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KLEBSIELLA PNEUMONIAE – TAXONOMY, OCCURRENCE, IDENTIFICATION, VIRULENCE FACTORS AND PATHOGENICITY

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Abstract. Gram-negative bacilli *Klebsiella pneumoniae* are among the most important pathogens responsible for healthcare-associated infections (HAIs). These bacteria often have high pathogenic and epidemic potential, contributing to infection outbreaks worldwide. *K. pneumoniae* is part of the natural microbiota of humans. At the same time, as an opportunistic microorganism, when the host organism is weakened, it can cause serious infections such as pneumonia, urinary tract infections, septic infections and intra-organ abscesses. Widespread distribution in nature and exceptional adaptability provide *K. pneumoniae* with the opportunity to master new niches in the hospital environment, which poses a threat to hospitalized patients. Also, the bacteria are increasingly causing life-threatening infections in the non-hospital environment. The pathogenicity of *K. pneumoniae* is determined by the presence of many virulence factors such as capsular polysaccharide (CPS, K antigen), lipopolysaccharid (LPS, O antigen), fimbrial and non-fimbrial adhesins, siderophores (aerobactin, enterobactin, salmochelin and yersiniabactin), heat-stabile and heat-labile enterotoxins, cytotoxins and biofilm-forming ability. Currently, hypervirulent strains of *K. pneumoniae* (hvKp) equipped with new virulence traits constitute a significant danger. The paper presents these bacteria concerning the global threat arising from the dynamic spread of hvKp strains in hospitals in Poland and worldwide.

Keywords: identification, *Klebsiella pneumoniae*, occurrence, pathogenicity, virulence factors

1. Introduction

The Gram-negative bacilli *Klebsiella pneumoniae* are an opportunistic pathogen with high pathogenic and epidemic potential, contributing to infection outbreaks worldwide. *K. pneumoniae* are the etiological factors of respiratory tract infections, mainly pneumonia, meningitis, septic infections and difficult-to-treat urinary tract infections. Increasing drug resistance, high mortality among patients infected with this pathogen and difficulties in treating the infection resulted in the World Health Organization (WHO) including these bacteria on the list of one of the most dangerous pathogens in the world (WHO 2024).

1.1. General characteristics of *Klebsiella* genus

1.1.1. Nomenclature and taxonomy

The generic name *Klebsiella* comes from the surname of the German microbiologist Edwin Klebs (1834–1913). Bacteria was first isolated in 1882 by Carl Friedländer from a patient who died of pneumonia (Grimont and Grimont 2015). The first species belonging to the genus *Klebsiella* described by Karl von Frisch was the bacterium *Klebsiella rhinoscleromatis* isolated from a patient with scleroma (Grimont and Grimont 2015).

In routine microbiological practice, exploitation of the 16S rRNA gene as a molecular marker led to the correction of previous findings regarding the taxonomy of bacilli belonging to the genus *Klebsiella* (Ma *et al.* 2021). This gene contains conserved regions (regions common to many bacteria) and species-specific regions, which allows precise identification of the genus or species of an isolated bacterial strain based on comparing determined sequences with sequences available in public databases (Srinivasan *et al.* 2015). According to the current state of scientific knowledge acquired in the course of many molecular studies, it has been shown that other genes with evolutionarily conserved sequences, for example, selected housekeeping genes, including the *rpo*B gene encoding the β subunit of RNA

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polymerase, are also characterized by a high potential differentiating (He *et al.* 2016). Currently, the identification of various groups of bacteria is carried out based on phenotypic and genotypic features based on rRNA coding sequences or sequenced genomes, additionally supplemented with information obtained from the amino acid structure of proteins performing critical functions in cells (Kim *et al.* 2021). On this basis, changes in the taxonomy of gamma-proteobacteria were proposed in 2016, the name of the *Enterobacteriales* order was modified, and a new division of families separated from the *Enterobacteriaceae* family, which included genus *Klebsiella* was introduced within the *Enterobacterales* order novum (Adeolu *et al.* 2016). Based on the findings, the monotypic order *Enterobacteriales*, containing one family of *Enterobacteriaceae*, was transformed into the polytypic order *Enterobacterales* order novum, consisting of seven new families, including *Enterobacteriaceae*, *Erwiniaceae*, *Pectobacteriaceae*, *Yersiniaceae*, *Hafniaceae*, *Morganellaceae* and *Budviciaceae* (Adeolu *et al.* 2016). It was also agreed that bacteria previously classified to the *Enterobacteriaceae* family, including species of the *Klebsiella* genus, will now be classified as a taxon in the order *Enterobacterales* (Fig. 1) (Adeolu *et al.* 2016; Schoch and Karsch-Mizrachi 2020).

In the course of the conducted phylogenetic studies, considering the latest divisions of microorganisms within the *Klebsiella* genus, a new species, *Klebsiella aerogenes,* appeared, referred to as a nomenclature (homotypic) synonym as *Klebsiella mobilis* (Szewczyk 2019). These bacteria were first described in 1885 by Theodor Escherich as "*Bacterium lactis aerogenes*", then renamed "*Bacillus aerogenes*" in 1896 by Walther Kruse, then "*Aerobacter aerogenes*" and finally named in 1960 by Estenio Hormaeche and Peter Geoffrey Edwards as *Enterobacter aerogenes* (Tindall *et al.* 2017). *Klebsiella mobilis* is an opportunistic pathogen responsible for nosocomial infections (Szewczyk 2019).

The genus *Klebsiella* also contains species previously counted in other taxonomic groups, including *Klebsiella oxytoca* and *Klebsiella ozaenae* (Tachibana *et al.* 2022; Yang *et al.* 2022). The first was isolated from sour milk and first described in 1886 by Carl Flügge as "*Bacillus oxytocus perniciosus*", then renamed in 1923 by David Hendricks Bergey as "*Aerobacter oxytocum*" and finally named *K. oxytoca* by Hans Lautrop in 1956 (Yang *et al.* 2022). The species name *K. oxytoca* comes from the Greek language and consists of the two elements "*oxus*", meaning "*sour*", and "*tokos*", meaning "*production*" (Yang *et al.* 2022). The other species included in the genus *Klebsiella* was *K. ozaenae*. These bacteria were observed in 1893 by Rudolf Abel in the nasal discharge of patients with ozena, or chronic atrophic malodorous rhinitis. Initially, these capsulated bacteria were known as "*Bacillus mucosus ozaenae*" and finally changed its name to *K. ozenae* (Tachibana *et al.* 2022). Another species included in the *Klebsiella* genus was *Klebsiella granulomatis* with the former name "*Donovania granulomatis*", otherwise called "*Calymmatobacterium granulomatis*" – the etiological agent of inguinal granuloma (donovanosis), i.e. an infectious granulomatous disease affecting the genitals and groin (Belda Junior 2020). The genus *Klebsiella* has also been enriched with a new species, *Klebsiella variicola*, isolated mainly from elements of edible plants such as roots, leaves and banana stem (Latin *Musa* spp.), corn shoots (Latin *Zea mays L.*), rice roots (Latin *Oryza sativa L.*) (Ma *et al.* 2021). In 2001, three other species of *Klebsiella*, namely *Klebsiella ornitynolytica*, *Klebsiella planticola*, and *Klebsiella terrigena* isolated from the environment previously classified as "*Klebsiella-like organisms*" were transferred to the newly created genus *Raoultella* (Kimura *et al.* 2014; Ma *et al.* 2021).

There are several taxonomic classification systems of rods belonging to the genus *Klebsiella* in use in the world, including the Cowan classification system introduced in 1960, the Bascomb classification system introduced in 1971, and the Ørskov classification system introduced in 1984 (Grimont and Grimont 2015). Most scientific teams rely on the classification developed by Ørskov (Grimont and Grimont 2015). Currently, the genus *Klebsiella* includes 22 species (Table I).

Domein: *Bacteria*
Type: *Proteobacteria*
Class: *Gammaproteobacteria*
Order: *Enterobacterales*
Family: *Enterobacteriaceae*
Genus: *Klebsiella*
Species: *Klebsiella pneumoniae*

Fig. 1. The current taxonomic position of the species *K. pneumoniae*.
Own graphic design according to (Adeolu *et al.* 2016; Schoch and Karsch-Mizrachi 2020; Dong *et al.* 2022).

Table I.
Clinical significance of selected *Klebsiella* species presented in alphabetical order

