https://doi.org/10.48047/AFJBS.6.2.2024.4200-4207

African Journal of Biological Sciences

Journal homepage: http://www.afjbs.com

Research Paper Open Access

Emergence of Pseudomonas aeruginosa with Difficult-to-Treat Resistance

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Article History

Volume 6, Issue 2, Apr-Aug 2024

Received: 1 August 2024

Accepted: 15 August 2024

Published: 17 August 2024

doi:10.48047/AFJBS.6.2.2024.4200-4207

Abstract: Antimicrobial resistance (AMR) is a critical global health threat, with a disproportionate burden on low- and middle-income countries where resistant infections occur at rates up to 60%, compared to 17% in high-income countries. Among multidrug-resistant (MDR) pathogens, *Pseudomonas aeruginosa* has emerged as a significant challenge, particularly in intensive care units (ICUs), where critically ill patients face heightened risks of infection due to invasive procedures, prolonged hospital stays, and extensive antibiotic use. This opportunistic Gram-negative pathogen causes severe infections, including respiratory tract infections, urinary tract infections, hospital-acquired pneumonia, and bacteremia, often resulting in treatment failures due to its intrinsic and acquired resistance mechanisms. The emergence of difficult-to-treat resistance (DTR) in *P. aeruginosa* is driven by diverse mechanisms, such as altered outer membrane permeability, efflux pump overexpression, antibiotic-inactivating enzymes, and biofilm formation. These mechanisms not only limit the efficacy of existing antimicrobial agents but also complicate therapeutic decision-making, especially for extensively drug-resistant (XDR) and pan-drug-resistant (PDR) strains. Recent advances in research highlight the potential of combining antibacterial agents with antibiofilm strategies to overcome these challenges, offering promising results in preclinical studies. This review examines the molecular underpinnings of DTR in *P. aeruginosa*, its clinical and epidemiological impact, and the innovative therapeutic approaches under development. By emphasizing the need for improved surveillance, targeted antimicrobial stewardship, and novel treatment strategies, this article underscores the urgent global effort required to combat the threat posed by DTR *P. aeruginosa* infections.

Keywords: *Pseudomonas aeruginosa, Resistance*

Introduction.

Pseudomonas aeruginosa, a Gram-negative, opportunistic pathogen, has emerged as a major cause of healthcare-associated infections (HAIs) worldwide, particularly in immunocompromised patients. It is a frequent cause of pneumonia, bloodstream infections, urinary tract infections, and surgical site infections, often associated with high morbidity and mortality rates. The bacterium's intrinsic resistance to many antibiotics, coupled with its ability to acquire additional resistance mechanisms, makes it a challenging pathogen to treat in clinical settings [1,2].

The intrinsic resistance of *P. aeruginosa* is largely attributed to its low outer membrane permeability and the presence of efflux pumps, which actively expel antibiotics from the bacterial cell. These mechanisms are complemented by adaptive resistance, such as biofilm formation, which shields bacterial communities from antibiotics and the host immune response. Biofilms are particularly problematic in chronic infections, such as those in cystic fibrosis patients, where they contribute to persistent and difficult-to-treat infections [3,4].

A growing concern is the rise of multidrug-resistant (MDR), extensively drug-resistant (XDR), and pan-drug-resistant (PDR) strains of *P. aeruginosa*. These strains exhibit resistance to nearly all available antibiotic classes, including β-lactams, aminoglycosides, and fluoroquinolones, leaving few therapeutic options for infected patients. This resistance has been linked to the acquisition of mobile genetic elements, such as plasmids and integrons, which carry resistance genes [5].

In healthcare settings, intensive care units (ICUs) are hotspots for *P. aeruginosa* infections. ICU patients are particularly vulnerable due to invasive procedures, mechanical ventilation, and prolonged hospital stays, which increase the risk of infection. Additionally, the widespread use of broad-spectrum antibiotics in these settings creates selective pressure that promotes the emergence of resistant *P. aeruginosa* strains [6].

Diagnostic delays often exacerbate the management of *P. aeruginosa* infections. Conventional diagnostic techniques are time-consuming and may fail to identify resistance patterns promptly. Rapid molecular diagnostics that detect specific resistance genes or mechanisms offer promise but are not yet widely implemented, particularly in low-resource settings where the burden of antimicrobial resistance is greatest [7].

