I., Forcelledo, L., Rosete, Y., García-Prieto, E., Escudero, D., Fernández, J., 2021. Spread of OXA-48-producing *Klebsiella pneumoniae* among COVID-19-infected patients: The storm after the storm. *J. Infect. Public Health* 14 (1), 50–52. <https://doi.org/10.1016/j.jiph.2020.11.001>.
- Geser, N., Stephan, R., Korczak, B.M., Beutin, L., Hächler, H., 2012. Molecular identification of extended-spectrum- $\beta$ -lactamase genes from Enterobacteriaceae isolated from healthy human carriers in Switzerland. *Antimicrob. Agents Chemother.* 56 (3), 1609–1612. <https://doi.org/10.1128/aac.05539-11>.
- Hansen, D.S., Gottschau, A., Kolmos, H.J., 1998. Epidemiology of *Klebsiella bacteremia*: a case control study using *Escherichia coli* bacteremia as control. *J. Hosp. Infect.* 38 (2), 119–132. [https://doi.org/10.1016/s0195-6701\(98\)90065-2](https://doi.org/10.1016/s0195-6701(98)90065-2).
- Harris, A.D., Kotetishvili, M., Shurland, S., Johnson, J.A., Morris, J.G., Nemoy, L.L., Johnson, J.K., 2007. How important is patient-to-patient transmission in extended-spectrum  $\beta$ -lactamase *Escherichia coli* acquisition. *Am. J. Infect. Control* 35 (2), 97–101. <https://doi.org/10.1016/j.ajic.2006.09.011>.
- Hartmann, A., Locatelli, A., Amoureux, L., Depret, G., Jolivet, C., Gueneau, E., Neuwirth, C., 2012. Occurrence of CTX-M producing *Escherichia coli* in soils, cattle, and farm environment in France (Burgundy region). *Front. Microbiol.* 3, 83. <https://doi.org/10.3389/fmicb.2012.00083>.
- Hilty, M., Betsch, B.Y., Bögli-Stuber, K., Heiniger, N., Stadler, M., Küffer, M., Kronenberg, A., Rohrer, C., Aebi, S., Endimiani, A., Droz, S., 2012. Transmission dynamics of extended-spectrum  $\beta$ -lactamase-producing Enterobacteriaceae in the tertiary care hospital and the household setting. *Clin. Infect. Dis.* 55 (7), 967–975. <https://doi.org/10.1093/cid/cis581>.
- Hudson, C.M., Bent, Z.W., Meagher, R.J., Williams, K.P., 2014. Resistance determinants and mobile genetic elements of an NDM-1-encoding *Klebsiella pneumoniae* strain. *PloS One* 9 (6), e99209. <https://doi.org/10.1371/journal.pone.0099209>.
- Huijbers, P.M., Blaak, H., de Jong, M.C., Graat, E.A., Vandenbroucke-Grauls, C.M., de Roda Husman, A.M., 2015. Role of the environment in the transmission of antimicrobial resistance to humans: a review. *Environ. Sci. Technol.* 49 (20), 11993–12004. <https://doi.org/10.1021/acs.est.5b02566>.
- Hung, C.H., Kuo, C.F., Wang, C.H., Wu, C.M., Tsao, N., 2011. Experimental phage therapy in treating *Klebsiella pneumoniae*-mediated liver abscesses and bacteremia in mice. *Antimicrob. Agents Chemother.* 55 (4), 1358–1365. <https://doi.org/10.1128/aac.01123-10>.
- Issakhanian, L., Behzadi, P., 2019. Antimicrobial agents and urinary tract infections. *Curr. Pharm. Des.* 25 (12), 1409–1423.
- Jacoby, G.A., 2009. AmpC  $\beta$ -Lactamases. *Clin. Microbiol. Rev.* 22 (1), 161–182. <https://doi.org/10.1128/cmr.00036-08>.
- Kamal, F., Williams, G., Akbar, H., Khan, M.A., Kadaria, D., 2017. *Klebsiella pneumoniae* liver abscess: a case report and review of literature. *Cureus* 9 (1). <https://doi.org/10.7759/cureus.970>.
- Karamatakis, T., Tsergouli, K., Behzadi, P., 2023. Carbapenem-resistant *Klebsiella pneumoniae*: virulence factors, molecular epidemiology and latest updates in treatment options. *Antibiotics* 12 (2), 234.