Species	Special features	References
1. *K. aerogenes*	The opportunistic pathogen, an etiological agent of nosocomial infections, present in various sewage wastes, chemicals and soil. Commercially important bacterium, „preeminent producer of hydrogen" produced by anaerobic fermentation, used as a substrate in molasses experiments, and a common cause of spoilage in maple sap and syrup.	(Tindall *et al.* 2017)
2. *K. africana*	The bacillus isolated from the asymptomatic carriage of the inhabitants of Kenya and Senegal, mainly an opportunistic pathogen.	(McDougall *et al.* 2021)
3. *K. granulomatis*	The etiological agent of inguinal granuloma (donovanosis), an infectious disease occurring in tropical and subtropical regions of Southeast Asia, India, Africa and Central America. The diagnosis of donovanosis is based on the history taking, the characteristic clinical picture (no changes in the lymph nodes) and the detection of the presence of vacuole in the tissue smear, the so-called Donovan bodies surrounding bacteria.	(Belda Junior 2020)
4. *K. grimontii*	A relatively common human pathogen isolated mainly in France, Germany and South Africa. It mainly causes bacteraemia and soft tissue infections.	(Passet and Brisse 2018)
5. *K. huaxensis*	The opportunistic pathogen. The etiological agent of urinary tract infections (UTIs).	(Hu *et al.* 2019)
6. *K. indica*	The opportunistic pathogen. Relatively little described in the scientific literature.	(Gujarati *et al.* 2020)
7. *K. kielensis*	The opportunistic pathogen. Relatively little described in the scientific literature.	(Schoch and Karsch-Mizrachi *et al.* 2020)
8. *K. michiganensis*	The opportunistic pathogen. First detected in Michigan. The bacterium was first isolated in Europe from blood and rectal swabs from an immunosuppressed patient.	(Seiffert *et al.* 2019)
9. *K. milletis*	The opportunistic pathogen. Bacillus mainly transmitted by food.	(Alves *et al.* 2006)
10. *K. oxytoca*	The second important species pathogenic for humans after *K. pneumoniae.* Isolated from pneumonia, and UTIs. Common cause of nosocomial infections in neonatal wards.	(Neog *et al.* 2021)
11. *K. pasteurii*	The opportunistic pathogen. Isolated from human and animals stool samples such as cows and turtles.	(Merla, Brisse *et al.* 2019)
12. *K. pneumoniae* subsp. *ozaenae*	The etiological factor of ozena - chronic, atrophic rhinitis, causing halitosis.	(Tachibana *et al.* 2022)
13. *K. pneumoniae* subsp. *pneumoniae*	The most frequently isolated in about 95% of all *Klebsiella* strains. An opportunistic pathogen. Isolated from: sepsis, endotoxic shock, pneumonia, lung abscesses, infections of the urinary, digestive and biliary tracts. In addition, it causes inflammation of the sinuses, middle ear, inflammation of soft tissues, osteomyelitis, and meningitis in newborns.	(Ali *et al.* 2022)
14. *K. pneumoniae* subsp. *rhinoscleromatis*	Frisch's bacillus, the etiological agent of heart disease („rhinoscleroma") known as „Slavic leprosy", a chronic infectious granulomatous disease of the respiratory tract covering mainly the nasal cavity, as well as the oral cavity, pharynx, larynx, trachea, and bronchi; is now very rare in Poland.	(Fusconi *et al.* 2018)
15. *K. quasipneumoniae*	Originally thought to be largely confined to agriculture. However, it may be responsible for causing disease in humans.	(Mathers *et al.* 2019)
16. *K. quasipneumoniae* subsp. *quasipneumoniae.*	The name derives from „*quasipneumoniae*" which means almost like „*pneumoniae*". The opportunistic pathogen. Pathogenicity as in *K. pneumoniae*, mainly the etiological agent of pneumonia.	(Brisse *et al.* 2014)
17. *K. quasipneumoniae* subsp. *similipneumoniae*	Name derived from „*similis*" which means similar to „*pneumoniae*". The opportunistic pathogen. Pathogenicity as in *K. pneumoniae*, mainly the etiological agent of pneumonia.	(Brisse *et al.* 2014)
18. *K. quasivariicola*	The opportunistic pathogen. First time isolated from a wound.	(Long *et al.* 2017)
19. *K. senegalensis*	The opportunistic pathogen. First detected in Senegal. Mainly foodborne pathogen.	(Alves *et al.* 2006)
20. *K. spallanzanii*	The opportunistic pathogen. Mainly isolated from human urine, cow feces and farms. cow feces and farms.	(Merla, Brisse *et al.* 2019)
21. *K. steroids*	The opportunistic pathogen. Relatively little described in the scientific literature.	(Schoch, Karsch-Mizrachi *et al.* 2020)
22. *K. variicola*	These rods account for less than 10% of *Klebsiella* clinical isolates previously classified as *K. pneumoniae*. Hypervirulent isolates have been identified, and colistin-resistant isolates of this species are also reported. Abundant in the environment (mainly rivers), edible plants, e.g. root, leaves, banana stem, sugar cane stem, corn shoots, rice roots. The etiological agent of mastitis in cattle.	(Rodriquez-Medina *et al.* 2019)

1.1.2. Occurrence

Bacteria of the genus *Klebsiella* are microorganisms widely distributed in the natural environment (Navon-Venezia *et al.* 2017; Khan *et al.* 2019; Huang *et al.* 2020). Moreover, *Klebsiella* is part of the microbiota in humans and various animals (dogs, cats, horses and pigs) (Navon-Venezia *et al.* 2017). *Klebsiella,* excreted in human and animal feces, is commonly found in soil, groundwater, surface and seawater, and on various plants such as banana, corn, rice and sorghum (Khan *et al.* 2017; Huang *et al.* 2020). These bacteria are also a component of industrial sewage (Navon-Venezia *et al.* 2017). The high adaptability of many species of *Klebsiella* bacilli, mainly *K. pneumoniae*, enables them to colonize hospital environments where multidrug-resistant hospital strains are selected. Recent studies show these strains present in the hospital environment, primarily in anesthesiology, intensive care, cardiology, neurosurgery, and neonatal departments. In patients and medical staff, these bacteria are part of the physiological permanent or transient microbiota (Ali *et al.* 2022). Outside the hospital, premature infants, newborns, older adults, as well as immunocompromised patients and alcoholics, are most at risk for infections caused by *Klebsiella* bacilli, in particular *K. pneumoniae* (Chang *et al.* 2021).

2. Characteristics of the *Klebsiella pneumoniae* species

2.1. Morphology, growth conditions, culture and biochemical profile

Klebsiella pneumoniae (formerly called Friedländer's bacilli) is a cylindrical, capsulated, ciliated, non-spore-forming bacterium measuring 0.3 to 1 µm in width and 0.6 to 6 µm in length (Fig. 2) (Ali *et al.* 2022). Some clinical strains of *K. pneumoniae* may be equipped with a single flagellum, which determines motility. The presence of these flagella is considered a virulence factor (Carabarin-Lima *et al.* 2016). *K. pneumoniae* in culture microscope preparations are arranged in pairs or short chains, the cells generally joining poles (Szewczyk 2019).

K. pneumoniae are facultative anaerobes that grow at an optimal temperature of 37°C. These bacilli survive in the inanimate environment in a wide temperature range (12°C–42°C). The ability to break down glucose, especially at high temperatures (up to 44.5°C), gives bacteria an advantage in non-living environments by providing energy for key life processes, supporting biofilm production and enabling adaptation. As a result, bacteria can survive longer in harsh environments, enhancing their ability to infect new hosts (Mason and Ztirich 1987; Centeleghe *et al.* 2023; Horng *et al.* 2023).

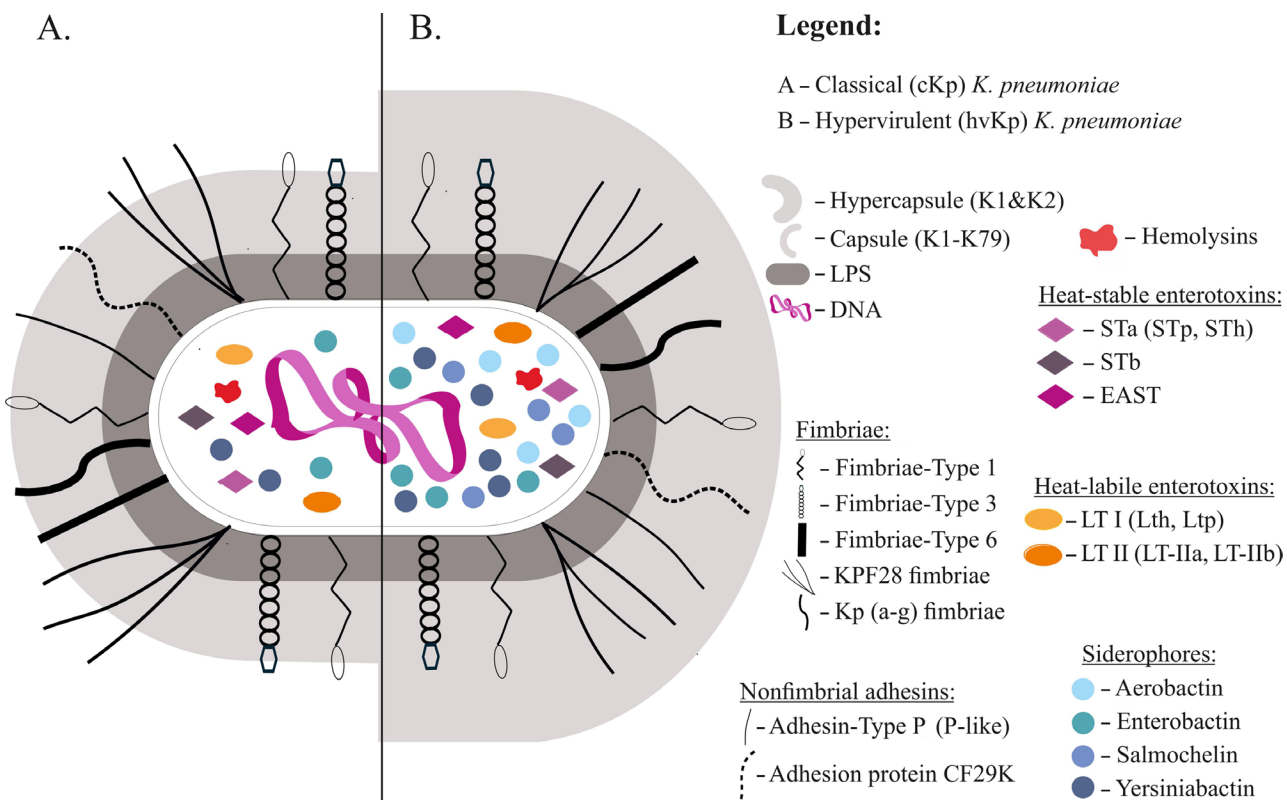

Fig. 2. Schematic representation of the differences in cell morphology of classical (cKp) and hypervirulent (hvKp) *K. pneumoniae,* taking into account virulence factors.

Own graphic design according to (Paczosa & Mecsas 2016; Ali *et al.* 2022; Dai and Hu 2022).