Treatment of *P. aeruginosa* infections increasingly relies on combination therapy and novel therapeutic approaches. Strategies include the use of β-lactam/β-lactamase inhibitor combinations, polymyxins, and adjunctive therapies such as bacteriophage therapy. Experimental approaches targeting biofilm disruption, quorum sensing, and efflux pumps have shown promise in preclinical studies but require further clinical evaluation [8,9].

Addressing the global challenge posed by *P. aeruginosa* requires a multifaceted approach. Enhanced surveillance systems, antimicrobial stewardship programs, and the development of novel therapeutic agents are critical to mitigating its impact. Research into innovative diagnostic tools and treatment modalities, along with improved understanding of resistance mechanisms and host-pathogen interactions, will be pivotal in combating this formidable pathogen [10].

A significant research gap in the study of *Pseudomonas aeruginosa* with difficult-to-treat resistance (DTR) lies in understanding the dynamic interplay between biofilm-associated resistance mechanisms and the host immune response in clinical settings. While the role of biofilm formation in antimicrobial resistance is well-documented, there is limited knowledge about how biofilms interact with the immune system under different host conditions, particularly in critically ill or immunocompromised patients.

Furthermore, while combination therapies involving antibiofilm and antimicrobial agents have shown promise in vitro, their effectiveness and safety in clinical settings remain underexplored. There is a need for robust

clinical trials to assess these strategies in real-world scenarios, especially in ICU environments where the burden of *P. aeruginosa* infections is highest.

Additionally, the development and validation of rapid, cost-effective diagnostic tools for detecting DTR *P. aeruginosa* and its associated resistance mechanisms represent another critical gap. Such tools could enable timely, targeted interventions, particularly in resource-limited settings where AMR surveillance and management are challenging.

Addressing these gaps would enhance the understanding of resistance dynamics, improve patient outcomes, and inform the development of innovative therapeutic and diagnostic approaches.

The Major Pathogenesis Factors of *Pseudomonas aeruginosa* and Therapeutics

Pseudomonas aeruginosa is a versatile pathogen with a broad arsenal of virulence factors contributing to its pathogenesis. Among these, its ability to adhere to and invade host tissues is central to establishing infection. This is facilitated by pili and flagella, which allow attachment to epithelial cells, and the secretion of enzymes such as elastases and proteases that degrade host tissues and evade immune responses [11]. The pathogen's adaptability to diverse environments further enhances its ability to colonize and persist in both the host and healthcare environments, making it a formidable threat in clinical settings [12].

A hallmark of *P. aeruginosa* infections is its production of biofilms, which are structured communities of bacteria encased in an extracellular polymeric matrix. Biofilm formation provides protection against antimicrobial agents and the host immune system, leading to chronic and recurrent infections. This property is particularly significant in medical device-related infections, such as those involving catheters and ventilators, where biofilms contribute to persistent inflammation and delayed healing [13].

Toxin production is another major virulence factor in *P. aeruginosa*. The bacterium secretes a variety of exotoxins, including exotoxin A, which inhibits protein synthesis in host cells, and pyocyanin, a redox-active pigment that induces oxidative stress and damages host tissues. These toxins contribute to the severity of infections and are particularly problematic in immunocompromised patients [14].

In addition to toxins, *P. aeruginosa* produces effector proteins delivered via the type III secretion system (T3SS), a needle-like apparatus that injects toxins directly into host cells. These proteins disrupt cellular signaling, impair immune responses, and promote tissue invasion. The T3SS is associated with acute infections and is a key target for therapeutic intervention [15].

Therapeutic strategies for *P. aeruginosa* infections face significant challenges due to the bacterium's intrinsic and acquired resistance mechanisms. Current treatments often rely on combination therapy, using antibiotics such as β-lactams, aminoglycosides, and polymyxins to overcome resistance. However, the emergence of multidrug-resistant (MDR) and extensively drug-resistant (XDR) strains has prompted the need for alternative approaches, including the use of β-lactamase inhibitors and synergistic drug combinations [16].

