



## Challenges and strategies in drug delivery systems for treatment of pulmonary infections



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### ARTICLE INFO

#### Keywords:

Drug delivery  
Nanomedicine  
Nanoparticles  
Anti-infectives  
Antibiotics  
Pulmonary infections  
Quorum sensing inhibitor  
*Pseudomonas aeruginosa*  
Biological barriers  
Biofilm  
Mucus

### ABSTRACT

Inhalation therapy has been reported as the most effective treatment for respiratory bacterial infections due to the increasing relevance of drug bioavailability. Drug delivery systems (DDS) have the capacity to overcome pulmonary biological barriers limiting the bioavailability of inhaled anti-infectives. This is important to eradicate bacterial infections and to prevent the development of bacterial resistance. Despite substantial efforts in the field, the current state-of-the-art often fails to achieve those goals, and we still observe increasing bacterial resistance. We give a brief insight on benefits and challenges in pulmonary delivery of anti-infectives. In the context of drug delivery development for pulmonary infections, particularly focusing on *Pseudomonas aeruginosa* (PA) infections, this mini review will critically discuss the main requirements, as well as the recent strategies of drug delivery system synthesis and preparation. Finally, interaction of DDS with crucial pulmonary biological barriers will be of great importance for the success of future applications of the developed DDS.

### 1. Introduction: Antimicrobial resistance and benefits of local delivery

Bacteria are present all around us. Most of them, e.g. bacteria in the intestines, are harmless and actually helpful; while others can cause infections, once they enter and colonize the host. Bacterial infectious diseases in humans, caused by dangerous pathogens, e.g. *Staphylococcus* [1,2], *Enterococcus* [3], or *Pseudomonas aeruginosa* (PA) [4,5], account for a significant proportion of global mortality [6–9]. In most cases, infected patients are treated with powerful antibiotics that are generally safe for fighting infectious diseases. Antibiotics are most preferably administered orally and/or intravenously [10,11]. For treatment of chronic infections, in particular, administration of high doses of antibiotics is frequently employed. Despite good therapeutic efficacy against infection, systemic delivery of antibiotics has some

disadvantages:

- (i) Adverse drug effects as well as cumulative and acute toxicity might occur with repeated use of antibiotics at high doses [12,13]. An example would be that repeated high doses of tobramycin can cause acute/chronic toxicity, in particular by reduction in glomerular filtration [14–16].
- (ii) Unnecessary use or accumulation of antibiotics in body sites without infection by harmful organisms (e.g., impacting the normal bacterial population of the colon) could lead to development of antibiotic resistance, further reducing effectiveness of antibiotics against bacteria [17].
- (iii) Most importantly, poor bioavailability of antibiotics in the infected region, which leads to sub-minimum inhibitory concentrations (MIC), can cause fast development of resistance [17,18].

**Abbreviations:** DDS, Drug delivery systems; MIC, minimum inhibitory concentration; PA, *Pseudomonas aeruginosa*; PBCS, pulmonary biopharmaceutics classification systems; QSI, quorum sensing inhibitors; APIs, active pharmaceutical ingredients; LC%, loading capacity; EE%, encapsulation efficacy; NPs, nanoparticles; PLGA, poly lactic-co-glycolic acid; wt%, weight percentage; MBEC, minimum biofilm eradicating concentration; PEG, polyethylene glycol; CF, cystic fibrosis

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<https://doi.org/10.1016/j.ejpb.2019.09.002>

Received 23 May 2019; Received in revised form 23 August 2019; Accepted 3 September 2019

Available online 04 September 2019

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**Table 1**  
Timeline of the discovery, introduction and resistance observed of antibiotics [17,22,23].

Antibiotic class	Year of discovery	Year of introduction	Year of resistance observed
$\beta$ -Lactams	1928	1938	1945
Sulfa drugs	1932	1936	1942
Aminoglycosides	1943	1946	1946
Tetracyclines	1944	1952	1950
Chloramphenicols	1946	1948	1955
Macrolides	1948	1951	1955
Fidaxomicin	1948	2011	1977
Glycopeptides	1953	1958	1960
Oxazolidinones	1955	2000	2001
Rifamycins	1957	1958	1962
Quinolones	1961	1968	1968
Streptogramins	1963	1998	1964
Lipopeptides	1986	2003	1987
Diarylquinolines	1997	2012	2006

While adverse drug effects may frequently be avoided by proper prescription of antibiotics [17,19], antimicrobial resistance has become one of the most pressing health threats. Infections from resistant bacteria are now too common, and some pathogens have even become resistant to multiple classes of antibiotics [7,17,20]. Table 1 summarizes the timeline of the discovery, introduction and observed resistance for antibiotic classes used to treat infections. Few new drug classes have been discovered and approved for clinical use since the discovery of penicillin, a member of the  $\beta$ -lactam class. In most cases, antimicrobial resistance has been observed shortly after the discovery of antibiotics, in some cases even before the year of introduction [21–23]. This problem might be primarily caused by incorrect and uncontrolled utilization of these antibiotics.

Gram-negative pathogens pose particular bacterial resistance problems. The most severe gram-negative infections and common pathogens are *Enterobacteriaceae*, *Pseudomonas aeruginosa* (PA), and *Acinetobacter*, as these include strains that are becoming resistant to nearly all anti-infective drugs that would be considered for treatment [7]. The same holds true, at least to some extent, for some of the gram-positive pathogens, e.g. *Staphylococcus* and *Enterococcus* [6]. The challenges in combatting gram-negative pathogens are: (i) they are highly efficient at keeping out drugs using their naturally sophisticated cell-wall structure. On the one hand, the outer membrane is a barrier for amphiphilic compounds which are usually necessary for water-solubility and penetrating the cytoplasmic membrane [24,25]. Multidrug-resistant pumps, in addition, efflux a wide variety of compounds that cross the outer membrane and chemically recognize molecules based mainly on polarity, preferring amphiphilic molecules [26,27]. On the other hand, penetration of hydrophilic actives is restricted by the inner membrane [25,28]. (ii) Moreover, bacterial mutations which modify proteins targeted by antibiotics cause inactivation of antimicrobial agents [29]. Mutations may also produce antibiotic-degrading and/or antibiotic-inactivating enzymes which could also account for resistance development [20,30]. Consequently, these natural biological properties create barriers that help gram-negative bacteria to become resistant.

As indicated in Fig. 1, the site of action of currently approved antibiotics is either located at the bacterial cell envelope or somewhere in the bacterial cytoplasm [9,22,31]. It is hence important that the antimicrobial molecules reach their target at high concentration to overcome potentially low drug susceptibility. As a result, topical delivery of antibiotic, e.g. in case of inhalation aerosols for the treatment of pulmonary infections, may be postulated to have two major advantages: First, higher drug concentrations at the site of infection will lead to more effective bacterial killing and decrease the risk of resistance development. Secondly, reduced exposure at non-infected body sites will reduce the risk of adverse drug effects, in particular compared to

systemic drug delivery [32–34].

## 2. Challenges to treatment of pulmonary infections

The opportunistic bacterial pathogen *Pseudomonas aeruginosa* is one of the leading causes of nosocomial pulmonary infections worldwide [35]. This pathogen is ranked second most prevalent among gram-negative pathogens reported to the National Nosocomial Infection Surveillance System [17,36]. Besides causing acute infections, PA is also accountable for debilitating chronic lung infections in immunocompromised patients. It is the most commonly isolated pathogen from cystic fibrosis sufferers, and considered the leading cause for morbidity and mortality in such patients [37,38]. Inhalation therapy has been reported as the most effective treatment for those mentioned respiratory bacterial infections, affording direct delivery to the disease site [34,37]. As a result, inhalation of antibiotics has been reported to reduce the frequency of exacerbations, decrease significantly airway bacterial density, recover pulmonary function, and most importantly improve quality of life for patients with pulmonary infection [39]. However, the current inhaled antibiotics are not showing maximum therapeutic efficacy to eradicate bacterial infection, as they still face some limitations and challenges:

- (i) Enhancing bioavailability of inhaled drugs from the lungs, however, still requires sufficient drug water solubility [40]. This is underlined by the fact that the total volume of the pulmonary lining fluid is small (ca. 150 mL) and distributed as a thin liquid film, (not more than 30  $\mu$ m) over the large epithelial surface area (140–160 m<sup>2</sup>) [41–43]. At the same time, the relatively low potency of current inhaled anti-infectives requires delivery of rather high doses (up to several 100 mg). For this reason, water solubility of inhaled anti-infectives is essential and highlights the need to design strategies to enhance water solubility significantly. This objective is the subject of ongoing discussions in the context of a pulmonary biopharmaceutics classification system (PBCS) [40].
- (ii) It is also essential to maintain the concentration of drug in the pulmonary lining fluid compartment above the MIC as long as possible [44]. This goal may be frustrated by systemic absorption across the air-blood barrier, and also by the efficient clearance mechanisms of the lungs, e.g. mucociliary [45] and macrophage clearance [46]. Furthermore, repeated use of high doses of antibiotics without controlled release and without specific targeting to the disease site could also permit toxicity to healthy lung cells [47,48].
- (iii) Recent clinical studies have revealed that current inhaled antibiotic formulations could only be fully proficient in terminating spread of the pathogen and reducing demolition of the airway tissues [49], not in fully eradicating the infection. As a gram-negative pathogen, PA is naturally resistant to many antibiotics for the reasons mentioned above. Moreover, pulmonary PA infections are complicated by the formation of PA biofilms. The latter are multi-cellular surface-attached and spatially oriented bacterial communities (described in Fig. 2), composed of bacterial cells in high metabolic outer regions and low metabolic/persisters central regions which are crucially accountable for the development of PA resistance [50–52]. As discussed in Fig. 1, antibiotics exert their mechanisms of action most efficiently on metabolically active bacterial cells [22]; as a result, the persisters bacterial cells in the dormant regions of biofilms foster biofilm survival and recurrent infections. Furthermore, the extracellular matrix in a biofilm, which is mainly composed of alginate, extracellular polymers, lipids and DNA, is a significant barrier to penetration of antimicrobial agents [53–55]. For instance, the effectiveness of aminoglycosides, in particular tobramycin (a positively charged antibiotic, widely used as first-line therapy in CF-related infections) has been shown to be decreased by strong interactions

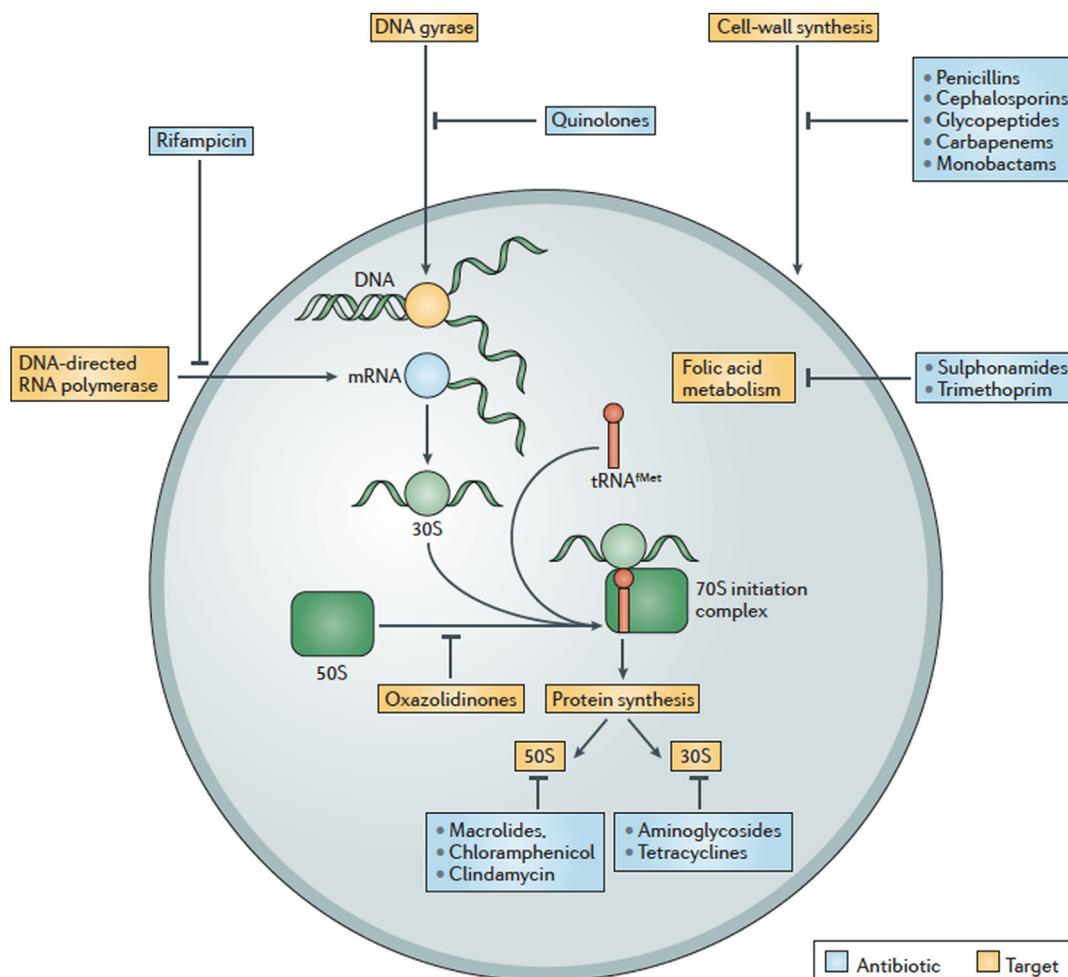


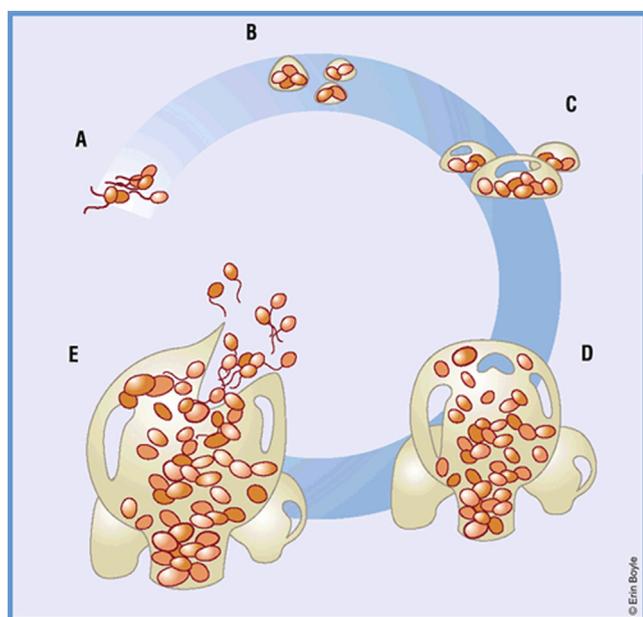
Fig. 1. Targets of antibiotics: The three most successful antibiotics hit targets including (i) the ribosome (which consists of 50S and 30S subunits), (ii) cell wall synthesis, (iii) and DNA gyrase or DNA topoisomerase. Reprinted with permission from Ref. [22].

between the drug and biofilm components, causing slow and incomplete penetration of the drug into the biofilm matrix [55,56]. Besides, the low pH in the surrounding infected environment and in the biofilm can protonate drugs like ciprofloxacin, enhancing interaction with alginate in the biofilm by charge interaction, further reducing free drug concentration at the site of action [57]. Consequently, antibiotic concentrations may not exceed the MIC, promoting micro-environmental pressure and further fostering biofilm formation, as well as generating drug-resistant bacterial sub-populations.