- Karamatakis, T., Tsergouli, K., Behzadi, P., 2024. Pan-genome plasticity and virulence factors: a natural treasure trove for *Acinetobacter baumannii*. *Antibiotics* 13 (3), 257.
- Kitchel, B., Rasheed, J.K., Patel, J.B., Srinivasan, A., Navon-Venezia, S., Carmeli, Y., Brolund, A., Giske, C.G., 2009 Aug. Molecular epidemiology of KPC-producing *Klebsiella pneumoniae* isolates in the United States: clonal expansion of multilocus sequence type 258. *Antimicrob. Agents Chemother.* 53 (8), 3365–3370. <https://doi.org/10.1128/aac.00126-09>.
- Lai, C.C., Wang, C.Y., Hsueh, P.R., 2020. Co-infections among patients with COVID-19: the need for combination therapy with non-anti-SARS-CoV-2 agents? *J. Microbiol., Immunol. Infect.* 53 (4), 505–512. <https://doi.org/10.1016/j.jmii.2020.05.013>.
- Lawrence, J.R., Korb, D.R., Hoyle, B.D., Costerton, J.W., Caldwell, D.E., 1991. Optical sectioning of microbial biofilms. *J. Bacteriol.* 173 (20), 6558–6567. <https://doi.org/10.1128/jb.173.20.6558-6567.1991>.
- Lear, G., Lewis, G.D., 2012. *Microbial Biofilms: Current Research and Applications*. Caister Academic Press, pp. 96–97.
- Lee, W.H., Choi, H.I., Hong, S.W., Kim, K.S., Cho, Y.S., Jeon, S.G., 2015. Vaccination with *Klebsiella pneumoniae*-derived extracellular vesicles protects against bacteria-induced lethality via both humoral and cellular immunity. *Exp. Mol. Med.* 47 (9), e183. <https://doi.org/10.1038/emm.2015.59>.
- Leverstein-van Hall, M.A., Dierikx, C.M., Stuart, J.C., Voets, G.M., van den Munckhof, M. P., van Essen-Zandbergen, A., et al., 2011 Jun. Dutch patients, retail chicken meat and poultry share the same ESBL genes, plasmids and strains. *Clin. Microbiol. Infect.* 17 (6), 873–880. <https://doi.org/10.1111/j.1469-0691.2011.03497.x>.
- Liakopoulos, A., Mevius, D., Ceccarelli, D., 2016. A review of SHV extended-spectrum  $\beta$ -lactamases: neglected yet ubiquitous. *Front. Microbiol.* 7, 1374. <https://doi.org/10.3389/fmicb.2016.01374>.
- Liu, C., Guo, J., 2018. Characteristics of ventilator-associated pneumonia due to hypervirulent *Klebsiella pneumoniae* genotype in genetic background for the elderly in two tertiary hospitals in China. *Antimicrob. Resist. Infect. Control* 7, 1–8. <https://doi.org/10.1186/s13756-018-0371-8>.
- Liu, C., Zhou, Y., Wang, L., Han, L., Lei, J.E., Ishaq, H.M., Nair, S.P., Xu, J., 2016. Photodynamic inactivation of *Klebsiella pneumoniae* biofilms and planktonic cells by 5-aminolevulinic acid and 5-aminolevulinic acid methyl ester. *Lasers Med. Sci.* 31, 557–565. <https://doi.org/10.1007/s10103-016-1891-1>.
- Li, Y., Kumar, S., Zhang, L., Wu, H., Wu, H., 2023. Characteristics of antibiotic resistance mechanisms and genes of *Klebsiella pneumoniae*. *Open Med.* 18 (1), 20230707. <https://doi.org/10.1515/med-2023-0707>.
- Li, Y.-H., Lau, P.C.Y., Lee, J.H., Ellen, R.P., Cvitkovitch, D.G., 2001. Natural Genetic Transformation of *Streptococcus mutans* Growing in Biofilms. *J. Bacteriol.* 183 (3), 897–908. <https://doi.org/10.1128/jb.183.3.897-908.2001>.
- Lu, T.K., Collins, J.J., 2007. Dispersing biofilms with engineered enzymatic bacteriophage. *Proc. Natl. Acad. Sci.* 104 (27), 11197–11202. <https://doi.org/10.1073/pnas.0704624104>.