Novel therapeutic options targeting *P. aeruginosa* virulence factors are gaining traction. Anti-biofilm agents, such as quorum sensing inhibitors, aim to disrupt bacterial communication and biofilm integrity. Similarly, inhibitors of the T3SS have shown promise in preclinical studies as a means to neutralize the pathogen's virulence without exerting selective pressure for resistance. These approaches represent a shift toward antivirulence strategies that disarm the pathogen rather than kill it directly [17].

Phage therapy, which uses bacteriophages to target and lyse *P. aeruginosa*, has also emerged as a potential treatment option. Phages are highly specific to their bacterial targets and can penetrate biofilms, making them particularly useful in treating chronic infections. Additionally, ongoing research into immunotherapy and host-directed therapies aims to enhance the host immune response against *P. aeruginosa*, offering a promising avenue for combating this challenging pathogen [18].

Pseudomonas aeruginosa Biofilm

Pseudomonas aeruginosa biofilms are one of the most critical factors contributing to its persistence and resistance in both environmental and clinical settings. Biofilms are structured microbial communities encased in an extracellular polymeric matrix (EPM) that adheres to surfaces or host tissues. This matrix is composed of

polysaccharides, proteins, and extracellular DNA, which provide a protective barrier against antimicrobial agents and the host immune system. The ability of *P. aeruginosa* to form biofilms allows it to thrive in harsh environments and significantly contributes to its role in chronic infections [19].

The biofilm life cycle consists of several stages: initial attachment, microcolony formation, maturation, and dispersion. Initial attachment is mediated by surface structures such as type IV pili and flagella, which enable motility and adhesion. As the biofilm matures, the bacteria produce EPM, which stabilizes the biofilm structure and promotes community resilience. Dispersion, the final stage, allows bacterial cells to detach and colonize new sites, facilitating the spread of infection [20].

Biofilm-associated *P. aeruginosa* infections are notoriously difficult to treat due to their inherent resistance to antibiotics. The EPM acts as a physical barrier that limits antibiotic penetration, while the cells within the biofilm exhibit a distinct metabolic state that reduces their susceptibility to antimicrobial agents. Additionally, the close proximity of bacterial cells within the biofilm promotes horizontal gene transfer, enabling the rapid spread of resistance genes [21].

In clinical settings, biofilm formation by *P. aeruginosa* is particularly problematic in infections associated with medical devices such as catheters, ventilators, and prosthetic implants. These biofilm-associated infections are often chronic, leading to prolonged hospital stays and increased healthcare costs. Furthermore, biofilm formation in the lungs of cystic fibrosis patients contributes to persistent respiratory infections and progressive lung damage, significantly affecting patient outcomes [22].

The regulation of biofilm formation in *P. aeruginosa* is controlled by complex signaling pathways, including quorum sensing (QS). QS involves the production and detection of small signaling molecules called autoinducers, which coordinate gene expression within the bacterial community. QS-regulated genes influence biofilm development, virulence factor production, and antibiotic resistance, making QS a critical target for therapeutic intervention [23].

Therapeutic strategies targeting *P. aeruginosa* biofilms are an area of active research. Approaches include the use of biofilm-disrupting agents, such as DNases and dispersin B, which degrade components of the EPM, as well as QS inhibitors that block bacterial communication. Combination therapies involving antibiotics and anti-biofilm agents have shown promise in preclinical studies, highlighting the potential of synergistic treatments to improve patient outcomes [24].

Phage therapy has also demonstrated potential in targeting *P. aeruginosa* biofilms. Bacteriophages can penetrate the biofilm matrix and specifically target bacterial cells, making them an attractive option for treating chronic biofilm-associated infections. Additionally, ongoing research into novel materials for medical devices that resist biofilm formation offers a promising avenue for preventing *P. aeruginosa* infections in healthcare settings [25].