In the pulmonary air space, the epithelia are covered with a layer of mucus which has hydrogel-like structure mainly composed of water, mucins (glycoproteins), DNA, proteins, lipids, and cell debris [58]. This mucosa represents the first landing spot and the primary site of entry for pathogens to interact with and colonize the host tissues [59]. Despite its barrier functions, mucus only insufficiently protects the exposed epithelia from external threats like pathogen colonization [59,60]. Neutrophils, macrophages, dendritic cells, natural killer cells, e.g. T and B lymphocytes, glycoproteins, effector peptides and proteins, e.g. defensins, complement, C-reactive protein, as well as pro-inflammatory chemokines and cytokines, which are of the innate and adaptive immune systems, are usually contained in mucosal epithelia to serve as host immune response to infections [59]. Once the pathogen, however, surpasses these natural defense systems, the mucosa may be a superior environment for bacterial infection and resistance development [61]. Notably, the thick and sticky mucus build-up in the lungs

makes cystic fibrosis (CF) sufferers more apposite to fast development of persistent bacterial infections. Despite its clinical importance, understanding of mucosal biofilm structure and behaviors of bacteria persisting in mucosal biofilms is incomplete. Nevertheless, it has been recognized that mucus-embedded biofilms persist for decades and cannot be wholly eradicated [62]. One might simply hypothesize that the naturally negatively charged matrix of mucus in addition to the extracellular matrix of biofilm would form a physically stronger barrier which might prohibit antibiotic penetration to the site of action [56,62,63]. Thus, bacteria that form mucosal biofilms are more difficult to eradicate by conventional inhaled therapy.

(iv) Despite a variety of available potent antibiotics, the attraction of potential clinical benefits, and aggressive efforts to develop new therapies and drugs, only a few antibiotics are approved for inhaled therapy to treat pulmonary infections [34,44,64]. For life-threatening PA infections associated with CF, only four inhaled antibiotics are approved for clinical use in Europe, including colistin (and its prodrug, colistin methanesulfonate), tobramycin, levofloxacin, and aztreonam (structures depicted in Fig. 3) [64,65]. Moreover, only a few drugs are currently in clinical trials for treatment of pulmonary infections [22], worrisome given the effort and time needed to complete clinical studies, and their moderate rates of success. As a consequence, it remains challenging to combat pulmonary infections using the limited portfolio of alternative antibiotics, once pathogens become resistant to one drug [64].



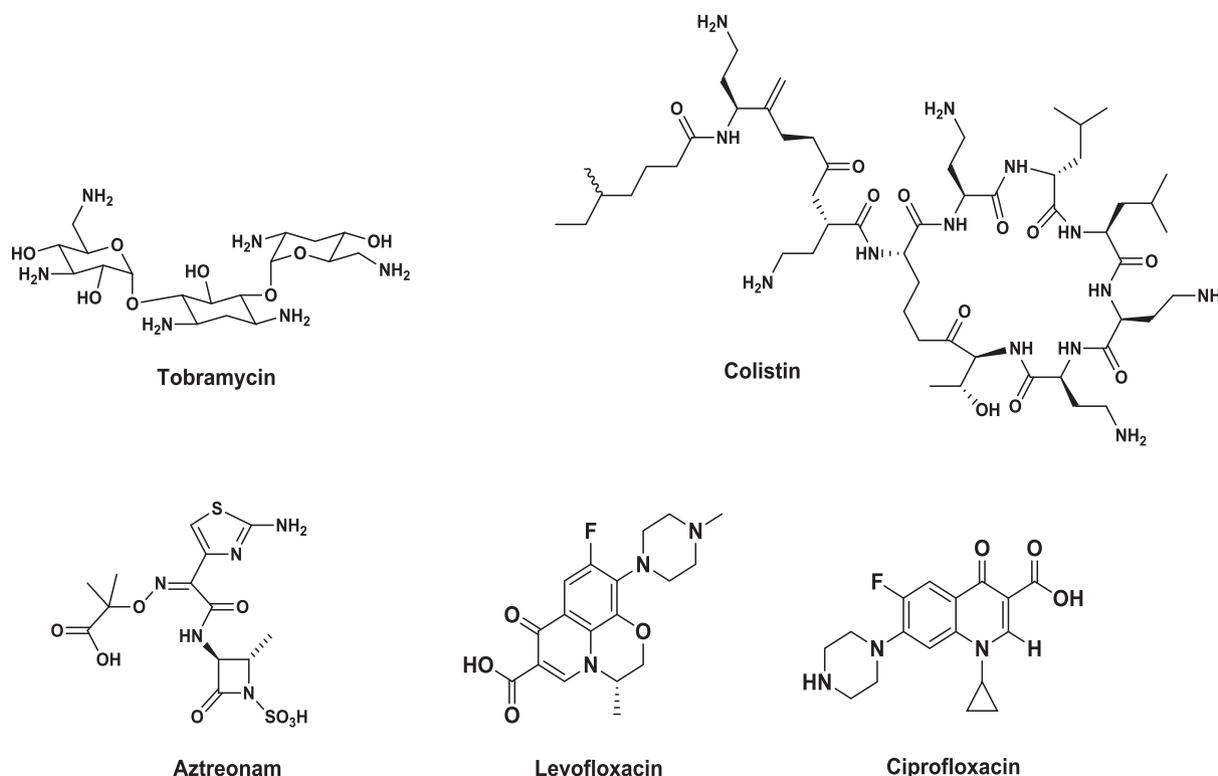
**Fig. 2.** Biofilm development (A) Planktonic bacteria attach reversibly to surface (B) irreversible adhesion to the surface, and effect of *quorum* sensing begins (C) Maturation phase: micro colony formation (D) extracellular matrix synthesis and biofilm maturation to reach maximum thickness (E) Dispersion/migration of planktonic bacteria from biofilm matrix. Reprinted with permission from Ref. [50].

Having considered the recognized challenges and knowledge gaps in combatting antimicrobial resistance, scientists have proposed and pursued different strategies to overcome or at least slow down development of resistance, thereby improving patient quality of life. The significant efforts to find new antimicrobials and strategies for

improving therapeutic efficacy of approved antibiotics are summarized in Fig. 4.

New antimicrobials have been discovered using a variety of advanced approaches, including phage therapy, immune therapy, and vaccination, as well as discovery and synthesis of new anti-infectives based on newly discovered and existing platforms. These approaches mainly aim to obtain more potent agents which could have better drug bioavailability at the site of action by better penetration through the bacterial cell wall and the surrounding environmental barriers, e.g. biofilms and cellular membranes [22,66–69]. These new actives tend to target species-specific proteins, enabling selectivity towards specific bacteria, and promoting lack of toxicity to host tissues, in agreement with the pioneering concept postulated by Erlich in 1906 who had referred to targeted drugs as ‘magic bullets’. Nevertheless, as stressed by R. Duncan (1997), “development of targeted drugs is inevitably a lengthy process, and breakthroughs are more frequently a dream rather than reality” [70]. One of the important reasons is that there is a limited number of exploited targets, out of nearly 200 conserved vital proteins in bacteria, that has been discovered and considered effective targeting for antibiotics [22], described in Fig. 1.

Despite intensive focus on discovery of new antimicrobials, the therapeutic value of such agents still has to be demonstrated clinically [22], recognizing the long, fraught pathway from preclinical studies to clinical success. In most cases, the antimicrobials have been designed to eradicate infection by interfering with bacterial growth, which intrinsically puts stress on bacteria and therefore might quickly lead to resistance development [71]. New approaches will have to deal with the same challenges faced by approved antibiotics. Taking a different view, the concept of pathoblocker such as e.g. *quorum* sensing inhibitors (QSI) may be considered a promising strategy to overcome the growing and challenging resistance problem. The QSI would not interfere with bacterial growth and therefore would avoid the stress caused by antibiotics that leads to resistance. Instead, they would prevent biofilm formation by inhibiting bacterial communications via *quorum* sensing and signal transduction systems which are suggested to mediate drug



**Fig. 3.** Structures of approved inhaled antibiotics for treatment of pulmonary infection in cystic fibrosis patients in Europe: colistin, tobramycin, levofloxacin, aztreonam. Ciprofloxacin is being studied in clinical trials.