- Madez, J.Y., Haenni, M., Nordmann, P., Poirel, L., 2017. Extended-spectrum  $\beta$ -lactamase/AmpC-and carbapenemase-producing Enterobacteriaceae in animals: a threat for humans? *Clin. Microbiol. Infect.* 23 (11), 826–833. <https://doi.org/10.1016/j.cmi.2017.01.013>.
- Martinez, J.L., 2009. Environmental pollution by antibiotics and by antibiotic resistance determinants. *Environ. Pollut.* 157 (11), 2893–2902. <https://doi.org/10.1016/j.envpol.2009.05.051>.
- Di Martino, P., Livrelli, V., Siro, D., Joly, B., Darfeuille-Michaud, A., 1996. A new fimbrial antigen harbored by CAZ-/SHV-4-producing *Klebsiella pneumoniae* strains involved in nosocomial infections. *Infect. Immun.* 64 (6), 2266–2273. <https://doi.org/10.1128/iai.64.6.2266-2273.1996>.
- Massey, B.W., Jayatilake, K., Meltzer, H.Y., 2020. Respiratory microbial co-infection with SARS-CoV-2. *Front. Microbiol.* 11, 2079. <https://doi.org/10.3389/fmicb.2020.02079>.
- Matatov, R., Goldhar, J., Skutelsky, E., Sechter, I., Perry, R., Podschun, R., et al., 1999. Inability of encapsulated *Klebsiella pneumoniae* to assemble functional type 1 fimbriae on their surface. *FEMS Microbiol. Lett.* 179 (1), 123–130. <https://doi.org/10.1111/j.1574-6968.1999.tb08717.x>.
- Mitharwal, S.M., Yaddanapudi, S., Bhardwaj, N., Gautam, V., Biswal, M., Yaddanapudi, L., 2016. Intensive care unit-acquired infections in a tertiary care hospital: An epidemiologic survey and influence on patient outcomes. *Am. J. Infect. Control* 44 (7), e113–e117. <https://doi.org/10.1016/j.ajic.2016.01.021>.
- Montrucchio, G., Corcione, S., Sales, G., Curtini, A., De Rosa, F.G., Brazzi, L., 2020. Carbapenem-resistant *Klebsiella pneumoniae* in ICU-admitted COVID-19 patients: Keep an eye on the ball. *J. Glob. Antimicrob. Resist.* 23, 398–400. <https://doi.org/10.1016/j.jgar.2020.11.004>.
- Niveditha, S., Pramodhini, S., Umadevi, S., Kumar, S., Stephen, S., 2012. The isolation and the biofilm formation of uropathogens in the patients with catheter associated urinary tract infections (UTIs). *J. Clin. Diagn. Res.: JC DR* 6 (9), 1478. <https://doi.org/10.7860/jcdr/2012/4367.2537>.
- Nordmann, P., Cuzon, G., Naas, T., 2009. The real threat of *Klebsiella pneumoniae* carbapenemase-producing bacteria. *Lancet Infect. Dis.* 9 (4), 228–236. [https://doi.org/10.1016/s1473-3099\(09\)70054-4](https://doi.org/10.1016/s1473-3099(09)70054-4).
- Nordmann, P., Poirel, L., 2014. The difficult-to-control spread of carbapenemase producers among Enterobacteriaceae worldwide. *Clin. Microbiol. Infect.* 20 (9), 821–830. <https://doi.org/10.1111/1469-0691.12719>.
- Opoku-Temeng, C., Kobayashi, S.D., DeLeo, F.R., 2019. *Klebsiella pneumoniae* capsule polysaccharide as a target for therapeutics and vaccines. *Comput. Struct. Biotechnol. J.* 17, 1360–1366. <https://doi.org/10.1016/j.csbj.2019.09.011>.
- Overdevest, I., Willemssen, I., Rijnsburger, M., Eustace, A., Xu, L., Hawkey, P., Heck, M., Savelkoul, P., Vandenbroucke-Grauls, C., van der Zwaluw, K., Huijsdens, X., 2011. Extended-spectrum  $\beta$ -lactamase genes of *Escherichia coli* in chicken meat and humans, The Netherlands. *Emerg. Infect. Dis.* 17 (7), 1216. <https://doi.org/10.3201/eid1707.110209>.
