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Influence of biofilm formation on the pathogenicity of *Klebsiella pneumoniae*

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Abstract

Klebsiella pneumoniae, a gram-negative bacterium which was long considered as an opportunistic pathogen has diverged itself to become a serious nosocomial and community transmissible microbe. One reason for this increase in pathogenesis can be attributed to its biofilm formation. Although extensive research has implicated the correlation between the microbe's capacity to form biofilms and how it influences *K. pneumoniae* drug resistance to antimicrobial agents, the mechanism and direct effect of the biofilm on multi-drug resistance is yet to be fully elucidated. This review sheds light on the interplay between biofilms and other virulence factors, highlighting the importance of biofilms in pathogenesis.

Key words: *Klebsiella pneumoniae,* biofilm, antibiotic resistance, capsule, adhesion proteins.

K*lebsiella pneumoniae* is a Gram-negative, encapsulated nosocomial bacterium first isolated in 1882 by Friedländer and named in 1886[6]. It thrives in soil, water, vegetation, and human mucosal surfaces (oropharynx, gastrointestinal tract) and causes infections such as bacteremia, pneumonia, urinary tract infections, meningitis, and surgical site infections.[13,21,28,38,40] Vulnerable groups, including newborns, the elderly, and immuno-compromised individuals, are particularly affected[5,46]. Though the pathogen infects various parts of the human body, its primary infection site is the respiratory tract[5,55]. *K. pneumoniae* strains are categorized as opportunistic, hypervirulent (hyKp), or multidrug-resistant (MDR)[56]. Classic *K. pneumoniae* (cKp) is commonly linked to nosocomial infections. Its epidemiology has evolved significantly, with increasing

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resistance, virulence, and transmissibility leading to its inclusion in the ESKAPE pathogens—six bacteria responsible for most hospital-acquired infections[19,33,42].

Antibiotic resistance, first noted in the 1940s, remains a critical challenge, with multidrug-resistant (MDR) *K. pneumoniae* strains causing severe nosocomial outbreaks and limited treatment options[1,38]. MDRstrains, particularly those producing extended-spectrum beta-lactamases (ESBL) hydrolyse cephalosporins and monobactams but are inhibited by clavulanate[38,47].Further, the detection of hypervirulent strains in community-acquired infections is alarming[9,12,31,48]. In 2019, *K. pneumoniae* was linked to over 600,000 AMR-related deaths, ranking third globally in AMR mortality[35].

A master of biofilm formation, *K. pneumoniae* uses this virulence trait to shield itself from antibiotics and immune responses. Biofilms, bacterial communities embedded in extracellular matrices composed of polysaccharides, proteins, enzymes, and nucleic acids, enable attachment to surface and protect bacteria from external stressors[7,8,29]. Approximately 65-80% of bacterial infections are biofilm-related. Biofilm which are regulated by chromosomally encoded genes protect the bacteria through multiple mechanism including restricted antibiotic penetration, reduced bacterial growth and help in immune evasion[10,16,29,49,56].

Understanding the role of biofilm and its intricate relationship with other virulence factors in providing increased resistance and pathogenicity of *K. pneumonia* strains are of utmost importance as this will reveal strategies to combat the spread of this pathogenic organism. This review will explore into the biofilm formation and its correlation with other virulence factors.

Biofilm formation :

K. pneumoniae has been reported to be able to grow *in vitro* as a biofilm since the end of the 1980s[27]. But clear evidence of an *in vivo* biofilm was provided only in 1992 by Reid et al., who investigated some bladder epithelial cells of a spinal cord injured patient with an asymptomatic urinary tract infection caused by *K. pneumoniae* by scanning electron microscopy[41]. *K. pneumoniae* produces a thick extracellular biofilm that facilitates bacterial adhesion to both living and non-living surfaces. This biofilm acts as a protective barrier, impeding the penetration of antibiotics and thereby diminishing their effectiveness against bacteria[53]. The ability to protect bacteria is due to the composition of the extracellular matrix of the biofilm which is composed of various sugar moieties, proteins, and nucleic acids[30,39]. Studieshave shown that male patients are at a higher risk of *Klebsiella* infection than female patients[2,36]. This increased susceptibility has been linked to lifestyle factors, such as smoking and alcoholism, which are more prevalent among men[37]. Patients under the age of 40 generally have stronger immune systems, creating greater challenges for *K. pneumoniae* to overcome the host's immunity[23]. In contrast, older individuals are at a higher risk of *K. pneumoniae* infection because of the increased prevalence of comorbid conditions associated with aging[32]. Seifi and coworkersobserved that the majority