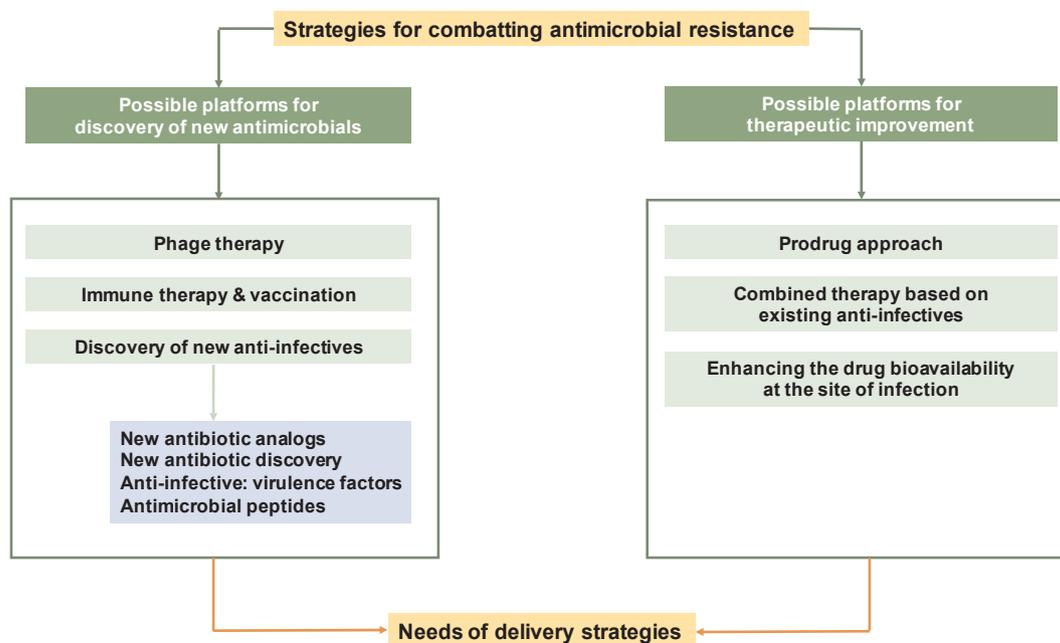


Fig. 4. Flow chart indicates different strategies for combatting antimicrobial resistance, and the needs of delivery strategies.

resistance [72]. Upon being treated with QSI, the biofilm structure would not grow strongly. The bacteria would thus not form persister cells, and would be more sensitive to antibiotics. The approach has shown some promising results in previous studies, especially when combining with approved antibiotics; efficacy against bacterial biofilms has been increased significantly [73,74]. However, most of the discovered QSI compounds have poor water-solubility, limiting bioavailability and thus therapeutic efficacy of these molecules, and impeding their administration by inhalation [75].

Attracted by the obvious clinical benefits of approved antibiotics, scientists have tried to improve their therapeutic efficacy to overcome bacterial resistance. Based on approved agents, prodrugs designed to act only in the targeted site have been synthesized [22,76]. This approach was expected to prevent toxicity caused by the antibiotic itself, e.g. for colistin and some of its prodrugs [77,78]. The hoped-for reduction in resistance development has, however, not been convincingly proved. Alternatively, combining antibiotics which would hit different targets is hypothesized to overcome antibiotic tolerance to individual antibiotics that often leads to treatment failure [79–82]. Moreover, to enhance drug concentration directly at the site of action, breaking biological barriers such as mucus by concurrent administration of mucolytic N-acetylcysteine has been also considered and successfully applied [83,84]. However, these aims remain challenging to achieve due to the range of physicochemical properties and pharmaceutical characteristics of the active pharmaceutical ingredients [80].

Taking together the discussions above, there are a variety of approaches, some progress, and remaining limitations to the discovery and development of strategies against antibiotic resistant bacterial infections. Whether focusing on the discovery of new antimicrobials or on improving the therapeutic effects of approved compounds, one crucial element in combatting bacterial resistance is enhancing the bioavailability of the drugs at the infection site. Thus, there is a need for efficient delivery strategies, which could accomplish the correctly sustained distribution of antimicrobials in the infected regions at high concentrations.

### 3. Drug delivery systems aim for treatment of pulmonary infections

#### 3.1. Advantages of drug delivery systems in treatment of pulmonary infections

As discussed above, pulmonary delivery of antibiotics has shown increasing relevance for treatment of respiratory bacterial infections compared to conventional (e.g. oral or intravenous) administration. Investigators hypothesize that pulmonary delivery offers less risk of systemic serious adverse effects, improved antibiotic bioavailability, and bio-distribution to targeted lung sites. These hypotheses appear promising, but pulmonary drug delivery is in general challenging for the aforementioned reasons. To address those challenges, appropriate formulations of drugs with pharmaceutical excipients, or drug delivery systems (DDS), are hence required.

Drug formulation strategies depend upon the physicochemical properties of drug molecules and their intended application. Successful DDS must address the limitations in therapeutic efficacy observed in many approved and investigational active pharmaceutical ingredients (APIs) which arise from (i) poor water-solubility, (ii) difficulty in delivering the molecules to pulmonary environment (e.g., reduced *in vivo* half-life/stability), and (iii) the potential to induce high toxicity [85,86]. In the field of drug delivery, nanotechnology has in particular attracted remarkable attention. It involves engineering drug-loaded nanostructures and nanomaterials with diameter between 10 and 1000 nm, for improving API performance [87]. The enhanced surface area of nanoparticles can increase dissolution rate of poorly water-soluble drugs, and if the polymer carrier is chosen carefully, the nanoparticle can also protect unstable molecules from degradation in the presence of enzymes, as well as minimize the possible adverse effects by controlling the drug release profile [88]. Nanotechnology-enabled antibiotic delivery could increase solubility of poorly water-soluble drugs in the thin pulmonary lining fluid, and could prevent fast drug clearance because the nanoparticle is below optimum diameter for mucociliary clearance [58,89]. Retention of the antibiotic in the thin fluid of the pulmonary lining, while avoiding or restricting mucociliary clearance and alveolar macrophages [90], can improve pulmonary drug bioavailability. Polymer-drug affinity can impact the release rate from the nanocarrier, and can be achieved by structural design of the

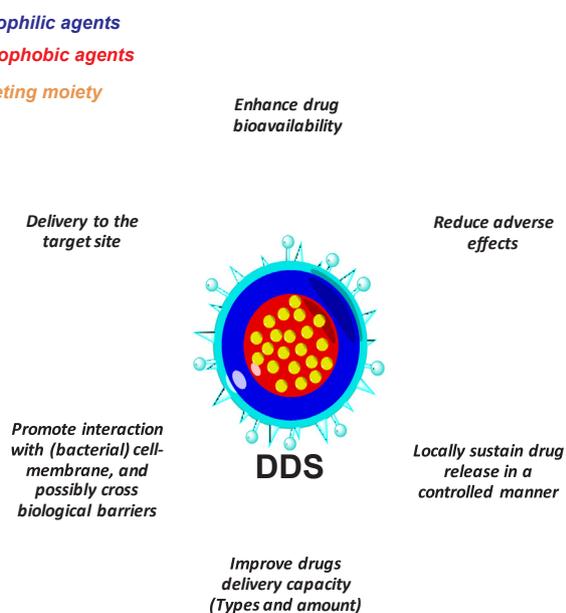


Fig. 5. Advantages of drug delivery systems.

polymeric excipient, and by adjusting the method of preparing the drug-loaded carriers. In addition to the achievement of temporal and spatial site-specific delivery, nanomedicine may also allow administration of a sustainably sufficient dose in a controlled release manner [91–93]. Especially for delivery of an antibiotic, its concentration would be maintained above the MIC value for a longer time [44]. Design and engineering of excipients used in nanomedicine could also offer better affinity towards the bacterial cell envelope. More importantly, despite the controversy and lack of clinical evidence, nanomedicine is believed to improve drug transport across biological barriers, e.g. biofilm and/or mucus, to deliver drugs more directly to the persister bacteria, which would possibly improve antibacterial activity, thereby reducing the potential for bacterial resistance and recurrent infections [94,95]. Lastly, nanomedicines could be designed to efficiently deliver established or emerging drug molecules, or even a combination of different functional actives in a targeted manner, creating multifunctional carrier systems (an example of the carrier structure is shown in Fig. 5). This flexibility of nanotechnology in drug delivery offers additional possibilities to combat bacterial resistance, and in particular pulmonary bacterial infections. The advantages of using DDS are summarized in Fig. 5.