- O'Toole, G.A., Kolter, R., 1998. Flagellar and twitching motility are necessary for *Pseudomonas aeruginosa* biofilm development. *Mol. Microbiol.* 30 (2), 295–304. <https://doi.org/10.1046/j.1365-2958.1998.01062.x>.
- Paczosa, M.K., Mecas, J., 2016. *Klebsiella pneumoniae*: going on the offense with a strong defense. *Microbiol. Mol. Biol. Rev.* 80 (3), 629–661. <https://doi.org/10.1128/mmr.00078-15>.
- Perovic, O., Singh-Moodley, A., Dusé, A., Bamford, C., Elliott, G., Swe-Han, K.S., Kularatne, R., Lowman, W., Whitelaw, A., Nana, T., Wadula, J., 2014. National sentinel site surveillance for antimicrobial resistance in *Klebsiella pneumoniae* isolates in South Africa, 2010–2012. *South Afr. Med. J.* 104 (8), 563–568. <https://doi.org/10.7196/samj.7617>.
- Pirofski, L.A., Casadevall, A., 2002. The meaning of microbial exposure, infection, colonisation, and disease in clinical practice. *Lancet Infect. Dis.* 2 (10), 628–635. [https://doi.org/10.1016/s1473-3099\(02\)00398-5](https://doi.org/10.1016/s1473-3099(02)00398-5).
- Pitout, J.D., Laupland, K.B., 2008. Extended-spectrum  $\beta$ -lactamase-producing Enterobacteriaceae: an emerging public-health concern. *Lancet Infect. Dis.* 8 (3), 159–166. [https://doi.org/10.1016/S1473-3099\(08\)70041-0](https://doi.org/10.1016/S1473-3099(08)70041-0).
- Podschun, R., Ullmann, U., 1998. *Klebsiella* spp. as nosocomial pathogens: epidemiology, taxonomy, typing methods, and pathogenicity factors. *Clin. Microbiol. Rev.* 11 (4), 589–603. <https://doi.org/10.1128/cmr.11.4.589>.
- Rasmussen, B.A., Bush, K., 1997 Feb. Carbapenem-hydrolyzing beta-lactamases. *Antimicrob. Agents Chemother.* 41 (2), 223–232. <https://doi.org/10.1128/aac.41.2.223>.
- Reid, G., Charbonneau-Smith, R.N., Lam, D., Kang, Y.S., Lacerte, M., Hayes, K.C., 1992. Bacterial biofilm formation in the urinary bladder of spinal cord injured patients. *Spinal Cord* 30 (10), 711–717. <https://doi.org/10.1038/sc.1992.138>.
- Sahly, H., Navon-Venezia, S., Roesler, L., Hay, A., Carmeli, Y., Podschun, R., et al., 2008. Extended-Spectrum  $\beta$ -Lactamase Production Is Associated with an Increase in Cell Invasion and Expression of Fimbrial Adhesins in *Klebsiella pneumoniae*. *Antimicrob. Agents Chemother.* 52 (9), 3029–3034. <https://doi.org/10.1128/aac.00010-08>.
- Said, K.B., Alsolami, A., Moussa, S., Alfouzan, F., Bashir, A.I., Rashidi, M., Aborans, R., Taha, T.E., Almansour, H., Alazmi, M., Al-Otaibi, A., 2022. COVID-19 clinical profiles and fatality rates in hospitalized patients reveal case aggravation and selective co-infection by limited gram-negative bacteria. *Int. J. Environ. Res. Public Health* 19 (9), 5270. <https://doi.org/10.3390/ijerph19095270>.

- Sathyavathy, K., Madhusudhan, B.K., 2020. Review on clinical diseases caused by klebsiella. *J. Pharm. Res. Int.* 32 (21), 12–19. <https://doi.org/10.9734/jpri/2020/v32i2130745>.
- Singhai, M., Malik, A., Shahid, M., Malik, M., Goyal, R., 2012. A study on device-related infections with special reference to biofilm production and antibiotic resistance. *J. Glob. Infect. Dis.* 4 (4), 193. <https://doi.org/10.4103/0974-777x.103896>.