of *K. pneumoniae* isolates (93.6%) demonstrated biofilm-producing capability, while a minority (6.4%) were non-biofilm producers[46]. This finding highlights the importance of biofilm formation as a key virulence factor in *K. pneumoniae*. Various other virulence factors, including genes coding for capsules, antibiotic resistance genes, and adhesion proteins, have been shown to exert their influence on biofilm formation.

Correlation between capsule and biofilm formation :

Capsular polysaccharides from *K. pneumoniae* not only play a role in biofilm formation but also exhibit anti-biofilm activity against other bacterial species. This ability provides *K. pneumoniae* with a competitive edge in mixed microbial communities, allowing it to dominate environments shared with other bacteria[17]. Several genes involved in capsule production have been shown to positively influence biofilm formation. Insertional mutants of *wza* and *wzc*genes play key roles in capsule formation and impaired biofilm synthesis. Additionally, *treC* mutants showed impaired capsule and biofilm synthesis. In contrast, *sugE* mutants showed elevated biofilm and capsule production which could be related to increased transcription of the *rmpA*gene[57]. In contrast, loss of capsule production of the *wbaP* gene enhanced biofilm formation in isolates, although capsule production was negatively regulated[20]. Previous studies have also reported that capsular formation can diminish the adhesion of bacteria to different substrates and, in turn, reduce biofilm formation[17,45]. The positive and negative correlations between capsule and biofilm formation further highlight the complex interplay between these two heterogeneous structures.

Correlation between adhesion proteins and biofilm formation :

Fimbrial formation, mediated by type 3 fimbriae, is known to aid biofilm formation. Type 3 fimbrial genes have been shown to regulate biofilms in a *K. pneumoniae* strain on a fosmid library screen[50]. Mutants of mrkA have shown an impaired ability to attach to substrates and form reduced biofilms[34]. The FimK protein has been shown to reduce the levels of type 1 fimbriae, thereby abating biofilm formation. Loss of fimK, on the other hand, has shown increased fimbrial and biofilm formation and was shown to efficiently localize on mice urinary tract through increased biofilm[25]. Several other genes have also been implicated in the regulation of biofilm formation. Isogenic mutants of oxyR were highly sensitive to oxidative stress, as well as reduced expression of adhesion proteins and biofilm production, indicating that multiple virulence factors are dependent on the expression of OxyR protein[24]. Transcriptional regulators, such as LysR and LuxR, have been identified as genes which regulate biofilm formation in bacteria based on signature-tagged mutagenesis[22]. LuxS protein, a molecule which plays a key role in quorum sensing, was shown to regulate biofilm formation, as mutants of autoinducer molecules greatly affected the architecture of the biofilm, although there was an apparent increase in biofilm biomass[14].

Correlation between lipopolysaccharide and biofilm formation :

Lipopolysaccharide (LPS), a vital

component of the outer membrane of gram-negative bacteria, such as *K. pneumoniae*, plays a significant role in biofilm formation. Studies, including that by Balestrino and coworkers, have shown that LPS facilitates the initial attachment of *K. pneumoniae* to abiotic surfaces, making it a crucial factor in the early stages of biofilm development[7]. This underscores LPS's dual role of LPS in both the structural integrity and biofilm-related pathogenicity of *K. pneumoniae*. The authors demonstrated that *K. pneumoniae*mutant strains lacking genes involved in LPS biosynthesis (such as the *wbbM*gene) or transport (such as the *wzm* gene) exhibited delayed biofilm formation. The authors hypothesised that the charge of LPS is essential for the proper folding of type 1 pili, which may help explain the impaired biofilm formation observed in these mutants. This suggests that LPS not only contributes to surface attachment but also plays a role in the structural integrity required for effective biofilm development[7,45].

