Phage Lysins for Fighting Bacterial Respiratory Infections: A New Generation of Antimicrobials

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Lower respiratory tract infections and tuberculosis are responsible for the death of about 4.5 million people each year and are the main causes of mortality in children under 5 years of age. *Streptococcus pneumoniae* is the most common bacterial pathogen associated with severe pneumonia, although other Gram-positive and Gram-negative bacteria are involved in respiratory infections as well. The ability of these pathogens to persist and produce infection under the appropriate conditions is also associated with their capacity to form biofilms in the respiratory mucous membranes. Adding to the difficulty of treating biofilm-forming bacteria with antibiotics, many of these strains are becoming multidrug resistant, and thus the alternative therapeutics available for combating this kind of infections are rapidly depleting. Given these concerns, it is urgent to consider other unconventional strategies and, in this regard, phage lysins represent an attractive resource to circumvent some of the current issues in infection treatment. When added exogenously, lysins break specific bonds of the peptidoglycan and have potent bactericidal effects against susceptible bacteria. These enzymes possess interesting features, including that they do not trigger an adverse immune response and raise of resistance is very unlikely. Although Gram-negative bacteria had been considered refractory to these compounds, strategies to overcome this drawback have been developed recently. In this review we describe the most relevant *in vitro* and *in vivo* results obtained to date with lysins against bacterial respiratory pathogens.

**Keywords:** phage lysins, pneumonia, respiratory infection, antibacterials, antibiotic resistance, endolysins

**THE IMPACT OF BACTERIAL RESPIRATORY DISEASES ON HUMAN HEALTH**

Lower respiratory tract infections remain the most deadly communicable diseases, and caused 3.2 million deaths worldwide in 2015 (1). Tuberculosis is still to date among the top 10 death causes, and community-acquired pneumonia is the single largest bacterial infectious cause of death in children worldwide (2). *Streptococcus pneumoniae* (pneumococcus) accounts for most of the bacterial pneumonia cases in children, followed by *Haemophilus influenzae* type b, and other bacterial pathogens: *Streptococcus pyogenes* (group A *Streptococcus*), non-typeable *H. influenzae*, *Staphylococcus aureus*, *Mycoplasma pneumoniae*, *Moraxella catarrhalis*, and *Klebsiella pneumoniae* (3). Pneumococcus is also a common cause of community-acquired pneumonia in elderly patients with comorbidities (4). On the other hand, hospital-acquired pneumonia and ventilator-associated pneumonia are among the leading nosocomial infections worldwide, with an increasing frequency of multidrug resistant (MDR) Gram-negative bacteria (G−) as the bacteriologic cause (5).
Indeed, antimicrobial resistance (AMR) and associated morbidity and mortality have been increasing globally. A recent study estimated that AMR could produce 10 million deaths a year by 2050 (6), although this prediction should be taken with care (7). Accordingly, economic simulations predict that the world will suffer an annual shortfall loss of between $1 and $3.4 trillion by 2030 because of AMR (8). In this scenario, the World Health Organization (WHO) has called for global action on AMR (9). This has encouraged several actions: (a) prevention and control actions in healthcare facilities (10); (b) widespread antimicrobial stewardship programs (11); (c) reduction of antibiotic use in livestock production and the environment (12); and (d) the search for alternatives to the currently used antibiotics (13), particularly against a group of MDR bacteria having a global impact (14). Among these priority pathogens, *S. pneumoniae*, *H. influenzae* and those referred to as “the ESKAPE bugs” (15), are of particular concern. Of note, *Mycobacterium tuberculosis* was not included in the above list as it is already in a globally established priority for which innovative new treatments are urgently needed (16). A few decades ago, phage therapy revived as an alternative to conventional antibiotics and, since the beginning of twenty-first century, phage lytic enzymes have also been extensively tested as antibacterials. This area of research is the focus of this review and the most relevant results of certain enzymes against respiratory pathogens will be discussed. Extensive details on the issue can be found in other recent reviews (17–26).