- Smet, A., Martel, A., Persoons, D., Dewulf, J., Heyndrickx, M., Cloeckaert, A., Praud, K., Claeyss, G., Catry, B., Herman, L., Haesebrouck, F., 2009. Comparative analysis of extended-spectrum- $\beta$ -lactamase-carrying plasmids from different members of Enterobacteriaceae isolated from poultry, pigs and humans: evidence for a shared  $\beta$ -lactam resistance gene pool? *J. Antimicrob. Chemother.* 63 (6), 1286–1288. <https://doi.org/10.1093/jac/dkp101>.
- Smith Moland, E., 2003. Plasmid-mediated, carbapenem-hydrolysing  $\beta$ -lactamase, KPC-2, in *Klebsiella pneumoniae* isolates. *J. Antimicrob. Chemother.* 51 (3), 711–714. <https://doi.org/10.1093/jac/dkg124>.
- Stahlhut, S.G., Tchesnokova, V., Struve, C., Weissman, S.J., Chattopadhyay, S., Yakovenko, O., Aprikian, P., Sokurenko, E.V., Krogfelt, K.A., 2009. Comparative structure-function analysis of mannose-specific FimH adhesins from *Klebsiella pneumoniae* and *Escherichia coli*. *J. Bacteriol.* 191 (21), 6592–6601. <https://doi.org/10.1128/jb.00786-09>.
- Stoodley, P., Sauer, K., Davies, D.G., Costerton, J.W., 2002. Biofilms as complex differentiated communities. *Annu. Rev. Microbiol.* 56 (1), 187–209. <https://doi.org/10.1146/annurev.micro.56.012302.160705>.
- Tang, L.M., Chen, S.T., 1994. *Klebsiella pneumoniae* meningitis: prognostic factors. *Scand. J. Infect. Dis.* 26 (1), 95–102. <https://doi.org/10.3109/00365549409008596>.
- Thakuria, B., Singh, P., Agrawal, S., Asthana, V., 2013. Profile of infective microorganisms causing ventilator-associated pneumonia: a clinical study from resource limited intensive care unit. *J. Anaesthesiol. Clin. Pharmacol.* 29 (3), 361–366. <https://doi.org/10.4103/0970-9185.117111>.
- Tsai, S.S., Huang, J.C., Chen, S.T., Sun, J.H., Wang, C.C., Lin, S.F., Hsu, B.R., Lin, J.D., Huang, S.Y., Huang, Y.Y., 2010. Characteristics of *Klebsiella pneumoniae* bacteremia in community-acquired and nosocomial infections in diabetic patients. *Chang Gung Med J.* 33 (5), 532–539.
- Valverde, A., Grill, F., Coque, T.M., Pintado, V., Baquero, F., Cantón, R., Cobo, J., 2008. High rate of intestinal colonization with extended-spectrum- $\beta$ -lactamase-producing organisms in household contacts of infected community patients. *J. Clin. Microbiol.* 46 (8), 2796–2799. <https://doi.org/10.1128/jcm.01008-08>.
- Vinué, L., Sáenz, Y., Martínez, S., Somalo, S., Moreno, M.A., Torres, C., Zarazaga, M., 2009. Prevalence and diversity of extended-spectrum  $\beta$ -lactamases in faecal *Escherichia coli* isolates from healthy humans in Spain. *Clin. Microbiol. Infect.* 15 (10), 954–957. <https://doi.org/10.1111/j.1469-0691.2009.02803.x>.
- Voulgari, E., Zarkotou, O., Ranellou, K., Karageorgopoulos, D.E., Vriani, G., Mamali, V., Themeli-Digalaki, K., Tsakris, A., 2013. Outbreak of OXA-48 carbapenemase-producing *Klebsiella pneumoniae* in Greece involving an ST11 clone. *J. Antimicrob. Chemother.* 68 (1), 84–88. <https://doi.org/10.1093/jac/dks356>.
- Wailan, A.M., Paterson, D.L., 2014. The spread and acquisition of NDM-1: a multifactorial problem. *Expert Rev. anti-Infect. Ther.* 12 (1), 91–115. <https://doi.org/10.1586/14787210.2014.856756>.
- Walter, J., Haller, S., Quinten, C., Kärki, T., Zacher, B., Eckmanns, T., et al., 2018. Healthcare-associated pneumonia in acute care hospitals in European Union/European Economic Area countries: an analysis of data from a point prevalence survey, 2011 to 2012. *Eurosurveillance* 23 (32). <https://doi.org/10.2807/1560-7917.es.2018.23.32.1700843>.