### 3.2. Requirements for preparation of drug delivery systems

DDS are used to improve the therapeutic efficacy of drugs, moving the new therapy closer to clinical use. Thus, it is important first to develop strategies that have capacity to improve drug bioavailability, which means that the carrier systems can encapsulate drug at high loading capacity (w/w ratio, the calculation is shown in equation (i)) and good encapsulation efficacy (the calculation is shown in equation (ii)). This is because only a small amount of formulated drug can be delivered to the relatively limited amount of pulmonary fluid, and the greater the proportion of active drug, the higher the drug bioavailability can be. Furthermore, the carrier systems should also allow simultaneous delivery of diverse active cargoes which would provide complementary therapeutic effects [96,97]. Flexibility for further modification of the systems is important to potentially enhance interaction and affinity with the targeted sites of infection [88,98]. Those kinds of functionalization are also good approaches to further reduce the risk of adverse effects. Most importantly, the materials that are used for the preparation of such carriers should be nontoxic and

biodegradable and cleared after fulfilling their function *in vivo* [83,99–103]. DDS can therefore help to solve these several challenges to pulmonary drug delivery. Consequently, systems that are more sophisticated should be developed to fulfill all requirements that would help improve the therapeutic effects of drugs.

Treatment success in inhalation therapy needs appropriate deposition in the lungs, which is mediated by aerosol technologies, including devices and formulations [104]. Devices used to deliver therapeutic agents as aerosols, e.g. nebulizers, pressurized metered-dose inhaler, and dry powder inhalers, are readily available, which can be selected for specific demands of drug formulation deposition in the lungs and of treatment [104]. Suitable aerodynamic size ranges for airways and alveolar deposition and the properties of such devices are well-known and reported [105,106]. This review focuses on the requirements for the DDS and pulmonary biological barriers.

Nanotechnology is in general one such avenue, as a scientifically diverse discipline that encompasses engineering, materials science, physics, chemistry, and the biological sciences. Its use in the field of drug delivery has been developing remarkably, creating many strategic alternatives for preparing DDS [107,108]. The focus of this field has shifted from making simple, drug-loaded carriers, to engineering nanocarriers with new, desired properties to better control the delivery profile and overcome biological barriers, with specific targeting action, and even equipped for imaging, thus rendering them attractive for therapy. However, relatively few nanomedicines have reached patients, as those sophisticated systems often fail in preclinical studies for various reasons, including complexity of manufacturing. Reproducible multi-functionalization of the carrier system, which would offer a better therapeutic efficacy, is also difficult to achieve in large-scale production. Such advanced therapeutics are also likely to be more costly than established therapies [109,110]. Fig. 6 depicts the general requirements for development of DDS, involving a compromise between pharmaceutical and engineering requirements in the selection of materials. Thus, the search for pharmaceutical excipients which qualify all requirements remains challenging. DDS development is especially challenging for anti-infective delivery systems, as high doses of such drugs are frequently required [110]. Therefore, it is necessary to have facile strategies, ideally using polymers that are already in approved formulations, for developing high antibiotic loading capacity carrier systems, and that still allow further modification for advanced therapeutic improvement.

DDS can profoundly improve therapeutic effects once high drug loading capacity of such carriers is achieved. Because of the limited capacity for delivery of formulated materials to the lungs, optimization of drug loading capacity is critical to consider in the process of developing DDS.

The drug loading capacity (LC %) and encapsulation efficacy (EE %) are calculated as the equations below:

$$EE\% = \frac{\text{Weight of encapsulated drug in NPs}}{\text{Initial weight of used drug}} \times 100 \quad (i)$$

$$LC\% = \frac{\text{Weight of drug in NPs}}{\text{Weight of NPs}} \times 100 \quad (ii)$$

“Weight of nanoparticles (NPs)” is calculated as (Weight of NPs = Weight of polymeric materials + Weight of encapsulated drug in NPs).

EE% indicates the efficiency of the drug loading procedure, control and maximization of which is important in order to produce reproducible formulations with minimal waste of valuable drug. LC% indicates the weight of drug as a percentage of the total DDS weight, which is needed in calculating the drug dose administered. Notably, the LC% is crucial in pulmonary delivery, since only a finite amount of formulated drug can be applied to the lung, e.g. by inhalation of dry powder. While EE% can frequently be maximized by optimizing process parameters, achievement of sufficiently high LC% remains challenging,

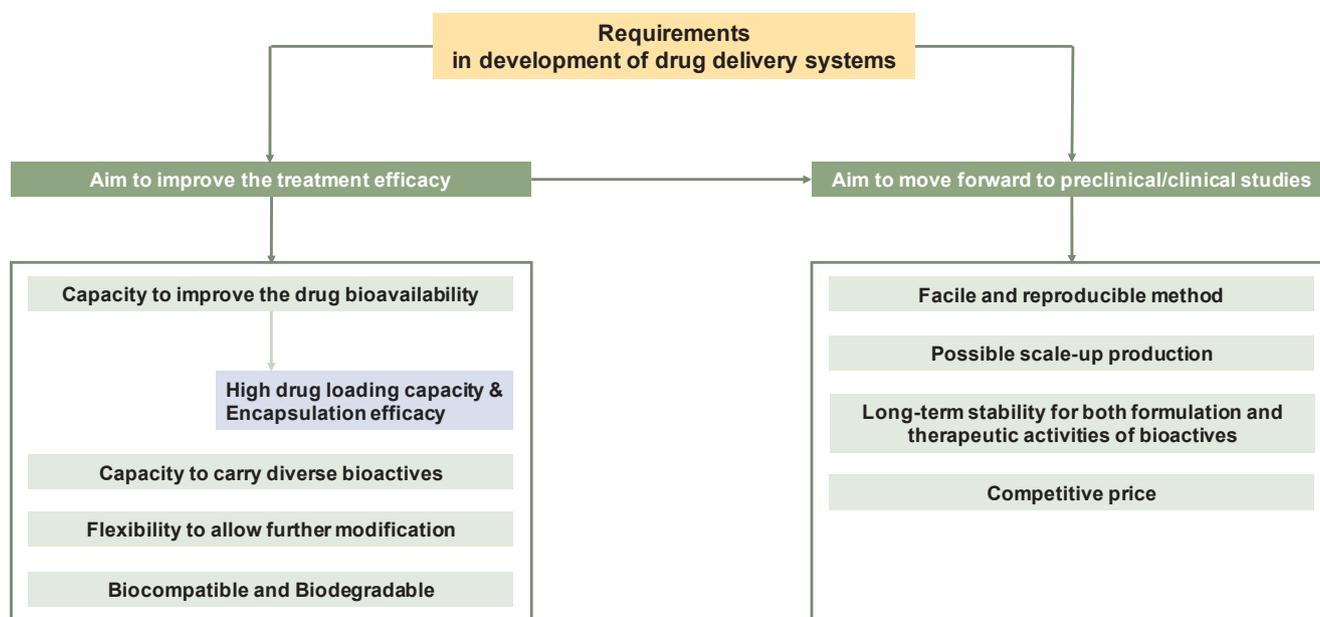


Fig. 6. General requirements in development of drug delivery systems.

sometimes causing problems even in preclinical studies. Thus, LC% is one of the first factors that needs to be improved to obtain a good DDS.

### 3.3. Recent development of anti-infective delivery systems for treatment of pulmonary infections

Recognizing the advantages of local delivery, inhaled antibiotic therapy has been used to treat chronic respiratory infection since the 1940s [111]. The earliest formulations were not explicitly designed for inhalation, so they caused significant bronchial irritation. A major advancement of such development took place in 1997, when the FDA first approved a designed formulation for inhalation, which was tobramycin for use in PA infected patients with cystic fibrosis [112,113]. The approach has shown significant clinical benefits in terms of lung function improvement and reduction of hospitalization in CF patients. Subsequently, dry powder DDS formulations were developed to enhance delivery of antibiotics to the lungs [114]. This strategy allowed notable decreases in dose of antibiotic per application [115]. This development has been considered as a very promising approach to improve pulmonary antibiotic safety profiles, and avoid fast development of resistance. Interest in the use of such formulations has been not only for infections associated with CF, but also for other lower respiratory tract infections [116,117].