**GENERAL CHARACTERISTICS OF LYSINS**

Endolysins, or more simply lysins, are phage-encoded enzymes capable of hydrolyzing the bacterial cell wall (CW) and that are synthesized at the end of the phage replication cycle. The peptidoglycan (PG) polymer is the basic component of the CW, and is composed of chains of a disaccharide repeat made up of N-acetylmuramic acid and N-acetylmuramyl peptide, linked by β(1→4) glycosidic bonds. Glycan strands are cross-linked by tetra/pentapeptide side arms attached to muramic acid residues through amide bonds. Lysins are usually classified as glycosidases [glucosaminidases, transglycosylases, and lysozymes (or muramidases)], if they break any of the bonds of the glycan chain, N-acetylmuramoyl-L-alanine amidases (NAM-amidases), if they break the amide bonds between the glycan strands and peptide chains, or endopeptidases if they hydrolyze different bonds within peptide chains. When purified lysins are added exogenously, their CW-degrading activity can lead to rapid osmotic lysis and bacterial death. The enzymatic activity of lysins was the basis for their exploration as antibacterial agents and they were also named “enzymbiotics” (27). Lysins possess several advantages over antibiotics: (a) they rapidly kill bacteria, practically upon contact; (b) they can be specific to the target pathogen, particularly against Gram-positive (G+) bacteria (28–31), which allows to preserve the normal microbiota (32); (c) development of resistance seems very unlikely (33, 34), probably because these enzymes directly target an essential and well-conserved structural component such as the PG, which cannot be easily modified without compromising fitness (35); (d) with few exceptions (36, 37), lysins are active independently of the bacterial physiological state (38, 39); (e) they are effective against MDR bacteria (20, 34, 40–42); (f) they can act synergistically with other lysins or antibiotics and thus theoretically reduce the development of resistance while increasing therapeutic efficiency; and (g) lysins are also effective killing colonizing pathogens growing on mucosal surfaces and/or in biofilms (Tables 1, 2).

Lysins encoded by phages infecting G+ bacteria generally display a modular structure, comprising one or more catalytic domains (CDs) and one or more CW binding domains (CWBD). Although the species specificity of a lysin is generally assigned to its CWBD, there are some data suggesting that combined interactions of CD and CWBD with unknown CW receptors may play a significant role (129). On the other hand, phages from G– bacteria usually encode globular lysins with a single CD, with several exceptions (31, 111, 128).

Concerning their systemic, therapeutic use, it has been alleged that lysins, as foreign proteins, could be expected to trigger the production of neutralizing antibodies that might hinder their antibacterial action in subsequent administrations. However, early studies addressing this potential drawback, strongly suggested that highly immune serum slows down—but does not block—lysins (46, 130). Pre-clinical and clinical trials with the antistaphylococcal lysin SAL-1 have been performed in animal models and, lately, in humans. An immune response was indeed elicited after repeated intravenous injections of SAL200, as demonstrated by the presence of specific antibodies and reduced C3 complement levels in the animal blood samples (80). Still, pharmacokinetic, pharmacodynamic, and tolerance studies of SAL200 in monkeys and humans did not show any serious adverse effects or clinically significant alterations even at the highest dose tested (81, 82). Anyhow, host immune responses to specific lysin formulations must always be considered concerning safety and improving the therapeutic potential of lysins.

The antibacterial efficacy of lysins can be improved by several means including: (a) replacement of certain amino acids to modify the net charge of the enzyme (53, 131) or allow dimerization (132); (b) deletion of entire domains (75, 133); (c) construction of chimeric proteins by domain shuffling (41); (d) fusion to cationic peptides (or other domains) to render lysins capable to cross the outer membrane (OM), a widely recognized drawback of lysin therapy against G– bacteria (122, 134, 135), or to increase CW affinity (136); (e) co-administration of lysins with membrane destabilizing agents (EDTA, carvacrol, etc.), especially in G– pathogens (53, 112).