These bacteria are catalase-positive and indole-negative, produce urease, ferment lactose, produce lysine decarboxylase, do not produce ornithine decarboxylase, reduce nitrates to nitrites, do not produce deoxyribonucleases (DNases), use malonic acid and citrate as a carbon source, are oxidase-negative, do not cause deamination of phenylalanine (Brisse *et al.* 2014; Mączyńska 2015; Szewczyk 2019). Broth cultures of *K. pneumoniae* are uniformly turbid with a ring or a characteristic film located on the surface of the culture. Like other species of *Enterobacterales*, *K. pneumoniae* grow well and abundantly on solid substrates, forming characteristic mucous, shiny, convex, smooth, grey-white colonies (Murray *et al.* 2022). *K. pneumoniae* bacilli cultures can be performed on non-selective media such as tryptic soy agar (TSA) and blood agar (BA), as well as on selective-differentiating (selective) media such as: (1) MacConkey agar containing selective factors (crystal violet and sodium deoxycholate) and a differentiating factor (lactose) – *K. pneumoniae* form pink colonies, (2) eosin-methylene blue agar (EMB), medium containing selective factors (eosin and methylene blue) and differentiation factors (glucose and/or sucrose) – *K. pneumoniae* form blue-black colonies, (3) as well as Drigalski medium containing selective factors (crystal violet and sodium deoxycholate) (4) and bromothymol blue agar (BTB), differentiation medium on which the distinguishing of bacilli from the family Enterobacteriaceae is based on the ability to ferment the lactose in the presence of bromothymol blue – *K. pneumoniae* form yellow colonies (Brisse *et al.* 2014; Szewczyk 2019).

2.2. Species identification

Various microbiological methods are used to identify the species of *K. pneumoniae* bacilli, from microscopic techniques through traditional phenotypic methods to advanced molecular analyses (Grimont and Grimont 2015; Cheng *et al.* 2018; Froböse *et al.* 2020). During the cultivation of bacilli on solid media containing carbohydrates, the isolation and initial classification of bacteria into the *Klebsiella* genus is facilitated by the visible mucous appearance of bacterial colonies as a result of the production of a multi-sugar bacterial coating capsular polysaccharide (CPS) by *K. pneumoniae* strains (Szewczyk 2019; Murray *et al.* 2022). The bacterial capsule of *K. pneumoniae* can be visualized using various staining techniques, including the negative-positive method (Burri-Gins) using Chinese ink and alkaline fuchsin. Identification of *K. pneumoniae* strains characterized by the ability to create a hypermucoviscosity (HM) phenotype typical of highly pathogenic isolates is carried out using the string test (Eisenmenger *et al.* 2021). Identification of *K. pneumoniae* is based on traditional bacteriological methods involving the analysis of biochemical features using manual methods or standardized sets, e.g. API® 20E strips, or automatic methods using compact systems for identifying bacteria with the simultaneous determination of antimicrobial susceptibility of microorganisms, for example using the VITEK® 2 Compact system (Master *et al.* 2013). According to Monnet *et al.* (1991), for conventional methods, incorrect identification was made in 13% of the *K. pneumoniae* strains tested (Monnet *et al.* 1991). Precise identification to the species level can be performed using highly specialized techniques using matrix-assisted laser description/ionization time-of-flight mass spectrometry (MALDI-TOF-MS) (Váradi *et al.* 2017).

Among the molecular methods used for species identification of *K. pneumoniae*, the polymerase chain reaction (PCR) method is commonly used (Järvinen *et al.* 2009). For the molecular identification of *K. pneumoniae*, as well as typing of the most pathogenic strains, numerous genetic methods based on the multiplex-PCR technique have been developed, aiming to detect genes encoding pathogenicity factors, as well as to determine the serotype of the envelope (Chen *et al.* 2014; Fonseca *et al.* 2017). Multiplex-PCR has a high sensitivity and test specificity of over 90% (Dessajan and Timsit 2024). PCR also forms the basis of other techniques used in *K. pneumoniae* identification/differentiation based on regions of the *rrn* operon. These techniques use a variety of methods, including amplification of a variable region within the gene encoding 16S or 23S rRNA, amplification of polymorphic sequences located between the genes encoding 16S and 23S rRNA (Internal Transcribed Spacer-PCR, or ITS-PCR) (Liu *et al.* 2008) and Real-Time PCR for detecting *K. pneumoniae* with *rmp*A or *mag*A genes associated with the hypermucoviscosity phenotype (Hartman *et al.* 2009). The Real-Time PCR technique has high sensitivity and specificity (Hartman *et al.* 2009). Droplet digiatal (ddPCR) is used to detect *K. pneumoniae* in stool samples (Feng *et al.* 2024).

Another innovation is the Loop-Mediated Isothermal Amplification (LAMP) method (Poirier *et al.* 2021). LAMP (like the method with PCR) uses technology based on amplification and detecting specific DNA sequences. It relies on the isothermal amplification reaction of nucleic acids. The LAMP method is highly specific, as six primers (3 pairs) are used in the reaction, and amplification of genetic material occurs only if the primers recognize 6 to 8 specific DNA sequences of the pathogen under study (Poirier *et al.* 2021). The LAMP technique found particular application in a study by Poirier *et al.* (2021), who identified three target genes (*yha*I, *eps*L and *xcp*W) common to *K. pneumoniae* isolates from both China and Europe and designed LAMP assays for detecting *K. pneumoniae* in clinical

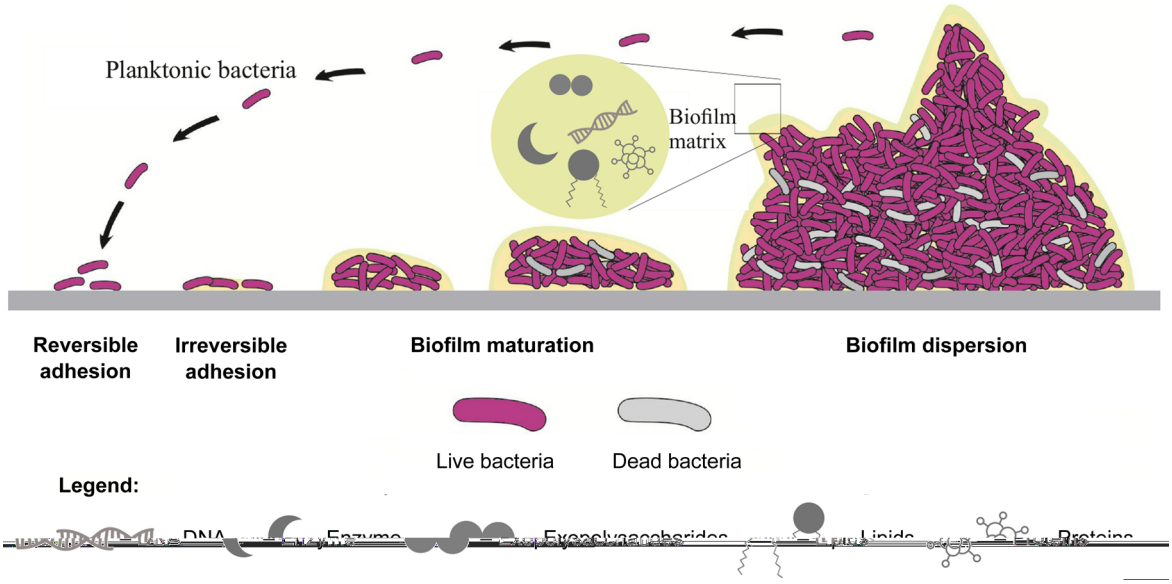

Fig. 3. Schematic representation of the subsequent stages of bacterial biofilm formation and its extracellular polymeric substance (EPS).
Own graphic design according to (Zhao *et al.* 2023).

samples (Poirier *et al.* 2021). In turn, Dong *et al.* (2015) described the LAMP method for rapid detection of the synthesis of the envelope polysaccharide regulating the *rcs*A gene from *K. pneumoniae* (Dong *et al.* 2015). The LAMP method is also being used to detect carbapenem resistance genes (bla_{KPC}, bla_{NDM-1}, $bla_{OXA-48-like}$, $bla_{IMP-1\ group}$, and bla_{VIM}) in *K. pneumoniae* (Poirier *et al.* 2021; Kim *et al.* 2022).

In addition to PCR-based methods, methods on Sanger sequencing, next-generation sequencing (NGS) and whole-genome sequencing (WGS) are used for proper molecular identification of *K. pneumoniae* species (Nafea *et al.* 2024). The main advantage of NGS over the conventional method is the simultaneous use of many genetic markers with high-resolution genetic data (Nafea *et al.* 2024). In turn, the high resolution of WGS analyses can provide information on the origin of bacteria, their routes of transmission, and biological traits (e.g., serotype). WGS also enables the identification of virulence genes and antibiotic-resistance genes. WGS analyses are suitable for introduction into routine laboratory testing. With comparative analysis of the entire genome, WGS could become the primary typing method used for early detection of epidemic outbreaks and monitoring the dynamics of the spread of a given pathogen (Nafea *et al.* 2024).

Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR-Cas) is increasingly used in microbiology. One of the first applications of this method was the typing of bacterial strains. CRISSP-Cas can also help develop new antimicrobial strategies (Barrangou *et al.* 2016; Ding *et al.* 2020). These techniques may find application in advanced studies of *K. pneumoniae.*

Molecular methods are increasingly being used in the identification of *K. pneumoniae* over classical phenotypic methods because they are independent of culture conditions, are more reproducibly sensitive and allow for shorter waiting times for results. The versatility of these methods is due to their applicability to the examination of virtually any biological material with minor adjustments to laboratory procedures. The limitations of these methods are limited availability and the inability to distinguish whether specific genetic material comes from live or dead bacteria.

2.3. Pathogenicity

Klebsiella spp. constitute a heterogeneous group of closely related enteric bacilli. *Klebsiella* rods are commonly found in hospital and non-hospital environments. They are part of the KESC subgroup (*Klebsiella*, *Enterobacter*, *Serratia* and *Citrobacter* subgroup), which collects genera with the closest relatedness and a similar biochemical profile (Szewczyk 2019). Microorganisms classified as KESC are characterized by multidrug resistance (MDR), and the presence of factors that enable them to survive freely in the hospital environment makes them a frequent cause of nosocomial infections (Szewczyk 2019). The species of the *Klebsiella* genus that most often causes infections in humans is *K. pneumoniae*. The second most frequently isolated species from clinical materials is *K. oxytoca*. The remaining *Klebsiella* species, much less frequently, may also be the etiological factor of infections (Chang *et al.* 2021).