In this mini review, we focus only on respiratory PA associated infections and strategies for treatment of such diseases. The most common dry powder inhalation antibiotics considered for PA infection are tobramycin [114] and colistin [117]. The dry powder form of ciprofloxacin has also been investigated in a Phase III randomized study and appeared to be favorable for treatment of pulmonary PA infections [118]. However, in many cases of severe and resistant infections, e.g. those associated with CF patients, the efficacy of the dry powder, in particular tobramycin dry powder, is still limited by the ability to achieve sufficient concentration levels at the site of infection [119]. It is noted that the drug loading capacity of the dry powder antibiotic, in particular in the case of tobramycin, is rather high (~50% w/w ratio) [120,121]. Hence, limited efficacy can be attributed to rapid clearance of the drug, its poor ability to permeate mucus and biofilms, and inactivation of the drug through binding interactions in these environments. Those problems could be explained in part by the particle sizes of dry powder antibiotics, usually produced by spray-drying, which typically have diameters in the micron range (1–10  $\mu\text{m}$ ) and are not

highly uniform [120]. Therefore, novel strategies for improving antibiotic delivery could enhance the activity of those vital antibiotics. In addition to developing delivery systems for available, potent antibiotics, there are opportunities to combine into the delivery systems novel anti-infectives by taking advantage of recent drug delivery techniques to further enhance the therapeutic effects of established antibiotics.

As discussed above, although nanotechnology has been aggressively pursued in drug delivery, simple approaches should still be advanced with the aim to achieve higher and faster rate of translation into clinical use. There is an uncountable number of studies aiming to prepare the vital antibiotic-loaded carrier systems. Popular carrier systems used include (i) liposomes [122,123]; (ii) microemulsions and nanoemulsions [122]; (iii) solid lipid NPs [23,124]; (iv) polymeric particles, including particles made from synthetic polymers, e.g. silica particles, poly lactic-co-glycolic acid (PLGA) particles, as well as made from natural polymers, e.g. chitosan derivatives or alginate [90,125–127]. Metallic NPs, e.g. silver, gold, titanium dioxide NPs, are known to have antimicrobial properties, and also widely developed and applied to prevent bacterial colonization and eradicate microbial biofilms [121]. However, considering the need for long-term administration, especially for CF-related infections, these non-biodegradable materials are not preferred for inhalation therapy. Table 2 summarizes representatives of antibiotic-loaded liposomal and particulate systems that are developed for inhalation therapy to treat PA associated infections. There is a vast number of publications concerning the preparation of antibiotic-loaded carrier systems, in which the EE% was reported carefully. LC%, one of the essential factors deciding the success of a DDS, was not always explicitly reported; thus, in Table 2 LC% is presented as the maximum obtained values where it was reported in the corresponding publications.

Some liposomal carrier systems prepared by conventional techniques are capable of loading a high percentage of antibiotic, up to nearly 60%. Such loading capacity is promising for improving antibiotic bioavailability at the targeted site, which in turn enhances the therapeutic effect. In addition to enhanced bioavailability, these investigations indicate that the adverse effects caused by antimicrobials in the lungs cells were significantly reduced when using drug loaded liposomes compared to inhaled free drug solutions. However, these antibiotic-loaded liposomal formulations only improved the therapeutic effect slightly as measured by decrease in MIC. Retention times in the

**Table 2**  
Summary of representative delivery systems of inhaled antibiotics for treatment of PA infection. Drug, production method, major excipients and loading capacity (LC%) are highlighted. LC% is presented as the maximum value reported in the corresponding publications.

Representative liposomal delivery systems of inhaled antibiotics aimed for PA infections treatment		Major excipients	LC%	Ref.
Drug	Production method			
Amikacin (Arikace®)	N/A <sup>(a)</sup>	N/A	~60%	[128]
Tobramycin	Membrane extrusion	1,2-Distearoyl-sn-glycero-3-phosphocholine, cholesterol	~60%	[129]
Ciprofloxacin (Lipoquin® and Pulmaquin®)	Membrane extrusion	Poly sorbate 20, 0.4% (w/v), hydrogenated soy phosphatidylcholine, cholesterol	16–33%	[130,131]
Colistin	Sonication/ Membrane extrusion	1,2-dipalmitoyl-sn-glycero-3-phosphocholine, cholesterol, 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine	12–55%	[132]
<b>Representative polymeric delivery systems of inhaled antibiotics aimed for PA infections treatment</b>				
Drug	Production method	Major excipients	LC%	Ref.
Levofloxacin	Lipid-coated nanoparticles via an emulsification-solvent-evaporation method followed by spray drying	PLGA, PVA, phosphatidylcholine, L-leucine	< 1.1%	[133]
Tobramycin	Nanoparticle suspension by the emulsion/solvent diffusion method followed by spray drying	PLGA, PVA, chitosan, alginate, lactose	< 2%	[134]
Tobramycin	Polyplexes	Alginate, chitosan	< 9%	[119]
Tobramycin	Polyplexes	Starch, chitosan	~3%	[127]
Colistin	Polyplexes	Starch, chitosan	17%	[127]
Gentamicin	Emulsion/solvent diffusion	PLGA, PVA	< 2%	[135]
Ciprofloxacin	Polyplexes	Chitosan crosslinked with sodium triphosphosphate	< 5%	[136]
Ciprofloxacin	Polyplexes	Chitosan, oxidized $\beta$ -cyclodextrin	~9%	[90]
<b>Representative polymer-antibiotic conjugates aimed for PA infections treatment</b>				
Drug	Synthesis	Polymer(s)	LC%	Ref.
Tobramycin	PEGylation by CDI conversion of amine to amide	Polyethylene Glycol (PEG)	~8.0% by molecular weight	[137]
Colistin	Copper-mediated photo induced living radical polymerization	Poly [Poly (Ethylene Glycol) Methyl Ether Acrylate]	24% by molecular weight	[138]
Ciprofloxacin	TsCl activation of carboxyl group	Hydroxyethyl cellulose/Hydroxypropyl cellulose	50% by wt%	[139]
Tobramycin	Thiolation of amino groups by N-acetylcysteine	Chitosan	~11.4% by degree of substitution	[140]
Ciprofloxacin	Living cationic/ring opening polymerization	Poly(2-oxazoline)s and Polyethylene Glycol	~13% by molecular weight	[141]
Ciprofloxacin	RAFT polymerization	Methylacrylate derivative polymer	30–35% by molecular weight	[142]

<sup>(a)</sup> N/A: not available.

infected regions were not remarkably improved, which may be a result of fast antibiotic release from the liposomal formulation. In most cases, cumulative antibiotic release reached 100% in a biologically relevant medium in a short time (10 min to 2 h) [143,144]. This burst effect is often seen in drug-loaded liposomes due to the non-specific binding of drug molecules and liposomal excipients. A further modification, e.g. surface coating and/or crosslinking, would possibly prevent such problems. The modification makes liposomes preparation method less facile and could also affect the stability of the system [145,146]. The same holds true for the antibiotic-loaded systems produced from microemulsions and nanoemulsions as well as solid lipid NP technologies, but LC% values in these cases are not as high as those obtained from liposomal formulations.

Polymeric NPs appear to be promising candidates to better prevent burst release, as binding between drug and excipients, either covalent or noncovalent, can be flexibly tuned by changing polymer properties and particle preparation methods. This approach does permit better controlled release profiles of encapsulated drugs. Furthermore, polymeric carrier systems may allow further modification to achieve more desired DDS properties, thus rendering them attractive for nanomedicines. Nevertheless, the LC% can be a considerable limitation when using these DDS for treatment of pulmonary infections, which is in most cases of inhalation therapy lower than 5% [143]. Consequently, polymeric nanoparticle-based DDS have not yet reached patients.

Particle size is another design parameter, which can affect the therapeutic effect of DDS. The importance of LC% is recognized, so optimizing such value might also increase the size of the carrier systems. Thus, while true nanomaterials are considered smaller than 100 nm in size, the so-called submicron range (i.e., 100–900 nm) is often used in nanomedicine, which may provide better opportunities to carry higher amount of drug [87]. For this reason, liposome and polymeric particulates with maximum recorded drug LC% usually have diameters > 500 nm, which are actually not favorable for crossing biological barriers, and become better targets for natural clearance mechanisms [87,147]. Thus, site-specific delivery of therapeutics will remain a distant vision unless drug carrier systems are designed that can cross biological barriers, which are, in the case of extracellular infections, biofilms. Such biological barriers significantly contribute to the failure of those DDS.