**LYSINS AGAINST GRAM-POSITIVE BACTERIA**

*Streptococcus pneumoniae*

The key aspect of the *S. pneumoniae* system is the role of the aminoacohol choline in the enzymatic activity of the bacterial autolysin LytA, and the pneumococcal phage lysins. Choline forms part of the (lipo)teichoic acids and constitutes an absolute CW substrate. This peculiarity explains the extreme specificity...
| Species                  | Lysin/phage          | Susceptible bacteria tested                      | Methodology used                                      | Acc. No.; comments                                      | References |
|-------------------------|----------------------|--------------------------------------------------|-------------------------------------------------------|--------------------------------------------------------|------------|
| **S. pneumoniae**       |                      |                                                  |                                                       |                                                       |            |
| Pal/Dp-1                | Pneumococci and relatives | Biofilm; synergy with Cpl-1                      | Colonization and sepsis (mice)                        | O03979                                                | (40, 43–45) |
| Cpl-1/Cp-1              | Pneumococci and relatives | Biofilm; synergy with Pal and antibiotics; cell culture | Colonization, otitis, pneumonia, sepsis (mice)         | P15057                                                | (43–52)    |
| LytA                    | Pneumococci and relatives | Biofilm                                          | Sepsis (mice)                                         | P06653; major autolysin                                | (45, 53)   |
| Cpl-7/Cp-7              | Streptococci; other G+ | Biofilm                                          | Colonization (mice), pneumococcal infection (zebrafish) | P19385                                                | (45, 53)   |
| Cpl-7S                  | Streptococci; other G+ | Cell culture                                     | Colonization (mice), pneumococcal infection (zebrafish) | Engineered protein                                   | (51, 53)   |
| Cpl-711                 | Pneumococci and relatives | Biofilm; synergy with antibiotics; cell culture | Colonization and sepsis (mice), pneumococcal infection (zebrafish) | Chimera of Cpl-7 and Cpl-1                           | (41, 51, 54) |
| PL3                     | Pneumococci and relatives | Biofilm                                          | Pneumococcal infection (zebrafish)                    | Chimera of Pal and LytA                               | (38)       |
| **S. pyogenes (GAS)**   |                      |                                                  |                                                       |                                                       |            |
| PyG/C1                  | GAS and other streptococci | Biofilm; cell culture (intracellular killing of GAS) | Colonization (mice)                                  | J7M5V6                                                | (27, 55–57) |
| PyG/MGAS315              | GAS and other streptococci |                                                | Sepsis (mice)                                         | AAM79913                                              | (58)       |
| **S. agalactiae (GBS)** |                      |                                                  |                                                       |                                                       |            |
| PyGBS                   | GAS, GBS and other streptococci |                                                | Colonization (mice)                                  | Q5MY96                                                | (59, 60)   |
| **S. aureus**           |                      |                                                  |                                                       |                                                       |            |
| Lysostaphin             | Staphylococci        | Biofilm; synergy with LysK; CHAP and antibiotics; controlled release | Sepsis and colonization (mice, rats)                  | P10547; from S. simulans                              | (35, 61–72) |
| LysK/K                  | Staphylococci        | Biofilm; complex with polycationic peptides      | Colonization (mice)                                  | Q6Y7T6                                                | (61, 73, 74) |
| CHAPK                   | Staphylococci        | Biofilm; synergy with lysostaphin; controlled release | Colonization (mice)                                  | CHAP domain of LySK                                   | (64, 75–78) |
| ClyS                    | Staphylococci        | Synergy with oxacillin and vancomycin            | Colonization and septiemia (mice)                     | Chimera of Twort phage lysis (O56788) and ϕNM3 phage lysis (Q2FWV2) | (79)       |