K. pneumoniae is responsible for most (about 95%) severe human infections (Murray *et al.* 2022). Risk fac-

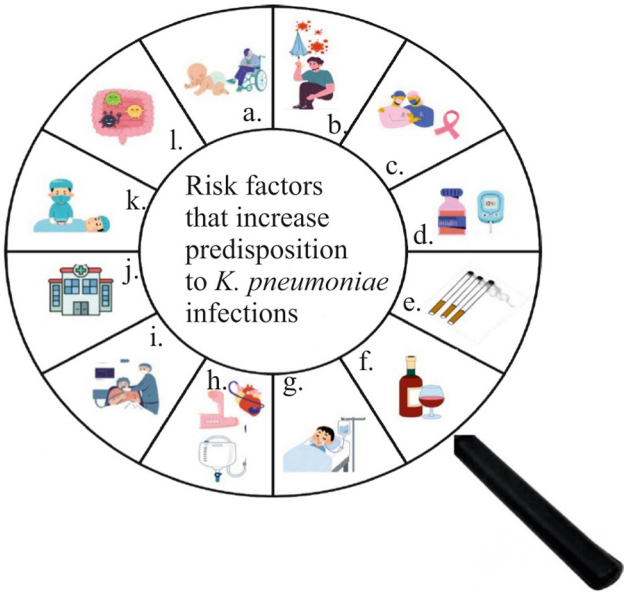

Fig. 4. Schematic representation of the risk factors that increase predisposition to *K. pneumoniae* infection: a – age (premature babies, newborns, elderly people), b – lowered immunity, c – debilitating diseases (cancer), d – concomitant diseases (e.g. diabetes), e – smoking, f – alcoholism, g – frequent or long-term hospitalization, h – use of vascular and urological catheters, drains and other implants, i – assisted breathing, j – stay in nursing homes, k – surgical interventions in the abdominal cavity, l – colonization of the gastrointestinal tract by hospital strains.

Own graphic design according to (Mączyńska 2015).

tors predisposing to *K. pneumoniae* infections include age (premature infants, newborns, the elderly), reduced immunity, debilitating diseases (cancer), frequent or prolonged hospitalization, assisted breathing, surgical interventions in the abdominal cavity, use of catheters (vascular, urological) drains and other implants, alcoholism, smoking, residence in nursing homes, colonization of the gastrointestinal tract by hospital strains (Fig. 4) (Mączyńska 2015).

Klebsiella can cause both healthcare-acquired infections (HAIs) and community-acquired infections (CAIs) (Chang *et al.* 2021). HAIs usually affect premature and frail neonates, older adults, and immunocompromised patients and include pneumonia, urinary tract infections (UTI), septic infections, endocarditis, central nervous system infections, purulent infections, wound infections, gastrointestinal infections associated with toxin production (Chang *et al.* 2021). CAIs include pneumonia, primary liver abscesses (PLA) combined with a characteristic invasive syndrome characterized by blood-borne infections spreading to other organs (bones and joints, eye, brain, lung, prostate, spleen) and rare infections occurring endemically (ozena, scleroderma and donovanosis) (Table I) (Fig. 5) (Fusconi *et al.* 2018; Belda Junior 2020; Tachibana *et al.* 2022).

K. pneumoniae is crucial in primary hospital-acquired pneumonia (HAPs) and community-acquired pneumonia (CAPs). In hospitalized patients, *K. pneumoniae* strains cause 7–14% of HAPs. The frequency of HAPs depends on the ward and condition of the patients and the intensity of invasive medical procedures (intubation, tracheotomy) associated with respiratory support (Mączyńska 2015). These factors increase the risk of pulmonary infections, including those associated with ventilator-acquired pneumonia (VAP), for which *K. pneumoniae* is also a significant etiologic agent. *K. pneumoniae* is generally the only *Enterobacteriaceae* causing 4–5% of out-of-hospital respiratory tract infections occurring most often in patients over 60 years of age, in poor health, often accompanying another severe underlying disease (e.g., diabetes, cardiovascular disease) but also much more common in smokers and alcohol abusers. These infections are characterized by a sudden onset, a severe course and a relatively high mortality rate. The characteristic symptom of the disease is the expectoration of a large amount of purulent, thick secretion, often colored by blood (Mączyńska 2015; Murray *et al.* 2022). The most important virulence factor of *K. pneumoniae* causing pneumonias is the polysaccharide envelope, which protects the bacteria from phagocytosis, and lysis by the complement system. It plays a crucial role in pathogenesis. Adhesins (especially fimbriae types 1 and 3) facilitate colonization of the airway epithelium

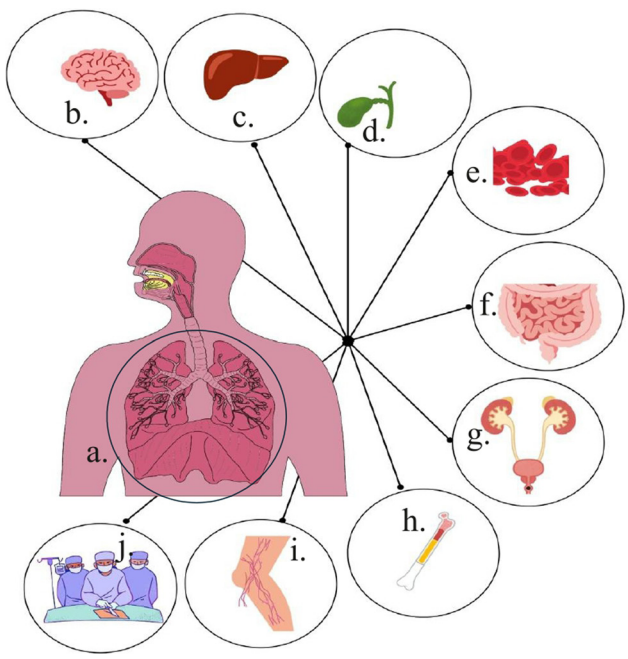

Fig. 5. Schematic representation of the pathogenicity of *K. pneumoniae*: a – pneumonia, b – central nervous system infections, c – primary liver abscess, d – cholecystitis, e – septic infections, f – gastrointestinal infections associated with toxin production, g – urinary tract infections, h – bone and joint infection, i – soft tissue infections, j – purulent and wound infections.

Own graphic design according to (Martinez and Baquero 2002; Chang *et al.* 2021; Ali *et al.* 2022).

and the production of mucus (exopolysaccharide) that is part of the biofilm structure that *K. pneumoniae* can form, for example, in the patient's lungs and endotracheal tubes or tracheostomy tubes, are also important in pneumonia (Alcántar-Curiel *et al.* 2013; Mączyńska 2015; Cader *et al.* 2020; Ochońska *et al.* 2021; Murray *et al.* 2022).

In addition to pneumonia, *K. pneumoniae* is often responsible for causing urinary tract infections – UTIs. *K. pneumoniae* causes 6–17% of hospital-acquired UTIs (Campana *et al.* 2017; Murray *et al.* 2022). UTIs can progress as pyelonephritis, typical bladder infections, but can also be recurrent and lead to permanent kidney changes. Among the most critical pathogenicity factors of uropathogenic *K. pneumoniae* strains are fimbriae, responsible for bacterial adhesion to urinary tract epithelial cells, which prevents the washout of microorganisms during micturition. The ability to produce urease and form biofilm structures on urinary catheters are also important virulence factors (Campana *et al.* 2017).

Septic infections caused by *K. pneumoniae* can manifest as asymptomatic bacteremia; these bacilli can also cause sepsis. The most common causes of sepsis are untreated urinary tract infections, respiratory tract infections and inflammation and obstruction of the intestines in immunocompromised patients (Carabarin-Lima *et al.* 2016).

A primary liver abscess (PLA) and the characteristic invasive syndrome are mainly caused by hvKp strains. The ability of hvKp strains to cause PLA with a tendency to spread to multiple tissues and organs (central nervous system, kidney, bones, eyes, lungs, prostate, skin and subcutaneous tissues, pancreas) is significant. The most common route of infection is bacterial translocation from the intestine. PLA caused by *K. pneumoniae* is localized in the right lobe of the liver and is limited in nature. Multiple abscesses may occur at different locations with greater or lesser frequency, commonly as liver abscesses, lung abscesses, biliary tract abscesses, skin and soft tissue abscesses, pleural abscesses, peritonitis, and inflammation of the external coating of the eye (*endophtalmitis*) (Ali *et al.* 2022). The rapid course of infection, having a poor prognosis and a tendency to develop generalized infection, is referred to as "*characteristic invasive syndrome*" (DIS) (Mączyńska 2015).

Among the isolates of *K. pneumoniae* are distinguished between classical *K. pneumoniae* (cKp) strains and hypervirulent *K. pneumoniae* (hvKp) strains (Fig. 2) (Russo *et al.* 2024). The cKp strains commonly cause infections in immunocompromised individuals. These are community-acquired pneumonia, UTIs, bacteremia or meningitis (Dai and Hu 2022). In addition, cKps strains are isolated from elderly patients and those with risk factors such as alcohol abuse and smoking (Russo *et al.* 2024). cKp is a group of *K. pneumoniae* that lacks hypercapsule, macromolecular exopolysaccharide or excessive siderophores and rarely causes disease in healthy individuals (except for UTIs), although it is MDR (Dai and Hu 2022). In contrast, hvKp are highly virulent strains responsible for community-acquired pneumonia characterized by a severe course and a high mortality rate reaching up to 40% in some regions of the world (Russo *et al.* 2024). As a result of the translocation process from the gut, strains of hvKP can spread to other organs and tissues (central nervous system, lungs, bones, prostate, skin and subcutaneous tissue) and also contribute to the formation of PLAs (Russo *et al.* 2024). hvKp is another type of *K. pneumoniae* that harbors hypercapsule, macromolecular exopolysaccharide, or highly active siderophores and induces infections in both immunocompromised and otherwise healthy individuals (Dai and Hu 2022).