- Wei, D.D., Chen, K.Q., Wang, L.H., 2016. Clinical and molecular characteristics of high virulent *Klebsiella pneumoniae* in infection in intensive care unit. *Chin. J. Nosocomiol* 26 (1), 5056–5059.
- Woerther, P.L., Burdet, C., Chachaty, E., Andremon, A., 2013. Trends in human fecal carriage of extended-spectrum  $\beta$ -lactamases in the community: toward the globalization of CTX-M. *Clin. Microbiol. Rev.* 26 (4), 744–758. <https://doi.org/10.1128/cmr.00023-13>.
- Woodford, N., Tierno, Jr.P.M., Young, K., Tysall, L., Palepou, M.F., Ward, E., Painter, R. E., Suber, D.F., Shungu, D., Silver, L.L., Inglima, K., 2004. Outbreak of *Klebsiella pneumoniae* producing a new carbapenem-hydrolyzing class A  $\beta$ -lactamase, KPC-3, in a New York medical center. *Antimicrob. Agents Chemother.* 48 (12), 4793–4799. <https://doi.org/10.1128/aac.48.12.4793-4799.2004>.
- Wood, S., Metcalf, D., Devine, D., Robinson, C., 2006. Erythrosine is a potential photosensitizer for the photodynamic therapy of oral plaque biofilms. *J. Antimicrob. Chemother.* 57 (4), 680–684. <https://doi.org/10.1093/jac/dkl021>.
- World Health Organization. Antimicrobial Resistance. Fact sheet N 194. Updated April 2015.
- Yahya, R.O., 2022. Problems Associated with Co-Infection by Multidrug-Resistant *Klebsiella pneumoniae* in COVID-19 Patients: A Review (MDPI). *Healthcare* 10 (12), 2412. <https://doi.org/10.3390/healthcare10122412>.
- Yigit, H., Queenan, A.M., Anderson, G.J., Domenech-Sanchez, A., Biddle, J.W., Steward, C.D., Alberti, S., Bush, K., Tenover, F.C., 2001. Novel carbapenem-hydrolyzing  $\beta$ -lactamase, KPC-1, from a carbapenem-resistant strain of *Klebsiella pneumoniae*. *Antimicrob. Agents Chemother.* 45 (4), 1151–1161. <https://doi.org/10.1128/aac.45.4.1151-1161.2001>.
- Yong, D., Toleman, M.A., Giske, C.G., Cho, H.S., Sundman, K., Lee, K., Walsh, T.R., 2009. Characterization of a new metallo- $\beta$ -lactamase gene, bla NDM-1, and a novel erythromycin esterase gene carried on a unique genetic structure in *Klebsiella pneumoniae* sequence type 14 from India. *Antimicrob. Agents Chemother.* 53 (12), 5046–5054. <https://doi.org/10.1128/aac.00774-09>.
- Zhou, F., Yu, T., Du, R., Fan, G., Liu, Y., Liu, Z., Xiang, J., Wang, Y., Song, B., Gu, X., Guan, L., 2020. Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. *Lancet* 395 (10229), 1054–1062. [https://doi.org/10.1016/s0140-6736\(20\)30566-3](https://doi.org/10.1016/s0140-6736(20)30566-3).
- Zhu, X., Ge, Y., Wu, T., Zhao, K., Chen, Y., Wu, B., Zhu, F., Zhu, B., Cui, L., 2020. Co-infection with respiratory pathogens among COVID-2019 cases. *Virus Res.* 285, 198005. <https://doi.org/10.1016/j.virusres.2020.198005>.
- Zhu, J., Sun, L., Ding, B., Yang, Y., Xu, X., Liu, W., Zhu, D., Yang, F., Zhang, H., Hu, F., 2016. Outbreak of NDM-1-producing *Klebsiella pneumoniae* ST76 and ST37 isolates in neonates. *Eur. J. Clin. Microbiol. Infect. Dis.* 35, 611–618. <https://doi.org/10.1007/s10096-016-2578-z>.
- Zhu, J., Wang, T., Chen, L., Du, H., 2021. Virulence factors in hypervirulent *Klebsiella pneumoniae*. *Front. Microbiol.* 12, 642484. <https://doi.org/10.3389/fmicb.2021.642484>.