Correlation between antibiotic resistance and biofilm formation :

Extensive studies have been conducted to determine the relationship between antibiotic resistance and biofilm formation. Interestingly, contrasting results were obtained. Anderl and coworkers investigated the penetration of antimicrobials through *K. pneumoniae* biofilms using an *in vitro* model. Their study showed that biofilms resisted killing by ampicillin and ciprofloxacin, despite prolonged exposure[4]. Ampicillin failed to penetrate wild-type biofilms but could infiltrate biofilms formed by β-lactamase-deficient mutants. Conversely, ciprofloxacin and a nonreactive tracer (chloride ion) rapidly diffused through biofilms. The findings demonstrated that the heightened resistance of both wild-type and mutant biofilms was not due to limited antibiotic diffusion but likely arose from other protective mechanisms. Samia and coworkers studied the response of *K. pneumoniae* strains isolated from medical devices to gentamicin, cefotaxime, and ciprofloxacin. They found that strains grown as biofilms were 10–25 times more resistant to these antibiotics than their planktonic counterparts were[43]. A 2012 analysis of 100 urine samples from catheterised UTI patients found that 80% of the biofilm-producing strains exhibited an MDR phenotype. These biofilm-positive isolates showed significantly higher resistance to nalidixic acid (93.3%), ampicillin (83.3%), cefotaxime (73.3%), and co-trimoxazole (80%) than biofilm non-producers, with resistance rates of 70%, 60%, 35%, and 60%, respectively[51].Sanchez and team later confirmed that MDR *K. pneumoniae* strains, especially those resistant to cephalosporins, form richer biofilms compared to susceptible strains[44]. It was emphasized that a strong correlation exists between the biofilm-forming ability of extensively drug-resistant (XDR) *K. pneumoniae* and its antibiotic resistance profile, highlighting biofilm formation as a significant factor in the pathogen's resistance mechanisms[54]. Cepas and colleagues explored the potential relationship between antimicrobial resistance and biofilm formation in *E. coli, K. pneumoniae,* and *P. aeruginosa*. They found no statistically significant link between general multidrug resistance (MDR) and biofilm production across these three gram-negative species. MDR isolates did not exhibit a greater propensity for biofilm formation than non-MDR isolates[11]. However, they identified specific correlations between

biofilm production and antibiotic resistance. Additionally, no statistically significant association between MDR phenotypes, biofilm formation, and ESBLproduction[11,18,52]. For instance, in *E. coli*, resistance to gentamicin and ceftazidime was linked to biofilm formation, whereas in *P. aeruginosa*, ciprofloxacin resistance was correlated with biofilm formation, and resistance to piperacillin-tazobactam and colistin was associated with biofilm production. This is particularly concerning because colistin is considered the last line of treatment for *K. pneumoniae* infection[26]. However, another study found no clear correlation between biofilm formation and clonal types of multidrug-resistant (MDR) bacteria[15]. Similarly, another study suggested that while antibiotic resistance and the ability to form biofilms are significant factors in the global spread of *K. pneumoniae,* the precise relationship between these two elements remains unclear and underexplored[3].

These studies highlight the complexity of the interplay between antibiotic resistance and biofilm formation, underscoring the need for more in-depth research to better understand how these factors contribute to bacterial pathogenicity and spread.

K. pneumoniae causes both community and hospital-acquired infections, with multidrug-resistant and biofilm-producing strains worsening patient outcomes. Biofilm formation, associated with 60-80% of bacterial infections, helps the pathogen evade immune responses and resist antibiotics, complicating treatment. Understanding the molecular mechanisms of biofilm formation and its link to antibiotic resistance is essential for new drug development. Key factors in biofilm formation include fimbriae, polysaccharides, quorum sensing, and efflux pumps. While drug-resistant strains often form stronger biofilms, the relationship is not always consistent. Novel therapies such as antibiotic combinations, antimicrobial peptides, nanoparticles, and phage therapy show promise, but further *in vivo* research and clinical trials are needed for wider application.

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