In general, single antibiotic loaded carrier systems do not decrease the dose of antibiotic used in the treatment of infection; the dosage may even be increased in some cases. Furthermore, while these DDS decrease bacterial susceptibility, the development of bacterial resistance remains challenging. Despite these pronounced limitations, the developed carrier systems have shown promising results in decreasing the viability of bacteria in biofilms, with efficacy exceeding that of the corresponding free antibiotics. Thus, there is the need to develop better antimicrobial delivery systems for combatting bacterial infections.

Taking advantage of DDS, advanced developments in nanotechnology, and novel anti-infectives, some studies have focused on advancing the carrier system and/or developing strategies for efficient co-delivery of multiple active agents, which consequently achieve enhanced, complementary therapeutic effects. We highlight several examples including: (i) polymyxin B containing polyion complex nanoparticles in which polymyxin B was complexed with different molecular weights of poly(styrene sulphonate). This development has shown 10,000-fold improvement in inhibitory effect against PA [148]; (ii) combination of tobramycin and a mucolytic agent, dornase alfa (DNase), achieved in a chitosan-alginate polyplex system, where such simultaneous delivery of the two active compounds improved the therapeutic effect of tobramycin in contact with cystic fibrosis sputum [119]. In another approach, chitosan nanoparticles were functionalized by alginate lyase to deliver ciprofloxacin [149]. Alginate lyase is applicable to the treatment of pulmonary infection by degrading the alginate component of PA biofilms [150]. These modified chitosan DDS were effective against PA biofilms (reduced biomass density was

confirmed using confocal microscopy); (iii) bismuth-ethanedithiol, a biofilm reducer, and tobramycin were co-loaded in a liposome system which showed a decrease in CFU count *in vivo* vs. the animals treated with free drug [129]; (iii) incorporation of farnesol, a natural QSI, and ciprofloxacin in a liposomal formulation exhibited a very interesting outcome. The minimum biofilm eradicating concentration (MBEC) value obtained using the co-delivery system was reported at 0.128 µg/mL of ciprofloxacin, essentially the same as the reported MIC value of ciprofloxacin for planktonic bacteria at 0.125 µg/mL [73]. The results of these studies were promising and point to the possibility of reducing the use of antibiotics when applying them in combination with other complementary agents, which is important to prevent antimicrobial resistance. However, more relevant *in vivo* data proving complete eradication of infection has not yet been reported. Furthermore, despite being a promising concept for combatting bacterial biofilms, formulation characteristics, including particulate characteristics (importantly the size and charge surface) and drug release profiles, have not been optimized.

We here also highlight several recent developments in biodegradable polymer-based DDS for pulmonary infection treatment focusing on the use of polysaccharides, in particular chitosan, which is heavily used due to its mucoadhesive and antimicrobial properties. Incorporating chitosan in formulations, e.g. chitosan based and chitosan coated PLGA particles [151,152], prolongs the bioavailability and possibly increases the performance of drugs both locally and systemically [153]. In addition to providing a mucoadhesive coating for nanoparticle DDS, chitosan can also be chemically modified for pulmonary biofilm infection treatments. Kenawy et al. synthesized an aminated chitosan functionalized with *p*-nitrobenzaldehyde [154]. This modified chitosan was not only active against biofilms, but also showed antioxidant and antimicrobial activity. The ability to select the degree of substitution of the *p*-nitrobenzaldehyde could be advantageous to optimize activity while minimizing cytotoxicity. This promising approach could also be explored in other amine functionalized biodegradable polymers. Lu et al. synthesized functionalized chitosan oligosaccharide derivatives able to release bactericidal nitric oxide [155]. This was accomplished by first degrading chitosan into oligomers with hydrogen peroxide and then functionalizing the primary amine groups with nitric oxide to form *N*-diazoniumdiolates, resulting in ≥ 99% killing of PA infections. It is important to note that nitric oxide release from such chitosan derivatives has dual activity against PA biofilms by both disrupting and eradicating the biomass whereas tobramycin alone is not known to alter biofilm physical properties [156]. PEG-substituted versions of these chitosan derivatives were also synthesized but were shown to be less effective [155]. In another approach, chitosan oligosaccharides can be directly conjugated to antibiotics through simple Schiff base chemistry followed by reduction of the labile imine bond. This approach has already been used with streptomycin, where the conjugate, COS-Strep, was proven to decrease PA biofilm mass more effectively than the unconjugated components alone or mixed [157]. Interestingly, Li et al. also found that this COS-Strep did not induce the MexX-MexY drug efflux pump, in contrast to free streptomycin. Although further investigation is needed, this suggests that this conjugation approach may help suppress drug resistance. These results suggest that polymer-anti-infective agent conjugates are promising, with increasing relevance in pulmonary DDS research.

Polymer-drug conjugates provide potential advantages for DDS: (i) increasing solubility and protecting actives from harsh biological environments; (ii) and being especially designed for stimulus responsive release. More importantly, development of novel anti-infectives is both costly and time consuming [158]. Therefore, using polymers to assist existing drugs is an attractive alternative. We summarize some representative polymer-anti-infective conjugates in Table 2. The synthetic polymer-anti-infective conjugating strategy has been limited to just a few antibiotics, e.g. ciprofloxacin, due to the demands of stability and functionality for conjugation to the polymer. Natural polysaccharide-

based materials like cellulose ethers or chitosan are advantageous due to their abundance of active functional groups for direct conjugation, and the ability to control the degree of substitution by simple stoichiometry. Moreover, often such conjugates have enhanced water-solubility vs. the original anti-infective. The use of benign and biodegradable polymers for conjugates is an especially emergent area and such systems are well documented in the treatment of pulmonary disease [159]. Biofilm antimicrobial activity can be improved by conjugation to PEG for example, as demonstrated in PA biofilms [137]. Conjugates that lower cytotoxicity have also been demonstrated. However, chemical conjugation does have disadvantages. Especially for natural polymers, harsh reaction conditions (e.g. high temperature, acidic reaction media etc.) can cause partial degradation, which can obviously have an impact on material properties [160,161]. Therefore, investigators of natural polymer-drug conjugation have attempted to utilize synthetic chemistry that is: (i) mild (i.e. minimizes or eliminates degradation); (ii) efficient (few synthetic steps); (iii) reproducible and scalable on an industrial level; (iv) moreover enhancing drug loading capacity and efficacy.

### 3.4. Interaction between drug delivery systems and pulmonary biological barriers

*P. aeruginosa* is an opportunistic pathogen which affects most people with a compromised immune defense, injury, or chronic diseases like CF or chronic obstructive pulmonary disease (COPD) [162]. The infection progression may be a result of the exposure and the following interplay between the bacteria and the host immune defense system. PA, thus, can cause either acute infection such as pneumonia, or chronic, persistent infections [163]. Acute pneumonia affects the deep lung and may even spread into the circulation by epi- and endothelial damage. Chronic PA infections instead persist in the small airways, e.g. the inflamed and widened bronchioles (bronchiectasis) of CF patients. For such chronic infections, biological lung barriers may prevent effective interruption of disease processes, despite having achieved successful accumulation of either drug molecules or the DDS specifically at the diseased sites. Particularly considering respiratory bacterial biofilm infection, biological barriers that limit drug transport include bacterial cell membranes, biofilms, mucus, mucosal biofilms, and pulmonary

immune regulators [164]. The crucial biological barriers to inhaled DDS are depicted in Fig. 7. As the ultimate aim is to treat biofilm infections while overcoming antimicrobial resistance, the antibiotic drug molecules should travel through all aforementioned barriers and be accumulated either in the bacterial membrane or in bacterial cytoplasm. However, free drug molecules usually fail to fully accomplish this aim, and DDS are thus needed. Although considerable research efforts have focused on incorporating multiple surface functionalities and moieties within the overall NP design and preparation, many of these strategies fail to successfully address these barriers successfully. A reinterpretation of conventional drug delivery systems is thus needed to successfully negotiate these impediments to a single carrier system. By successively understanding and addressing each of these biological barriers, appropriate design features could be rationally incorporated, creating a successful generation of particulate-based DDS.