(Continued)
| Species | Lysin/phage | Susceptible bacteria tested | Methodology used | Acc. No.; comments | References |
|---------|------------|-----------------------------|-----------------|--------------------|------------|
|         |            |                             | **In vitro**    | **in vivo**        |            |
| SAL-1/SAP-1 | Staphylococci | Biofilm | Bacteremia (mouse), toxicity and pharmacokinetics (rats, dogs, monkeys), pharmacokinetics and pharmacodynamics (healthy humans) | SAL200 is a drug formulation of SAL-1 | (80–84) |
| P128    | Staphylococci | Biofilm; cell culture; synergy with antibiotics | Colonization and sepsis (rats) | Chimera of Gp57 (Q6Y7R1) and lysostaphin; under clinical testing | (85–92) |
| LysGH15/GH15 | Staphylococci | Biofilm | Sepsis and pneumonia (mice) | D6QY02; under clinical testing | (93–97) |
| OF-301 (PlySs2)/S. suis 9/1591 prophage | S. aureus, S. pyogenes, S. pneumoniae; other G+ | Biofilm; synergy with antibiotics | Sepsis (mice) | M1NS67; under clinical testing | (33, 98, 99) |
| ClyF    | Staphylococci | Biofilm | Sepsis (mice) | Chimera of Ply187 (O56785) and PlySs2 | (100) |
| Mycobacterium sp. | LysB/Ms6 | Mycobacteria | Growth inhibition with surfactants | Q92X49; esterase | (101, 102) |
| LysB/Bx2 | Mycobacteria | Growth inhibition with surfactants | Q9F279; esterase | (101) |
| LysA/BTCU-1 | Mycobacteria | Cell culture | O64203; intracellular killing of M. smegmatis | (103) |
| LysB/BTCU-1 | Mycobacteria | Cell culture | R9B59; intracellular killing of M. smegmatis; esterase | (103) |
**TABLE 2 | Selected lysins active against Gram-negative bacteria.**

| Species | Lysin/phage | Susceptible bacteria | Methodology used | Acc. No.; comments | References |
|---------|-------------|----------------------|------------------|-------------------|------------|
| **P. aeruginosa** | Lys1521/B. amyloliquefaciens phage | G– | Activity on intact bacteria | Q94ML9 | (104–107) |
| | EL188/EL | G– | Activity on permeabilized bacteria | CAG27282 | (108–110) |
| | KZ144/ϕKZ | G– | Activity on permeabilized bacteria | AAL83045 | (108, 110) |
| | OBPGp279/OBP | G– | Activity on intact bacteria | YP_004958186 | (111) |
| | Art-175 | G– | Activity on intact bacteria | Chimera of KZ144 and SMAP-29 peptide | (34, 112) |
| | LysPA26/JD010 | G– | Activity on intact bacteria, biofilm | A0A1V0EFL1 | (113) |
| **A. baumannii** | LysAB2/ϕAB2 | G– and S. aureus | Activity on intact bacteria in vivo: sepsis (mice) | F1BCP4 | (114, 115) |
| | LysABP-01/ØABP-01 | G– | Activity on intact bacteria; synergy with colistin | KF548002 | (116) |
| | PlyAB1/Abp1 | A. baumannii | Activity on intact bacteria | YP_008058242 | (117) |
| | Ply307/RL-2015 | A. baumannii; otros G– | Activity on intact bacteria, biofilm in vivo: sepsis (mice) | AJG41873 | (36, 118) |
| | LysAB3/A. baumannii ATCC 17978 prophage | A. baumannii | Activity on intact bacteria | ABO12027 | (119) |
| | LysAB4/A. baumannii ATCC 17978 prophage | A. baumannii | Activity on intact bacteria | CP000521 | (119) |
| **E. coli** | Lysep3/Ep3 | E. coli, P. aeruginosa | Activity on permeabilized bacteria | A0A088FRS6 | (120) |
| | Lysep3-D8 | G–, Streptococcus sp. | Activity on intact bacteria | Chimera of Lysep3 and Lys1521 (Q94ML9) | (121) |
| | Colcin-lysep3 | E. coli | Activity on intact bacteria in vivo: intestinal infection | Chimera of Lysep3 and colicin A (Q47108) | (122) |
| | EndoT5/T5 | E. coli | Activity on permeabilized bacteria | Q6QGP7 | (123) |
| | PlyE146/E. coli 8.0569 prophage | G– | Activity on intact bacteria | EKK47578 | (37) |
| **K. pneumoniae** | K11gp3.5/K11 | G– | Activity on permeabilized bacteria | B3VCZ3 | (124) |
| | KP32gp15/KP32 | G– | Activity on permeabilized bacteria | D1L2U8 | (124) |
| | KP27 lysin/KP27 | G– | Activity on permeabilized bacteria; cell culture | K7NPX3 | (125) |
| **C. freundii** | CIP1 lysin/CIP1 | Citrobacter sp. | Activity on intact bacteria | A0A1B19XL3 | (126) |
| **S. maltophilia** | P28 | G– and some G+ | Activity on intact bacteria | Lytic enzyme from a bacteriocin system | (127) |
| **Burkholderia sp.** | AP3gp15/AP3 | G– | Activity on permeabilized bacteria | A0A1SSNV50 | (128) |
of CBPs for pneumococci. The first article reporting the use of a CBP as an enzymatic demonstrated the capacity of the NAM-amidase Pal to kill pneumococci of every serotype tested, including penicillin-resistant isolates (40). These results were confirmed in a mouse model of nasopharyngeal carriage (27). The Cpl-1 lysozyme has also been successfully tested in several in vitro assays and in different animal models of infection (46–48), and a synergistic effect was found when Cpl-1 was used together with several antibiotics (49, 50), or in combination with Pal (43, 44). The Cpl-7 lysozyme represents an exception to choline-recognizing pneumococcal lysins, since it harbors a different CWBD (138–140) that allows it to recognize and kill a broader range of bacteria. Moreover, the bactericidal effect of Cpl-7 has been improved in the engineered Cpl-7S by inverting the net charge of its CWBD (53). To date, the most powerful killing lysins tested against S. pneumoniae are nonetheless chimeric proteins: Cpl-711, a chimera of Cpl-7 and Cpl-1 (41), and PL3, a fusion protein between Pal and LytA [Table 1; (38)]. Treatment with Cpl-711 strongly reduced the attachment of S. pneumoniae to human epithelial cells, and a single intranasal dose of Cpl-711 significantly reduced nasopharyngeal colonization in a mouse model (51).