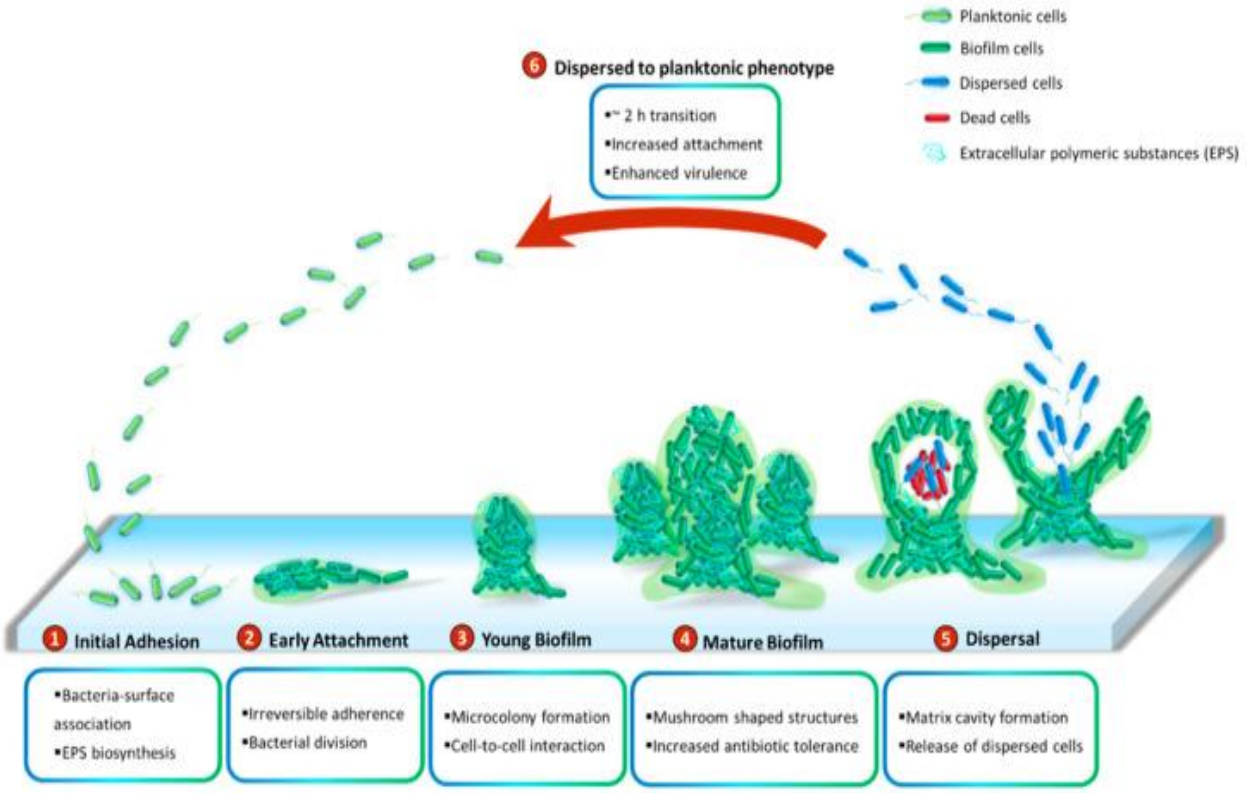

Figure 1. Cycle of *Pseudomonas aeruginosa* biofilm development.

Emergence of *Pseudomonas aeruginosa* with Difficult-to-Treat Resistance

The emergence of *Pseudomonas aeruginosa* strains with difficult-to-treat resistance (DTR) has become a significant concern in global healthcare. DTR refers to strains that exhibit resistance to almost all available antimicrobial agents, leaving limited therapeutic options. This phenomenon is driven by the pathogen's intrinsic resistance mechanisms and its ability to acquire additional resistance determinants through horizontal gene transfer. These DTR strains are associated with severe infections, including hospital-acquired pneumonia, bloodstream infections, and surgical site infections, often leading to treatment failure and poor patient outcomes [26].

One of the main contributors to DTR in *P. aeruginosa* is its ability to produce β-lactamases, including carbapenemases such as metallo-β-lactamases (MBLs). These enzymes inactivate β-lactam antibiotics, including carbapenems, which are often considered last-resort treatments for Gram-negative infections. The widespread dissemination of carbapenemase genes, often carried on mobile genetic elements like plasmids and transposons, has facilitated the rapid spread of resistance among *P. aeruginosa* strains globally [27].