2.4. Virulence factors

The pathogenicity of *K. pneumoniae* is determined by many virulence factors (Compain *et al.* 2014; de Souza *et al.* 2024). Their presence can lead to infection and antibiotic resistance. The major virulence factors playing an essential role in the pathogenesis of infections caused by *K. pneumoniae* are capsule polysaccharides (CPS, K-antigen) and lipopolysaccharides (LPS, O-antigen). These critical virulence factors help to enter the bloodstream and cause septic shock in the host. Fimbrial and non-fimbrial adhesins, siderophores (aerobactin, enterobactin, salmochelin and yersiniabactin), heat-stable and heat-labile enterotoxins, cytolysins and the ability to form a biofilm are also important (Fig. 2) (Ali *et al.* 2022). The genes encoding many virulence factors are located in the large mosaic virulence plasmid (pLVPK) in *K. pneumoniae* isolates (Mączyńska *et al.* 2015; Clegg and Murphy 2016).

2.4.1. Capsule polysaccharide (CPS)

The capsule polysaccharide (CPS) is the essential, well-known virulence factor of *K. pneumoniae,* forming a layer hermetically surrounding the bacterial cell wall (Ali *et al.* 2022). It is responsible for the initial interaction between bacteria and host. Moreover, the CPS is vital for the survival of *K. pneumoniae* in the host tissue, allowing the pathogen to escape phagocytosis (Ali *et al.* 2022). Structurally, *K. pneumoniae* CPS is a heteropolymer comprised of repeated sugar moieties of hexoses (D-glucose, D-galactose and D-mannose), deoxyhexoses (D-fucose and D-rhamnose) and glucuronic and galacturonic acids. The capsule may also include non-sugar components such as O-acyl, succinate, formate and pyruvate residues (Ali *et al.* 2022). The CPS of *K. pneumoniae* is characterized by the diversity of the structure of polysaccharides that build them and

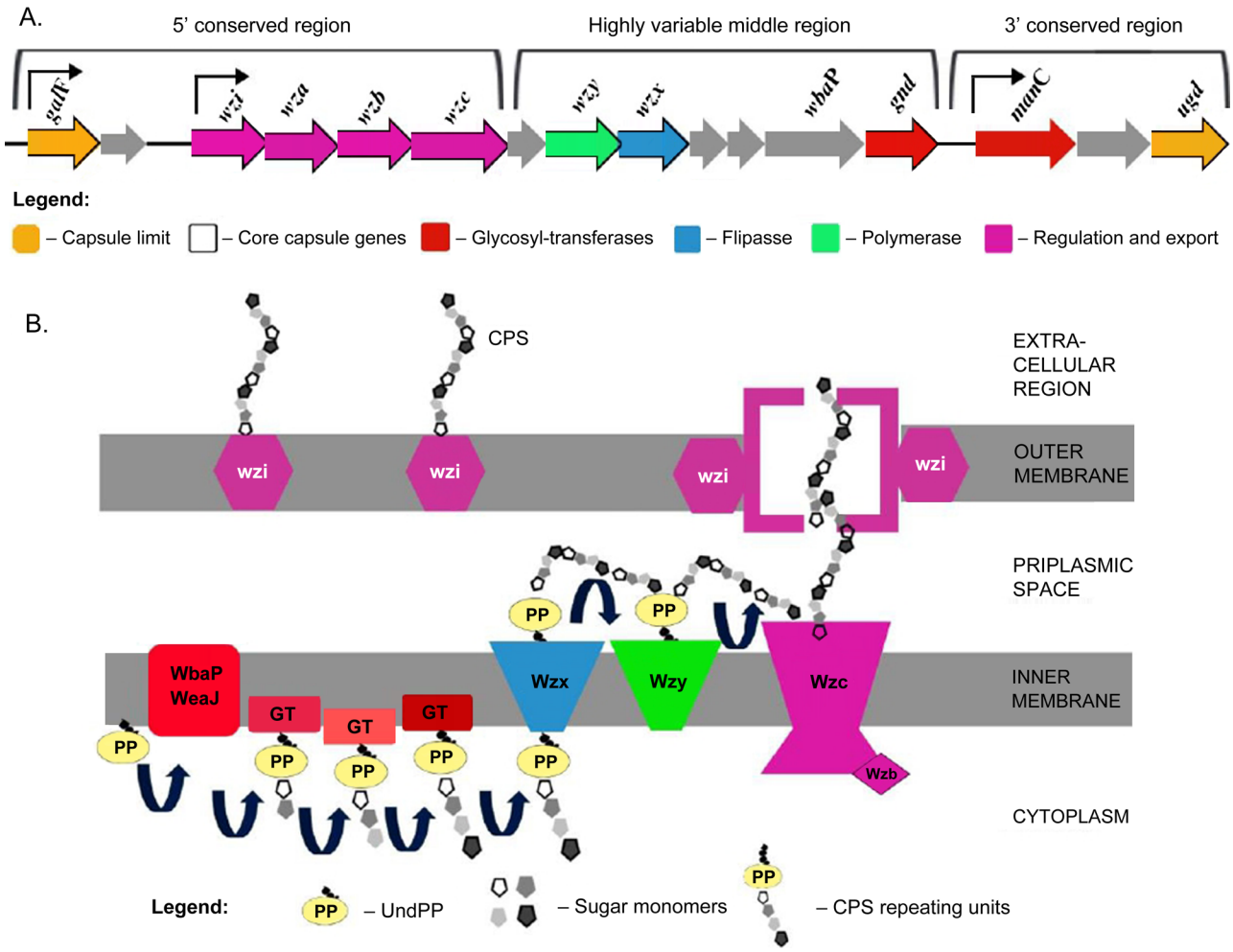

Fig. 6. (**A**) Schematic representation of the capsule (CPS) biosynthetic pathway in *K. pneumoniae.* Own graphic design according to (Patro and Rathinavelan 2019, Rendueles 2020, Patro *et al.* 2020),

(**B**) Scheme of the representative *locus* cps system of *K. pneumoniae* using the K1 serotype as an example. Own graphic design according to (Rendueles 2020, Patro *et al.* 2020).

high serological variability. Genes involved in capsule production are located on the chromosome's capsular polysaccharide synthesis (*cps*) region. The region of the *cps* cluster (from *gal*F to *ugd*) harbors over 20 genes, mainly driven by three promotors located upstream of genes, *gal*F, *wzi*, and *man*C, respectively (Fig. 6) (Zhu *et al.* 2020). A group of six genes mainly carries out the synthesis of the capsule at the 5' end of the *cps* operon (*gal*F, *cps*ACP, *wzi, wza, wzb, wzc*) and the *ugd* gene located at the 3' end (Shu *et al.* 2009; Wyres *et al.* 2016). The diversity of serotypes is the result of the action of various glycosyl-transferases (GTs), whose genes *wba*P, *wba*Z, *wca*N, wcaJ and *wca*O, are located in the middle part of the *cps* operon (Shu *et al.* 2009). The protein encoded by *wba*P mediated the first step in capsule biosynthesis. Glycosylation of the repeating unit is initiated by WbaP (when the initializing sugar linked to the undecaprenol-pyrophosphate – Und-PP – is galactose) or by WcaJ (when the initializing sugar linked to Und-PP is glucose). Current research has shown that some *wba*P mutations increased pathogenicity by increasing biofilm formation and invasion of bladder epithelial cells in urinary tract infections (UTIs) (Zhu *et al.* 2020). Several genes that regulate the biosynthesis of CPS sugar, *rml*A, *rml*B, *rml*C, *rml*D, *man*B and *man*C, are also located towards the end of the *cps* locus (Ali *et al.* 2022). Differences in *K. pneumoniae* capsule types result from changes in the nucleotide sequences of the *cps* locus and genes involved in CPS biosynthesis, assembly and translocation (Ali *et al.* 2022).

Enhanced capsule production in *K. pneumoniae* may result from the activity of other genes in addition to those located in the *cps* cluster: the capsular synthesis gene B (*rcsB*), the mucoid phenotype regulators A and A2 (*rmpA* and *rmpA2*) and *Klebsiella* virulence regulators (*kvrA* and *kvrB*) and *wzy-K1* (Zhu *et al.* 2020). Different combinations of these genes can result in the production of capsules with different structures. The *rmp*A or *rmp*A2 genes are found in 55–100% hvKp strains, while they are less frequently found in cKp

strains. RmpA regulates mucoid phenotype in pK100 and RmpB (Dai and Hu 2022). The expression of *rmp*A depends on RcsB, KvrA, and KvrB. The newly described regulators, *kvr*A and *kvr*B, affect the virulence of K1/K2 hvKp strains due to the activation of capsule gene expression, which is not present in cKp strains. Different from *rcs*B found in chromosome, both *rmp*A and *rmp*A2 could be located in plasmid or chromosome. Chromosomal *rmp*A (*c-rmp*A and *c-rmp*A2) are located in an integrative and conjugative element (ICEK*p1*) and are only found in < 50% *K. pneumoniae* strains of serotype K1 (Zhu *et al.* 2020; Dai and Hu 2022). Plasmidic *rmp*A (*p-rmp*A and *p-rmp*A2) are more prevalent. The *wzy*-K1 gene is specific to the K1 serotype of *K. pneumoniae*. The function of a *wzx* gene product is to transport the polymer from the cytoplasm to the periplasm. The Wzy protein is involved in the polymerization *via* a catch-and-release mechanism (Zhu *et al.* 2020). Proteins encoded by *wza* and *wzc* genes form a translocation complex responsible for assembling capsular polysaccharides and transporting them from the periplasm to the surface of the bacteria (Pan *et al.* 2013). The Wzb protein, as the cognate phosphatase of Wzc, combines with the catalytic domain on Wzc and, in turn, dephosphorylates Wzc (Zhu *et al*. 2020).