In DDS design, particle size, surface properties, morphology, and particle shape are frequently tuned to achieve better penetration through biological barriers [94]. Ideally, particles that would successfully penetrate through such obstacles would have these characteristics, including (i) small size, the smaller the better for the transport. The same particles must also be able to carry a significant drug load. The appropriate size range may be between 100 and 200 nm in order to satisfy the requirements for both transport and loading capacity; (ii) anti-fouling surface, which is covered with polyethylene glycol (PEG) or zwitterionic materials; (iii) smooth surface morphology. Within these requirements, spherical PEGylated NPs with size range < 200 nm are known to transport at high rates through biological barriers including mucus and biofilms [165,166]. Moreover, the small size range and neutral charge surface would elicit only a slow immune system response, leading to prolonged *in vivo* residence time [94]. However, having PEGylated or zwitterionic surface would simultaneously decrease or lose the potential of drug loading on the surface which is dependent on the degree of modification. In addition, the small size range would limit the drug loading capacity in the core side of the particles. Hence, the design of biological barriers penetrating NPs should find a compromise with the loading capacity of such systems.

The mucosal epithelia, e.g. airway epithelia, are covered with a retentive viscoelastic mucus layer, a three-dimensional macromolecular network with the ability to entrap and remove NPs in a size-dependent

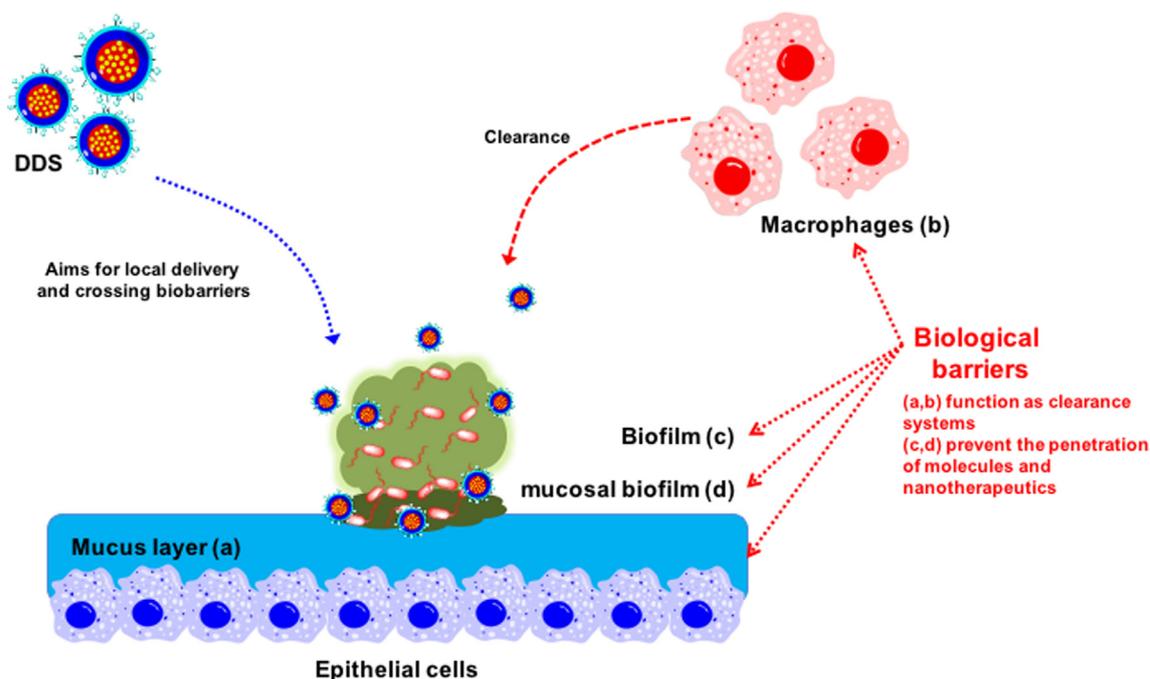


Fig. 7. Biological barriers to inhaled anti-infective-loaded drug delivery systems.

manner. Furthermore, the mucociliary clearance mechanism continuously propels mucus out of the lungs [58,89]. Additionally, such a mucus layer together with a biofilm/mucosal biofilm could also interact and adsorb the particles as well as molecules via electrostatic interactions [58]. Instead of fighting and overcoming these natural characteristics of mucus and biofilm/mucosal biofilm, those have been exploited as a method to prolong the residence time of DDS, in particular for positively charged NPs, e.g. chitosan based NPs [167,168]. Natural clearance is, in turn, inevitable (and essential, especially in the lungs) when applying foreign particulate materials *in vivo*. Clearance rate is thus another important factor, which helps to dictate *in vivo* residence time of the DDS. For those DDS used in inhalation therapy, the key regulators of pulmonary immunity, e.g. lung macrophages and dendritic cells, should be carefully taken into consideration [147,169]. Therefore, the dependence of macrophage uptake efficiency on particle size and surface properties should be investigated to predict *in vivo* performance. This will enable accurate recommendations for situations where these drug-loaded carrier systems can provide desired therapeutic results.

#### 4. Conclusion and expert opinion

This review discusses the benefits and challenges of pulmonary delivery of anti-infectives for treatment of pulmonary infections, and highlights the advantages of DDS used to address the significant problems in utilization of the anti-infectives, overcome biological barriers, and improve drug bioavailability at the infection site. In this regard, particularly focusing on PA infections, there are a vast number of existing strategies, and intensive efforts to discover novel platforms. However, only a few drugs have been clinically approved for such diseases. Recent DDS studies are mostly devoted to the delivery of relevant antibiotics, including tobramycin, colistin, levofloxacin, and ciprofloxacin, as well for simultaneous co-delivery of diverse bioactives. Liposome and polymer-based DDS are the most common strategies and platforms, which are biocompatible, biodegradable, and relatively stable in biologically relevant media, as well as being practical to manufacture. We describe in this review that these DDS are reported to have shown improved anti-infective delivery and efficacy. Notably, polymeric DDS offer possible tuning of physicochemical properties, with improved controlled release manner yet with limited LC% in most cases, while liposomal DDS have roughly the opposite advantages and disadvantages.

In many recent studies, structural design and synthesis of materials have been successfully employed to explore and maximize the drug loading capacity and the potential of co-loading diverse agents with the aim to combat bacterial infection and to prevent the development of bacterial resistance. Efficacy against bacterial infection at different stages was evaluated in different biologically relevant environments (e.g. presence of mucus or biofilm). Most interestingly, a complementary therapy against bacterial biofilm infection can be achieved by the simultaneous delivery of an antibiotic (ciprofloxacin) and a QSI (farnesol). As a result, the antimicrobial efficacy of the antibiotic could be enhanced, to eventually reduce the required dose of the antibiotic employed, and avoid development of bacterial resistance. In the light of rising antibiotic resistance, we expect such combined therapeutic strategies including innovative molecules beyond antibiotics (e.g. QSI or other anti-virulence strategies, immunomodulatory agents) to be a promising approach and worth further investigating to combat bacterial biofilm infections.

Polymer-anti-infective conjugates and modified polymers are a versatile approach to explore for applications in the treatment of pulmonary infection among many other diseases. They provide the opportunity to control release, and have already shown improved efficacy in eradication of biofilms *in vitro*. However, the challenge in implementing these DDS lies in optimization of the synthetic strategy. Further exploration into the modification of biodegradable polymers

using facile chemistry under controlled synthetic conditions will lead to expansion of this field.

The research reviewed here is promising and shows the possibility to enhance the efficacy of antibiotics when applying them with these delivery strategies and/or in a combination with other complementary actives. However, relevant *in vivo* data performed on validated *in vivo* models to prove the complete eradication of pulmonary infections has not been reported. Furthermore, despite creating a promising concept to combat bacterial biofilms, optimization of formulation characteristics is still required, in particular the LC%, *in vivo* respiratory biocompatibility at high doses, and the drug release profile.

Finally, we recommend studying the interaction of the future developed and innovated DDS with crucial pulmonary biological barriers: biofilm, mucus, and macrophages. These interactions should be carefully considered to better design and prepare the drug delivery systems to overcome these barriers as needed for successful applications.

#### Funding sources

This work has received funding from the European Union's Framework Programme for Research and Innovation Horizon 2020 (2014–2020) under the Marie Skłodowska-Curie Grant Agreement No. 642028.

#### Notes

The authors declare no competing financial interest.

#### Author contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

#### Acknowledgment

Brittany L.B. Nichols would like to acknowledge the German Fulbright Commission 2018-2019 for their funding.

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