**Staphylococcus aureus**

Although S. aureus is frequently carried asymptomatically in humans, it is also the cause of a variety of diseases and, particularly, methicillin-resistant strains (MRSA) are responsible for a great percentage of all infections, up to 80% in some countries (141). The S. aureus PG displays a characteristic pentaglycine interpeptide cross-linking the glycan strands (142). Most tested lysins in the S. aureus system contain two CDs (endopeptidase and NAM-amidase) together with an SH3b CWBD (61, 143, 144). Although the exact interaction between the CWBD and the structures to which these domains bind remains to be demonstrated in many cases, it has been proposed that some CWBDs recognize the pentaglycine peptide cross-bridge (145) or the CW-associated glycopolymers (79). Of note, the vast majority of studies reporting the therapeutic use of lysins are directed to fight S. aureus infections (20, 21). Together with lysostaphin (produced by *Staphylococcus simulans*), LysK and its derivatives seem to be the most lethal lysins against S. aureus, including MRSA (73, 76, 146, 147) as well as vancomycin-intermediate and -resistant isolates [see reference (21) and references therein]. Other examples of anti-staphylococcal lysins include several engineered proteins such as chimeric or truncated proteins (76, 85, 100, 148, 149) or fusion proteins with short cationic peptides able to cross the eukaryotic membrane and kill intracellular S. aureus (150, 151). Nevertheless, lysin-based studies that consider S. aureus as a respiratory pathogen are scarce and only include some decolonization assays (62, 63, 75, 85) and a single example of endolysin efficacy in a mouse S. aureus pneumonia model (93).

**Other Gram-Positive Pathogens and Mycobacteria**

*S. pyogenes* is a major causative agent of upper respiratory tract infections (152). The most relevant example of a lysin targeting this pathogen is PlyC, a peculiar multimeric enzyme that kills group A streptococci with high efficiency (27, 55). In addition, the ability of PlyC to penetrate respiratory tract epithelial cells to eliminate intracellular *S. pyogenes* cells has also been proven (56). This intracellular activity overcomes one of the major drawbacks of antibiotic therapy against streptococcal throat infections, which is bacterial self-protection by cellular invasion. Other lysins reported to kill *S. pyogenes* are PlyPy (58) and the broad range, pneumococcal phage-derived Cpl-7S (53). Besides, group B streptococci are known to cause severe pneumonia in newborns (153). At least one attempt has been conducted in mice toward oropharyngeal decolonization of group B streptococci using PlyGBS lysin (59).