Efflux pumps also play a critical role in DTR. *P. aeruginosa* possesses multiple efflux pump systems, such as MexAB-OprM, which actively expel antibiotics from the bacterial cell, reducing their intracellular concentrations. Overexpression of these pumps is commonly observed in resistant strains and is associated with resistance to multiple antibiotic classes, including fluoroquinolones, aminoglycosides, and β-lactams. This multidrug efflux capability is a key factor in the pathogen's ability to withstand diverse antimicrobial agents [28].

Additionally, alterations in porin proteins, such as OprD, further contribute to DTR. Porins are channels that facilitate the entry of antibiotics into the bacterial cell. Loss or downregulation of OprD reduces the uptake of

carbapenems, resulting in high-level resistance. This mechanism, in combination with efflux pumps and β-lactamases, creates a formidable resistance barrier that is difficult to overcome with current therapies [29].

DTR *P. aeruginosa* is particularly problematic in intensive care units (ICUs), where critically ill patients are at heightened risk of infection due to invasive procedures, mechanical ventilation, and prolonged antibiotic exposure. These factors create selective pressure for the emergence and persistence of resistant strains. Infections caused by DTR *P. aeruginosa* are associated with increased morbidity, mortality, and healthcare costs, underscoring the urgent need for effective prevention and treatment strategies [30].

Current treatment options for DTR *P. aeruginosa* are limited, often relying on polymyxins, such as colistin, as a last resort. However, polymyxin resistance is also emerging, driven by modifications to the bacterial outer membrane that reduce drug binding. Novel therapeutic approaches are being explored, including the use of β-lactam/β-lactamase inhibitor combinations, synergistic drug therapies, and adjunctive treatments such as bacteriophage therapy and immunomodulators [31].

The emergence of DTR *P. aeruginosa* highlights the critical need for improved antimicrobial stewardship, enhanced surveillance systems, and investment in the development of new therapeutic agents. Strategies to combat this threat should include targeting resistance mechanisms, disrupting biofilm formation, and addressing the underlying factors driving resistance, such as the misuse of antibiotics. Collaborative global efforts are essential to mitigate the impact of this challenging pathogen on public health [32].

Mechanisms of Resistance to Antibiotics in *Pseudomonas aeruginosa*

Pseudomonas aeruginosa is a highly adaptable pathogen capable of resisting a wide range of antibiotics through intrinsic, acquired, and adaptive mechanisms. Intrinsic resistance arises from the bacterium's structural and functional characteristics, such as low outer membrane permeability, the presence of efflux pumps, and the production of antibiotic-inactivating enzymes. These mechanisms provide a baseline resistance to many antibiotics and make *P. aeruginosa* inherently difficult to treat [33].

One of the most significant mechanisms of resistance in *P. aeruginosa* is the production of β-lactamases, which hydrolyze β-lactam antibiotics, rendering them ineffective. These include carbapenemases, such as metallo-β-lactamases (MBLs), which degrade carbapenems, a class of last-resort antibiotics. Genes encoding these enzymes are often carried on mobile genetic elements, facilitating their horizontal transfer among bacterial populations and contributing to the global spread of resistance [34].

Efflux pumps are another critical component of *P. aeruginosa*'s resistance arsenal. These transport proteins actively expel antibiotics and other toxic compounds from the bacterial cell, reducing their intracellular concentration. The MexAB-OprM efflux pump system, for instance, is responsible for resistance to multiple classes of antibiotics, including β-lactams, quinolones, and aminoglycosides. Overexpression of efflux pumps is frequently observed in multidrug-resistant (MDR) strains [35].

Alterations in porin channels, such as the loss or downregulation of OprD, also play a pivotal role in resistance. Porins are proteins in the outer membrane that facilitate the uptake of small molecules, including antibiotics. The absence of functional OprD reduces the entry of carbapenems into the bacterial cell, leading to carbapenem resistance. This mechanism often acts in conjunction with other resistance mechanisms, compounding the challenge of treatment [36].

Biofilm formation is another key resistance mechanism in *P. aeruginosa*. Bacteria within biofilms are encased in an extracellular polymeric matrix that provides a physical barrier to antibiotics and the host immune response. Biofilms also promote a metabolically dormant state, reducing the efficacy of antibiotics that target actively dividing cells. This resistance is particularly problematic in chronic infections and medical device-associated infections [37].