Hypercapsules can be regulated by the capsule A (*cps*A) and B (*cps*B) genes (Dai and Hu 2022). 70% of hvKp strains produce a hypercapsule composed of types K1 and K2. This kind is more stable than the typical capsule found in cKp strains, contributing to their increased virulence in hvKp strains. Other capsule types occur in cKp strains (Marr and Russo 2019). In *K. pneumoniae*, the capsule binds to the surface protein Wzi. Loss of this protein can reduce or lose virulence (Ali *et al.* 2022). There are 79 serotypes of capsulated *K. pneumoniae* strains (K1 to K79). The eight most common types have been described in hvKp strains: K1, K2, K5, K16, K20, K54, K57, and KN1 (Zhu *et al.* 2020). Recently, a classification scheme has been proposed based on the sequence of conserved *wzi* and/or *wzc* genes in the *cps* locus (Ali *et al*. 2022).

In *K. pneumoniae*, the WGS method is specifically used to identify *cps* locus variants (Wyres *et al*. 2016). In a study conducted by Wyres *et al*. (2016), among 2503 *K. pneumoniae* genomes, the diversity of capsid fusion loci (K-loci) was examined. The study included analysis of full-length K-locus nucleotide sequences and clustering protein-coding sequences to identify, annotate and compare K-locus structures. A total of 134 distinct K-locuses were identified, including 31 new types. Comparative analyses revealed 508 unique clusters of protein-coding genes that appear to reassort through homologous recombination. In addition, a high diversity of intra- and inter-locus nucleotides was detected among *wzi* and *wzc* genes. Based on the results, a standardized nomenclature for K loci was proposed, a reference database was presented, and a new software tool – Kaptive, was developed to automate the process of identifying K loci based on complete locus information extracted from the whole-genome sequence (https://github.com/katholt/Kaptive) (Wyres *et al.* 2016).

Assessment of the prevalence of specific serotypes in the *K. pneumoniae* population is valuable for epidemiological investigations. The prevalence of individual serogroups/serotypes depends on the geographical location, patient age, and changes over the years. Strains of different serotypes differ in their resistance to phagocytosis *in vitro* and their ability to activate the humoral response. Some serotypes are more frequently associated with human diseases and epidemics. Hypervirulent strains of *K. pneumoniae* (hvKp) with high virulence usually have the K1 or K2 envelope antigen (Ali *et al.* 2022). *K. pneumoniae* strains represent the same epidemic clone and have the same capsule type (Choi *et al.* 2020). Serotyping is not sufficient for epidemiological purposes due to its poor resolution. However, the synthesis of the bacterial capsule is determined by a set of *cps* genes located in the chromosome and plasmid. Thanks to knowledge of the allele sequence in the *cps* locus, the PCR method is now more widely used for their detection (Walker and Miller 2020; Ali *et al.* 2022).

2.4.2. Lipopolysaccharide (LPS)

Lipopolysaccharide (LPS), also called endotoxin, is an integral and essential component of the cell membrane of Gram-negative bacteria (Ali *et al.* 2022). LPS has strong cytotoxic, immunomodulatory and proinflammatory properties. LPS remains one of the most important pathogenic factors and the main antigen of the *K. pneumoniae* cell wall. It plays a role in the pathomechanism of infection, especially in endotoxic shock accompanying central nervous system infections, blood infections and pneumonia (Choi *et al.* 2020). It is connected with a massive release of LPS after bacterial cells lysis. Increased release of LPS can occur under the influence of various groups of antibiotics that cause lysis of bacterial cells or interfere with their function, including β-lactams (Eng *et al.* 1993; Kirikae *et al.* 1997; Holzheimer 2001).

LPS is a substance with a conservative structure consisting of three fundamental components, i.e. lipid A, the core oligosaccharide and a polysaccharide that determines antigenic specificity (chain O, somatic antigen O). Serological typing of *K. pneumoniae* is based on two main groups of antigens, i.e. the somatic polysaccharide O and the typical-specific capsular antigen K (Choi *et al.* 2020).

Lipid A is a crucial virulence factor responsible for the endotoxic effects of LPS (Navon-Venezia *et al.*

2017). It is recognized as the most structurally conserved region. Several enzymes encoded by the *lpx* gene cluster are involved in the synthesis of lipid A components. The host immune cell receptor, Toll-like receptor 4 (TLR4), recognizes and binds lipid A of LPS, which initiates a cascade of host immune reactions. Although modifications of the lipid A component help the pathogen escape recognition by the host immune cells by favoring the pathogen to establish the infection successfully (Ali *et al.* 2022). Lipid A is the hydrophobic part of endotoxin, responsible for anchoring the heteropolymer in the outer membrane of the host, thanks to which it creates a specific barrier that inhibits the penetration of substances, including antibiotics and detergents, into the microorganism while generating resistance to these compounds. In *K. pneumoniae*, the ineffective antimicrobial effect of colistin can occur through plasmid-mediated transfer of *mcr-1*, a resistance gene, causing modification of LPS lipid A and disruption of the interaction between polymyxins and lipid A (MacDermott-Opeskin *et al.* 2022). Acylation of lipopolysaccharides plays a key role in providing Gram-negative bacteria with some resistance to structural and intrinsic defense mechanisms, particularly the antibacterial properties of detergents (e.g., bile) and cationic defensins (Clements and Strugnell *et al.* 2022).

The core oligosaccharide component of the LPS connects lipid A to the terminal side chains called the O antigen. The genes encoding core oligosaccharides are located in the *waa* locus, and the ligase enzyme WaaL, which links the core structure to the antigen O chain, is encoded by *waa*L. The outer part of the LPS structure, O antigen, comprises multiple repeating units of oligosaccharides: glucose, galactose, mannose and ribose residues. Epidemiological investigations within a species are based on their structure. The *wb* gene cluster regulates the O antigen's synthesis, assembly and translocation. The variation in the oligosaccharide repeats underlies the LPS diversification structurally and functionally. So far, up to nine O *K. pneumoniae* antigens have been identified based on the composition of the sugar molecules (Ali *et al.* 2022).

2.4.3. Fimbrial and non-fimbrial adhesins

The adhesive properties of *K. pneumoniae* are also due to the possession of fimbrial and non-fimbrial adhesins. The fimbrial adhesins include type 1 mannose-sensitive (MS) fimbriae, type 3 mannose-resistant (MR) fimbriae, type 6 fimbriae, KPF-28 fimbriae. *Klebsiella pneumoniae* fimbriae with a fimbrin molecular mass of 28 kDa) and Kp (a-g) fimbriae (Klemm *et al.* 2000; Struve *et al.* 2009; Chen *et al.* 2011; Alcantal-Curiel *et al.* 2013; Mączyńska 2015; Alcantal-Curiel *et al.* 2018; Khonsari *et al.* 2021). Non-fibrillar adhesins include the non-fibrillar P-type adhesion factor and CF29K adhesion protein (CF29K adhesion factor) (Staniszewska *et al.* 2000; Chan *et al.* 2012; Hennequin *et al.* 2016).

Type 1 fimbriae are among the best characterized. They are expressed in about 90% of *K. pneumoniae* strains (Mączyńska 2015). They are mannose-sensitive hemagglutinins (MSHAs), forming long, thick, stiff filaments 1 to 2 μm long and about 7 nm in diameter (Chen *et al.* 2011). Type 1 fimbriae are protein heterocomplexes of the major fimbriae subunit (FimA) that form the protuberance's structure. Smaller subunits (FimB, FimC, FimD, FimE, FimF, FimG, FimH, FimK, FimS and FimX), in addition to adhesion functions, are responsible for protuberance elongation and stability (Alcantal-Curiel *et al.* 2013; Mączyńska 2015). Receptors for FimH are mannosides. The protein determining adhesion properties can be located at the top of the fimbriae and distributed along the spear's entire length (Alcantal-Curiel *et al.* 2013, Mączyńska 2015). The individual subunits of the fimbriae are linked by hydrophobic bonds and form a right-handed helix stabilized by hydrogen bonds. The structural and functional integrity of the elements formed is called the "*fimbriae-adhesin complex*" (Mączyńska 2015). A set of *fim* genes located in a chromosome or plasmid is responsible for the expression of type 1 fimbriae. Synthesis of type 1 fimbriae follows the "all-or-nothing" principle (Mączyńska 2015). Recent evidence shows that the expression of fimbriae's subunit genes responsible for turning on or off fimbriae synthesis can be directly influenced by oxygen availability, elevated temperature but also by the presence of sub-minimal inhibitory concentrations (sub-MICs) of an antibiotic (e.g. streptomycin), which can affect the production of longer fimbriae lacking the ability to bind mannose (Shibl *et al.* 1985; Klemm *et al.* 2000; Struve *et al.* 2009; Mączyńska 2015). Streptomycin induces fimbriae formation that is both functionally and morphologically abnormal. This may have resulted from amino acid substitutions in fimbrial proteins due to the misreading of mRNA by ribosomes (Shibl *et al.* 1985).

Type 3 fimbriae are expressed on the surface in more than 80% of *K. pneumoniae* strains (Murphy and Clegg 2012; Khonsari *et al.* 2021). These are protein heterocomplexes and are mannose-resistant hemagglutinins (MRHA). They form short and thin filaments about 2–4 nm wide and 0.5–2 μm long (Murphy and Clegg 2012). At least nine genes from the *mrk* cluster are required to express type 3 fimbriae. The *mrk* gene cluster can be located in chromosomal or plasmid DNA. The *mrk*A gene encodes the main structural subunit of the fimbriae, while the *mrk*D gene encodes the actual adhesin. This protein determines the specific interaction of the protuberance with the receptor. Smaller

fimbriae subunits MrkB, MrkC, and MrkD form the characteristic structure, and their genes *mrk*B, *mrk*C and *mrk*D regulate the spears' expression. The product of the *mrk*F gene stabilizes the fimbriae structure on the bacterial cell surface (Murphy and Clegg 2012; Alcantal-Curiel *et al.* 2013; Mączyńska 2015).