The acid-fast *M. tuberculosis* is still rather unexplored for the development of lysin-based therapy. This might be due to the peculiarity of *Mycobacterium* CW structure, which comprises a thick PG layer covalently attached to arabinogalactan sterified with mycolic acids (154). Because of this architecture, the lytic cassette of mycobacteriophages comprises two different lytic enzymes: a classical PG hydrolase (usually named LysA) and mycolyl-arabinogalactan esterase (LysB), which cleaves the ester bond linking mycolic acid to the arabinogalactan-PG layer. As a result, the mycolic acid layer detaches from the cell, rendering vulnerable to osmotic shock and, finally, lysis (155). Some in vitro assays have been conducted with both mycobacteriophage-derived hydrolases, yielding, in general, promising results that show either growth arrest (101) or a bactericidal effect (103), but further research is still required. The mycobacterial endolysins and their therapeutic potential have been recently reviewed (156).

**LYSINS AGAINST GRAM-NEGATIVE BACTERIA**

*Pseudomonas aeruginosa*

The first lysins tested against *P. aeruginosa*, for example, EL188, only killed bacteria when membrane permeabilizers (e.g., polycationic agents, EDTA) were co-administered (108, 109). Due to the potential difficulties of therapies based on the co-administration of lysins and permeabilizing agents, some of the most recent efforts have been directed toward the engineering of the enzymes themselves, giving rise to the “artilysin” concept (134). In this study, lysins were fused to cationic, antimicrobial peptides (AMPs), and these fusions were able to exert a permeabilizing activity that allowed them to cross *P. aeruginosa* OM to degrade the PG layer both in vitro and in vivo (134). Art-175 is an artilysin that was constructed by fusing lysin KZ144 and the sheep myeloid AMP 29 (SMAP-29), and further optimizing the thermostability of the resulting chimera by point mutation of several cysteine residues (34). Art-175 was able to efficiently kill either antibiotic-susceptible or MDR *P. aeruginosa* strains. Of note, Art-175 also controlled the appearance of persisters, i.e., bacterial subpopulations transiently tolerant to antibiotics that often appear upon antiinfective chemotherapy (157).

Despite the engineering efforts mentioned above, lysins able to lyse G- bacteria on their own are also currently available.
Typically, this intrinsic activity from without relies on non-enzymatic mechanisms, which were first described for the T4 phage lysozyme (158) and then in several *P. aeruginosa* phage lysins (159). These lysins harbor AMP-like elements (peptides with an amphipathic secondary structure and a positive net charge) that destabilize the OM. In some cases, as for T4 lysozyme, these regions account for the bactericidal activity of the enzyme to a higher extent than the enzymatic activity itself (158).

One of the first examples of a lysozyme with a natural cationic peptide exploited as an enzymbiotic was the *Bacillus amyloliquefaciens* phage lysin Lys1521, which was indeed able to lyse *P. aeruginosa* cells (104). Other examples of *P. aeruginosa* lysins with intrinsic anti-G− activity include OBPgp279 (124) and LysPA26 (113). Although active research is being performed to deal with the OM barrier issue, no extensive in vivo experimental evidence has been provided for the clearance, upon lysin treatment, of *P. aeruginosa* from respiratory infections.

**Acinetobacter baumannii**

In general, lysins against G− bacteria appear to be less specific than their G+ counterparts, possibly due to the (apparently) simpler organization of the former sacculi (160). This broader spectrum allows some lysins to kill several pathogenic genera, like the already mentioned lysin LysPA26, which besides *P. aeruginosa* can also lyse other G− pathogens such as *E. coli*, *K. pneumoniae* or *A. baumannii* (113), or Art-175, which also kills *A. baumannii* (112). This bacterium is a potential respiratory pathogen (particularly for immunocompromised and debilitated patients) that is receiving great attention in recent years due to its worrisome increased antibiotic resistance (161). Thus, several enzymbiotics have also been developed with emphasis in their *A. baumannii* killing capacity, such as LysAB3 and LysAB4 (119), PlyAB1 (117), and LysABP-01 (116).