Adaptive resistance mechanisms, such as the activation of stress response pathways, further enhance *P. aeruginosa*'s ability to survive antimicrobial treatments. For example, the activation of the AmpC pathway in response to β-lactam antibiotics leads to the overproduction of β-lactamase enzymes. Similarly, exposure to

sub-inhibitory concentrations of antibiotics can induce the expression of resistance genes and efflux pump systems, allowing the bacterium to survive under selective pressure [38].

The emergence of resistance in *P. aeruginosa* highlights the need for innovative approaches to counteract these mechanisms. Strategies such as combining antibiotics with efflux pump inhibitors, developing molecules that target specific resistance pathways, and using biofilm-disrupting agents are under investigation. Understanding the molecular basis of these resistance mechanisms is essential for the development of novel therapies to combat this highly resistant pathogen [39].

References

1. Centers for Disease Control and Prevention (CDC). Antibiotic Resistance Threats in the United States, 2019. Atlanta, GA: CDC; 2019.
2. Breidenstein EB, de la Fuente-Núñez C, Hancock RE. Pseudomonas aeruginosa: all roads lead to resistance. Trends Microbiol. 2011;19(8):419-426.
3. Lambert PA. Mechanisms of antibiotic resistance in Pseudomonas aeruginosa. J R Soc Med. 2002;95(Suppl 41):22-26.
4. Ciofu O, Tolker-Nielsen T, Jensen PØ, Wang H, Høiby N. Antimicrobial resistance, respiratory infections and role of biofilms in lung infections in cystic fibrosis patients. Adv Drug Deliv Rev. 2015;85:7-23.
5. Pitout JD, Nordmann P, Poirel L. Carbapenemase-producing Pseudomonas aeruginosa: a major threat to our health care systems. Clin Microbiol Rev. 2015;28(4):887-922.
6. Kollef MH, Golan Y, Micek ST, Shorr AF, Restrepo MI. Appraising contemporary strategies to combat multidrug-resistant Gram-negative bacterial infections–proceedings and data from the Gram-Negative Resistance Summit. Clin Infect Dis. 2011;53(Suppl 2):S33-S55.
7. van Belkum A, Dunne WM. Next-generation antimicrobial susceptibility testing. J Clin Microbiol. 2013;51(7):2018-2024.
8. Bassetti M, Righi E, Carnelutti A. Multidrug-resistant Pseudomonas aeruginosa: challenges and treatment options. Expert Rev Anti Infect Ther. 2018;16(8):601-608.
9. Tiwari V, Tiwari M. Phage therapy to combat multidrug-resistant Pseudomonas aeruginosa infections. Expert Rev Anti Infect Ther. 2020;18(9):831-835.
10. Tacconelli E, Carrara E, Savoldi A, et al. Discovery, research, and development of new antibiotics: the WHO priority list of antibiotic-resistant bacteria and tuberculosis. Lancet Infect Dis. 2018;18(3):318-327.
11. Gellatly SL, Hancock RE. Pseudomonas aeruginosa: new insights into pathogenesis and host defenses. Pathog Dis. 2013;67(3):159-173.
12. Moradali MF, Ghods S, Rehm BH. Pseudomonas aeruginosa lifestyle: a paradigm for adaptation, survival, and persistence. Front Cell Infect Microbiol. 2017;7:39.
13. Bjarnsholt T. The role of bacterial biofilms in chronic infections. APMIS Suppl. 2013;136:1-51.
14. Rybtke M, Hultqvist LD, Givskov M, Tolker-Nielsen T. Pseudomonas aeruginosa biofilm infections: community structure, antimicrobial tolerance, and immune response. J Mol Biol. 2015;427(23):3628-3645.
15. Hauser AR. The type III secretion system of Pseudomonas aeruginosa: infection by injection. Nat Rev Microbiol. 2009;7(9):654-665.
16. Bassetti M, Righi E, Vena A, Graziano E. New therapeutic options for Pseudomonas aeruginosa infections in the age of multidrug resistance. Curr Opin Infect Dis. 2018;31(6):492-502.
17. Rutherford ST, Bassler BL. Bacterial quorum sensing: its role in virulence and possibilities for its control. Cold Spring Harb Perspect Med. 2012;2(11):a012427.