Type 6 fimbriae are the longest, thick spears present in small numbers on the bacterial surface. Type 6 fimbriae have only been confirmed in the species *K. pneumoniae* subsp. *ozenae* and their role in pathogenicity is little understood (Darfeuille-Michaud *et al.* 1992; Mączyńska 2015).

KPF28 fimbriae (*Klebsiella pneumoniae* fimbriae with a fimbrin molecular mass of 28 kDa) are a long, thin, and flexible, about 4 to 5 nm in diameter and 0.5 to 2 mm long (Di Martino *et al.* 1996). The N-terminal amino acid sequence of the KPF-28 major fimbrial subunit showed no homology with type 1 and type 3 pili of *K. pneumoniae*. Still, it showed 61.7% identity with residues 6 to 19 of the N-terminal amino acid sequence of PapA, the Pap major pilus subunit expressed by uropathogenic *Escherichia coli* strains (UPEC) (Di Martino *et al.* 1996). In a study of *K. pneumoniae* responsible for nosocomial infections, KPF-28 was shown to be present in strains producing the extended-spectrum β-lactamase CAZ-5/SHV-4 (current name SHV-4) (Di Martino *et al.* 1996). KPF-28 fimbriae are plasmid-encoded, specifically in plasmid R, which contains $bla_{\text{SHV-4}}$ gene (Di Martino *et al.* 1996). A study by Di Martino *et al.* (1996) involving *K. pneumoniae* strain CF914-1 isolated from urine from a patient in ICU and 78 other *K. pneumoniae* isolates involved in nosocomial infections showed that fimbriae KPF-28 were present in *K. pneumoniae* strain CF914-1, as well as in vast majority (83%) of clinical *K. pneumoniae* strains producing SHV-4 extended-spectrum β-lactamase (DiMartino *et al.* 1996). Further studies on the occurrence of KPF28-type fimbriae in *K. pneumoniae* strains causing UTIs are needed.

Kp-type fimbriae (Kpa, Kpb, Kpc, Kpd, Kpe, Kpf and Kpg) are another seven types of fimbriae detected in *K. pneumoniae* (Wu *et al.* 2010). A study by researchers in Taiwan showed that Kp-type fimbriae are only found in *K. pneumoniae* strains with the K1 capsule antigen and increase the ability of strains to form a biofilm (Wu *et al.* 2010).

A non-fimbrial P-type adhesion factor is a protein heteropolymer that exhibits hemagglutination properties similar to the analogous properties of P fimbriae in *E. coli*. It has no fimbrial filament-forming subunits. The receptor for the non-fimbrial P-type adhesion factor is the globoside receptor α – D – Galp – (1 4) – D – Galp. The P-type factor involves bacterial adhesion to the epithelium of the urinary, gastrointestinal and respiratory tract (Staniszewska *et al.* 2000).

CF29K (nonfimbrial protein of 29 kDa) (CF29K adhesion factor) – nonfimbrial adhesion protein was found in *K. pneumoniae* strains characterized by high adhesion capacity to intestinal cell lines. It is encoded by the *cf*29A gene located on a plasmid that also contains the gene encoding TEM-5 β-lactamase. It shows high homology to the CS31A-L protein encoded by the *clp*G gene and produced by enterotoxigenic *E. coli* (ETEC) strains (Hennequin *et al.* 2016).

2.4.4. Siderophores

Siderophores such as aeroactin, enterobactin, salmochelin and yersiniabactin are virulence factors synthesized by *K. pneumoniae* (Farzand *et al.* 2021). Bacterial siderophores, called iron carriers, are low-molecular, organic chemical compounds of a non-protein and non-porphyrin nature, chelating iron ions and secreted extracellularly by some microorganisms to capture this element (Farzand *et al.* 2021). In bacterial cells, iron is an element necessary for the synthesis of cytochromes and ribonucleotide reductase, which are involved in the DNA synthesis process, as well as other enzymes. The survival of *K. pneumoniae* in the environment depends on siderophores to meet the demand for iron. They compete with the host for the available iron pool (Chhabra *et al.* 2020).

Enterobactin is the primary iron uptake system in *K. pneumoniae* and is the most commonly but not only siderophore synthesized in this bacterial species. Studies show that hypervirulent hvKp strains quantitatively produce more siderophores than cKp strains (Dai and Hu 2022). Enterobactin is catecholate. The chromosomal gene cluster *ent*ABCDEF and *fep*ABCDG encode its biosynthesis and transport. *ybt* and *fyu* genes encode transporters for the secretion of enterobactin, and *ybt*O encodes the uptake receptor of enterobactin. Lipocalin-2 (LCN2), known as neutrophil gelatinase-associated lipocalin (NGAL), is extremely important in immune processes and can bind and neutralize enterobactin. LCN2 participate in the regulation of cell aging, cell differentiation and modeling of the immune response (Xiao *et al.* 2017). In *K. pneumoniae* respiratory tract infection, LCN2 is up-regulated by the host. This lipocalin also has pro-inflammatory effects, leading to IL-8-mediated recruitment of neutrophils to the site of infection (Dai and Hu 2022).

Aerobactin is a citrate-hydroxamate siderophore found mainly in more than 90% hvKp strains, while it is less common (6%) in (cKp) strains. Aerobactin was present in hvKp-caused lung infections and is the dominant siderophore in hvKp strains. Aerobactin production is usually associated with hypercapsule, while *K. pneumoniae* with hypercapsule does not always contain aerobactin. The *iuc*ABCD gene cluster controls aerobactin synthesis, while its transport is determined

by *iut*A. They are often present in the same pLVPK-like plasmids carrying *p-rmp*A (Dai and Hu 2022). LCN2 does not neutralize aerobactin (Xiao *et al.* 2017).

Salmochelin is a c-glucosylated form of enterobactin and another siderophore in *K. pneumoniae*. Glucosylation is carried out by the *iro* gene cluster, *iro*ABCDE, which can be localized in a chromosome or plasmid. IroN contributes to the transport of iron-carrying salmochelin. Salmochelin is not neutralyzed by LCN2. This siderophore induces colonization of the nasopharyngeal cavity by *K. pneumoniae*, leading to pneumonia. Salmochelin, like aerobactin, is usually found in hvKp strains with a frequency of over 90% and only 2–4% in cKp strains (Dai and Hu 2022).

Yersiniabactin is another siderophore in *K. pneumoniae* whose production is likely due to horizontal gene transfer (HGT) genes from *Yersinia*. Yersiniabactin was found in 18% of cKp strains and 90% hvKp. Located in chromosome *irp* gene cluster encodes proteins for yersiniabactin synthesis. Yersiniabactin and enterobactin are highly expressed during lung infection, and LCN2 does not inhibit it *in vivo*. However, yersiniabactin alone cannot acquire the iron for *K. pneumoniae,* and the lack of the other three siderophores would prevent *K. pneumoniae* from colonizing the lungs (Holden and Bachman 2015; Dai and Hu 2022).

2.4.5. Heat-stable and heat-labile enterotoxins

Important extracellular pathogenicity factors of *K. pneumoniae* are also enterotoxins – protein toxins similar to enteroaggregative *E. coli* heat-stable enterotoxin 1 (EAST 1), heat-stabile (ST) and heat-labile (LT) enterotoxins (O'Ryan 2011). Plasmids carrying enterotoxin-coding genes acquired by multidrug-resistant strains may contribute to the emergence of epidemics. Bacterial diarrhea occurring in the hospital environment can cause the spread of bacteria, and strain ability to produce enterotoxins can be a factor that predisposes to cause an epidemic outbreak (O'Ryan 2011).

2.4.6. Hemolysins

Until recently, *Klebsiella* spp. were considered non-hemolytic, and only single papers of one research group from the 1980s described the hemolysin produced by *K. pneumoniae* and *K. oxytoca* (Barberis *et al.* 1986). According to them, these hemolysins are thiol-activated cytolysins and are supposed to belong to the TACY group. The cytolytic activity of the TACY group toxins is observed upon the addition of thiol compounds, for example, 2-mercaptoethanol or dithiothreitol (DTT). The hemolysin produced by these bacteria was named klebolysin, and it was established that it is inactivated in the presence of cholesterol and cross-reacts with antibodies directed against streptolysin O (Szramka 2001).

2.5. *K. pneumoniae* biofilm

Over the past few years, there has been a growing problem of infections caused by *K. pneumoniae* due to the bacteria's ability to form a biofilm (Dsouza *et al.* 2019). *K. pneumoniae* exhibits many pathogenic properties that facilitate their survival, spread in the hospital environment, and adhesion to biotic or abiotic surfaces (Piperaki *et al.* 2017). *K. pneumoniae* is characterized by a high ability to adhere and form biofilm structures, which plays an essential role in the colonization and persistence of these microorganisms on mucous membranes of the body and artificial surfaces of catheters, implants and others. Important pathogenic features of *K. pneumoniae* involved in biofilm formation include overproduction of mucus (hvKp strains), selection of strains with a specific type of envelope and transfer of adhesin genes in plasmids (Guerra *et al.* 2022).

Biofilm is defined as a complex organized multicellular, single- or multi-species structure in which bacterial cells are embedded in a matrix made of extracellular polymeric slime (EPS), where they adhere to each other and/or show adhesion to various surfaces (Piperaki *et al.* 2017). The phenomenon of biofilm formation is a process that occurs in several stages: reversible adhesion, irreversible adhesion, maturation and dispersion (Fig. 3) (Piperaki *et al.* 2017). Then, as a result of the movement of bacterial cells along with blood and other body fluids, the colonisation process of new niches begins, giving rise to a new biofilm. Mature biofilm structures are characterized by bacterial persister cells (PCs), which enable the renewal of the biofilm population (She *et al.* 2022). The biofilm formation process is controlled by "*quorum sensing*", a unique intercellular communication system regulated by chemicals called signaling molecules (Piperaki *et al.* 2017).