PlyF307 was capable of killing *A. baumannii* isolates, including MDR strains, both in planktonic and biofilm cultures (36) and represents the first example of an intact lysin with intrinsic anti-G− activity tested in a mammalian (mouse bacteremia) model. Unsurprisingly, it was later determined that such intrinsic activity from without partly resided in a cationic peptide located in the C-terminal domain of the lysin (118). Further studies revealed that this region contains sub-domain structural motifs with membrane permeabilizing ability, but lacking enzymatic activity; similar motifs have also been found in other lysins. For example, lysin LysAB2 (114) represents a broad-spectrum enzymbiotic, both active against G+ and G− bacteria (*A. baumannii*, *Escherichia coli* and, surprisingly, *S. aureus*). Based on its permeabilizing properties (114), AMPs based on the C-terminal region of LysAB2 have been synthesized and demonstrated high antimicrobial activity when tested in mice infected with *A. baumannii* (115).

**Other Gram-Negative Pathogens**

In spite of being a prominent member of the ESKAPE group (162), there are only few reports of lysins active against *K. pneumoniae*. As already mentioned, LysPA26 also showed bactericidal activity against *K. pneumoniae* (113). Consequently, it is conceivable that some of the other broad spectrum anti-G− lysins would kill *K. pneumoniae*. As for specific *Klebsiella* phage lysins, some examples of lysins with proven lytic activity are those from phages K11, KP32, and KP27 (124, 125, 163), but only KP32 and KP27 were tested for their anti-*Klebsiella* activity. Although usually associated with intestinal infections, *E. coli* is also a frequent cause of nosocomial pneumonia (164). Again, some of the other G− lysins are also active against *E. coli* (105, 113, 114, 116, 124). Specifically from an *E. coli* phage, Lyse3 lysin has demonstrated noticeable activity against permeabilized *E. coli* cells (120). Moreover, a chimeric construction between Lyse3 and a colicin was able to traverse the OM via specific recognition by OM transporters (122, 165).

**CONCLUDING REMARKS AND FUTURE TRENDS**

As MDR bacterial respiratory pathogens are increasingly prevalent, alternative therapeutics are urgently needed. Lysins represent more than a hope in this scenario and may be a perfect counterpart to therapies based on standard antibiotics. The potential for lysin development is seemingly endless. For example, thousands of putative lysins, many of which displaying novel domain architectures, have been recently described using bioinformatic techniques (166). All this huge amount of information, together with the crystal structures of lysins and a more detailed knowledge on the bacterial CW structure, will provide better insights to design and construct “tailor-made lysins” potentially directed against any desired pathogen. Drug delivery and other added-value systems involving lysins are now also being researched by setting up different approaches (167–170). Several polymers have been studied as potential drug release vehicles not only for research but also for clinical purposes. Particularly interesting is the case of poly(N-isopropylacrylamide) (PNIPAM) that has been used for the coadministration of the CHAP$_{k}$ lysin and lysostaphin through a thermally triggered release event (the temperature increase due to infection) (64).

Although a limited number of endolysins have entered clinical trials and some of them are already available in the market [reviewed in reference (18)], phages and phage-based products are subjected to strict regulatory measures (171). Moreover, in spite of their demonstrated specificity and lack of resistance development, the use of phage endolysins in humans raises several concerns. Among them, the relatively short plasma life of lysins, their immunogenicity and possible toxicity, the proinflammatory response to bacterial debris, and the difficulties to attack intracellular bacteria have been mentioned. Although only limited data of phage lysin interactions with the human body, e.g., pharmacokinetic/pharmacodynamic studies, have been published, it is encouraging that most (if not all) of the above mentioned potential limitations lack current experimental support (18, 23, 25). Although this scenario seems favorable toward hitting the clinic in the short term, further evidence is still due, especially when bacterial respiratory diseases—in particular, those caused by G− bacteria—are considered. Additional efforts
to cover the currently unmet therapeutic requirements are warranted.

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RV, EG, and PG wrote, edited, and approved the final manuscript.

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