18. Forti F, Roach DR, Cafora M, et al. Design of therapeutic phage cocktails to combat multidrug-resistant Pseudomonas aeruginosa. Cell Rep. 2018;24(3):338-348.
19. Costerton JW, Stewart PS, Greenberg EP. Bacterial biofilms: a common cause of persistent infections. Science. 1999;284(5418):1318-1322.
20. Flemming HC, Wingender J. The biofilm matrix. Nat Rev Microbiol. 2010;8(9):623-633.
21. Stewart PS, Franklin MJ. Physiological heterogeneity in biofilms. Nat Rev Microbiol. 2008;6(3):199-210.
22. Elborn JS. Cystic fibrosis. Lancet. 2016;388(10059):2519-2531.
23. Williams P, Cámara M. Quorum sensing and environmental adaptation in Pseudomonas aeruginosa. Nat Rev Microbiol. 2009;7(2):131-140.
24. Wu H, Moser C, Wang HZ, Høiby N, Song ZJ. Strategies for combating bacterial biofilm infections. Int J Oral Sci. 2015;7(1):1-7.
25. Pires DP, Cleto S, Sillankorva S, Azeredo J, Lu TK. Genetically engineered phages: a review of advances over the last decade. Microbiol Mol Biol Rev. 2016;80(3):523-543.
26. Bassetti M, Peghin M, Vena A, Giacobbe DR. Treatment of infections due to MDR Gram-negative bacteria. Front Med (Lausanne). 2019;6:74.
27. Livermore DM. Current epidemiology and growing resistance of Gram-negative pathogens. Korean J Intern Med. 2012;27(2):128-142.
28. Poole K. Efflux-mediated antimicrobial resistance. J Antimicrob Chemother. 2005;56(1):20-51.
29. Hocquet D, Vogne C, El Garch F, Vejux A, Gotoh N, Lee A, et al. Mex efflux pumps control resistance emergence in Pseudomonas aeruginosa submitted to sub-inhibitory doses of antibiotics. PLoS Pathog. 2011;7(6):e1002106.
30. Lister PD, Wolter DJ, Hanson ND. Antibacterial-resistant Pseudomonas aeruginosa: clinical impact and complex regulation of chromosomally encoded resistance mechanisms. Clin Microbiol Rev. 2009;22(4):582-610.
31. Tamma PD, Aitken SL, Bonomo RA, Mathers AJ, van Duin D, Clancy CJ. Infectious Diseases Society of America Guidance on the treatment of AmpC β-lactamase-producing Enterobacterales, carbapenem-resistant Acinetobacter baumannii, and Pseudomonas aeruginosa with difficult-to-treat resistance. Clin Infect Dis. 2021;72(2):e169-e183.
32. Tacconelli E, Carrara E, Savoldi A, et al. Discovery, research, and development of new antibiotics: the WHO priority list of antibiotic-resistant bacteria and tuberculosis. Lancet Infect Dis. 2018;18(3):318-327.
33. Lambert PA. Mechanisms of antibiotic resistance in Pseudomonas aeruginosa. J R Soc Med. 2002;95(Suppl 41):22-26.
34. Livermore DM. Current epidemiology and growing resistance of Gram-negative pathogens. Korean J Intern Med. 2012;27(2):128-142.
35. Poole K. Efflux-mediated antimicrobial resistance. J Antimicrob Chemother. 2005;56(1):20-51.
36. Hocquet D, Vogne C, El Garch F, et al. Mex efflux pumps control resistance emergence in Pseudomonas aeruginosa submitted to sub-inhibitory doses of antibiotics. PLoS Pathog. 2011;7(6):e1002106.
37. Stewart PS, Franklin MJ. Physiological heterogeneity in biofilms. Nat Rev Microbiol. 2008;6(3):199-210.
38. Quale J, Bratu S, Gupta J, Landman D. Interplay of efflux system overexpression and mutations in Pseudomonas aeruginosa isolates resistant to carbapenems. Antimicrob Agents Chemother. 2006;50(5):1633-1641.
39. Zgurskaya HI, López CA, Gnanakaran S. Permeability barrier of Gram-negative cell envelopes and approaches to bypass it. ACS Infect Dis. 2015;1(11):512-522.