Bacteria in biofilms, including pathogens such as *K. pneumoniae*, display highly developed adaptive capabilities that enable them to survive under challenging conditions, colonize new environments, and evade the host immune system (Piperaki *et al.* 2017; Dsouza *et al.* 2019; Thoraninsdottir *et al.* 2020; Guerra *et al.* 2022; Centeleghe *et al.* 2023). Mature biofilm provides a protective barrier against the effects of antibiotics and disinfectants. One of the possible mechanisms of antibiotic resistance is limited penetration of antibiotics into the bacterial cell and reduced metabolism of cells located inside the biofilm. Even if antibiotics reach the bacteria, the cells inside the biofilm are less metabolically active, making antibiotics that target rapidly dividing cells (e.g., β-lactams) less effective. Biofilm constitutes a physical barrier for immune cells such as neutrophils and macrophages, hindering their access to the bacteria inside (Piperaki *et al.* 2017; Dsouza *et al.* 2019; Thoraninsdottir *et al.* 2020; Guerra *et al.* 2022; Centeleghe

et al. 2023). The biofilm matrix can also bind components of the complement system, which hinders its activation and limits the effectiveness of the immune response. Bacteria in a biofilm can survive in conditions lethal to planktonic (free-swimming) bacteria. Low nutrient or oxygen availability is compensated by bacteria differentiating into different metabolic states. Currently, the phenomenon of biofilm formation is related mainly to the rapid development of biomaterials engineering (biomedical materials) and their extensive use in various fields of modern medicine (Chung *et al.* 2016; Piperaki *et al.* 2017; Zheng *et al.* 2018; Dsouza *et al.* 2019; Thoraninsdottir *et al.* 2020; Ochońska *et al.* 2021).

Biomaterial surfaces with biofilm formation are an essential reservoir of etiological agents of biomaterials associated infections (BAIs) (Chung *et al.* 2016; Piperaki *et al.* 2017; Thoraninsdottir *et al.* 2020). Targeted at improving patient comfort and function, the comprehensive use of biomaterials contributes to an increase in the frequency of the risk of developing BAIs. It is currently estimated that BAIs are responsible for approximately 65–80% of all infections occurring in humans and animals (Garcia and Percival 2011; Guerra *et al.* 2022). BAIs include device-related and non-device-related infections due to streptococci, staphylococci, Gram-negative bacteria and/or fungal infections (Jamal *et al.* 2018). A study of clinical strains of *K. pneumoniae* reported that 72.7% of tested isolates detected on medical devices were biofilm producers. However, they remained susceptible to different classes of antibiotics (Folliero *et al.* 2021). Another study confirmed the survival of *K. pneumoniae* on dry surfaces in biofilm (Centeleghe *et al.* 2023). The presence of viable but non-culturable (VBNC) bacteria indicated that *K. pneumoniae* could survive on surfaces for up to 4 weeks. It was possible to remove these bacteria from surfaces by mechanical wiping. The study proved the need for robust cleaning regimens in the hospital (Centeleghe *et al.* 2023). Another research showed that 44.4% of the tested clinical strains of *K. pneumoniae* could form biofilm on the surfaces of tracheostomy tubes made of polyethylene and polyvinyl chloride. The biofilms formed on the inner part of these surfaces were observed using scanning electron microscopy (SEM) after only 48 hours of exposure to a bacterial suspension at a concentration of 10^6 CFU/ml (Ochońska *et al.* 2021). A varied degree of biofilm formation by clinical *K. pneumoniae* strains was also found on venous catheters made of polyurethane and urinary catheters made of latex, polyvinyl chloride and silicone. In a study of the penetration of antibiotics (β-lactams, quinolones, aminoglycosides and trimethoprim) and disinfectants (chlorhexidine, ethacridine lactate, hydrogen peroxide, polyhexanidine, povidone-iodine and octenidine) into the biofilm formed by *K. pneumoniae*, the minimum inhibitory concentration (MIC) for bacteria in the biofilm was higher than in the planktonic form (Bartoszewicz *et al.* 2011). Preliminary analyses of the effect of erythromycin on the biofilm formed by *K. pneumoniae* strains showed that macrolides affect the synthesis of the AI-2 autoinducer system, for example, by reducing the expression level of *lux*S genes, blocking the autoinducer synthase enzyme or the signal molecule itself (Martínez and Baquero 2002). The search for agents that would prevent the formation of *K. pneumoniae* biofilm or cause its breakdown is still ongoing, e.g., studies involving attempts to coat catheters with various substances, e.g., silver (Mousavi *et al.* 2023) or to use specific antimicrobial preparations such as octenidine hydrochloride or sodium hypochlorite (Stoffel *et al.* 2020; Huang *et al.* 2022). Attempts are also being made to use some enzymes that eliminate slime or block the metabolic pathways of bacteria, leading to their multiplication and biofilm formation (e.g. DNA-ze, oxindole-L-alanine, a tryptophanase inhibitor regulating the disintegration of tryptophan to indole) (Mączyńska *et al.* 2015). In addition, research is being conducted on regulating *K. pneumoniae* biofilm production. New genes related to this process are being discovered, such as *lux*S – synthesis of autoinducer, *lux*R – coordination of biofilm formation steps, e.g. regulation of synthesis of various virulence factors (including "*quorum sensing*"), *fim*A – regulation of specific adhesion, *mag*A, *rmp*A, *rmp*A2 – regulation of mucus production in hvKp strains (Widmer *et al.* 2007; Mączyńska 2015).

Fimbrial and non-fimbrial adhesins also play a vital role in BAIs by *K. pneumoniae* (Alcántar-Curiel *et al.* 2013). These bacterial structures actively participate in adhesion to epithelial cells, which facilitates colonization, which is the first stage of infection. A thorough understanding of the structures involved in bacterial adhesion and invasion may contribute to the discovery of effective inhibitors of these processes, which will allow for effective treatment at the beginning of the disease, which will enable effective suppression of the disease development (Davies *et al.* 2009; Kalia 2013; Gopu *et al.* 2015; Ribeiro *et al.* 2015; Wang *et al.* 2022).

The main approaches to reduce biofilm development involve modifying the surface of materials to reduce microbial adhesion. In recent years, many research teams have focused on low-molecular-weight compounds (small molecules) capable of inhibiting biofilm development. In a study by Davies and Marques (2009), it was shown that cis-2-decylenic acid, produced by a strain of *Pseudomonas aeruginosa*, can eradicate the mature biofilm of various bacterial species including *K. pneumoniae* (Davies *et al.* 2009). In another study, new chemical entities (NCEs) with activity against *K. pneumoniae* and *Acinetobacter baumannii* could be used in new therapies for drug-resistant infections (Blasco and Piddock

2024). A promising strategy to combat BAIs is application coatings that exhibit bacteriostatic or bactericidal properties (Siddique and Muzammil 2020). Siddique and Muzammil (2020) demonstrated the efficacy of silver nanoparticles (AgNPs) as safe antimicrobial and antibiofilm compounds against MDR *K. pneumoniae* (Siddique and Muzammil 2020). Among the intensively researched biological methods of *K. pneumoniae* biofilm eradication, phagotherapy appears promising (Zurabov *et al.* 2023). Another strategy for biological control of *K. pneumoniae* biofilm is using enzymes targeting the polysaccharide matrix (matrix-targeting enzymes) (Ribeiro *et al.* 2015). Interference with the structure or degradation of the extracellular polymeric matrix of the biofilm can effectively weaken it or lead to its dispersal (Ribeiro *et al.* 2015).

Inhibition of quorum sensing (QS) systems, called Quorum Quenching (QQ), is now considered another promising strategy to combat biofilm-forming bacteria (Kalia 2013). Many substances of natural and synthetic origin with the function of quorum sensing inhibitors (QSIs) have become known and may have potential therapeutic applications. Plant compounds are considered one of the most important groups of QSIs due to their chemical structure similarities to acylated homoserine lactone (AHL) and their ability to degrade protein transcriptional regulators (LuxR/LasR) (Kalia 2013). A study by Gopu *et al.* (2015) showed the quorum quenching activity of anthocyanin malvidin from *Syzygium cumini* (L.) Skeels against *K. pneumoniae* (Gopu *et al.* 2015). Promising results were obtained for two molecules (3-methyl-2(5H)-furanone and 2-hydroxycinnamic acid) that can be developed as a complement to antibiotics (Cadavid and Echeverri 2019). A study by Ahmad *et al.* (2020) attempted to identify new inhibitors of SdiA (a homolog of the transcriptional regulator LuxR) of *K. pneumoniae* using various computational techniques (Ahmad *et al.* 2020). A study by Liu *et al.* (2020) showed that tea polyphenols can act as an effective QS inhibitor, enhance resistance to *K. pneumoniae* infection in a *Caenorhabditis elegans* model, and may serve as a novel antiviral agent to combat bacterial pathogens (Liu *et al.* 2020). A study by Wang *et al.* (2022) showed that chlorogenic acid (CA) may be an effective antimicrobial and antiviral compound that can target QS in hvKp infections, thus providing a new therapeutic direction for treating bacterial infections (Wang *et al.* 2022).

3. Conclusion

K. pneumoniae is an example of a microorganism that has evolved from a common opportunistic microorganism to one of the most dangerous pathogens causing serious healthcare-acquired infections – HAIs. Infections caused by this bacterium are characterized by a severe and progressive course, requiring prolonged hospitalization of patients. They are often challenging to treat due to the ease of acquiring new virulence and antibiotic-resistance traits by *K. pneumoniae.* The accumulation of virulence factors in bacterial strains of *K. pneumoniae* significantly impacts their ability to cause disease and survive in the host. Through mechanisms of horizontal gene transfer, regulation of gene expression, biofilm formation and increased envelope production, these bacteria can effectively evade the host immune system. In addition, the spread of virulence mechanisms is facilitated by the development of civilization and the faster and unrestricted movement of highly virulent *K. pneumoniae* strains. In the face of these threats, knowledge about *K. pneumoniae* should be continuously updated to capture the changing pathogenicity characteristics to prevent future infections.

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