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Endolysins of Bacteriophages as an Anti-Methicillin Resistant *Staphylococcus aureus* Infection in Children: A Narrative Review

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Abstract

Context: Spread of Methicillin-resistant *Staphylococcus aureus* (MRSA) can cause serious and sometimes fatal diseases especially in children. Outbreaks and increasing the prevalence of antibiotic-resistant MRSA at pediatric hospital calls for the development of novel preservation techniques. Endolysins and bacteriophages have been used successfully to control bacterial infections in children. Endolysins were considered as a useful treatment especially for the pathogens without disturbing the normal flora, the low chance of bacterial resistance, and their ability to kill colonizing pathogens on mucosal surfaces. Herein, we aimed to review the effectiveness of endolysins of bacteriophage for controlling methicillin-resistant *Staphylococcus aureus* infections in children.
Evidence Acquisition: This review was performed by searching studies indexed in international databases including PubMed, Scopus, Web of Science, Science Direct, as well as Google Scholar published from 2000 until 2016.
Results: Experimental data show that endolysins of *Staphylococcus aureus* bacteriophage can be used to combat methicillin-resistant *Staphylococcus aureus* infections in children.
Conclusions: Endolysins of bacteriophages could be effective for controlling a variety of methicillin-resistant *Staphylococcus aureus* infections.

Keywords: Review, Endolysins, Child, Infection Methicillin-Resistant *Staphylococcus aureus* (MRSA)

1. Context

Methicillin-resistant *S. aureus* (MRSA) causes skin and soft-tissue infections, pneumonia, osteomyelitis, septic arthritis, bacteremia, and other invasive diseases in children that were limited to patients that were hospitalized in the 1960s (1).

Antibiotic-resistant is a major challenge in current medicine that is related to all antibiotic prescriptions in pediatrics. Of these, 53% are related to children under the age of 4 and 70% of the children are infants who received at least 1 antibiotic during the first 200 days of life (2-4).

The unjustifiable, over use of antibiotics, and lack of involvement of pharmaceutical companies regarding development of new antimicrobial agents subsequently lead to propagation of resistant bacterial pathogens such as MRSA, especially in children (5-9).

With the increasing prevalence of antibiotic-resistant bacteria and high costs on health budgets, a novel alternative to antimicrobial agents is necessary to be expanded. Endolysins of phages are suggested that could prevent and/or treat the bacterial infections.

Endolysins and enzybiotics belonged to a group of enzymes that are encoded by phage at the end of lytic cycle, that lead to peptidoglycan hydrolases and progeny virions extrication.

MV-L from the phage MR11 is the first staphylococcal lysin that was experimented in a mouse infection model (10, 11).

Bacteriophages, viruses, and specially infecting bacteria, are composed of nucleic acid that encapsulated by many forms of protein coat (capsid). There are various forms of phages from filaments to highly complex structures consisting of a head and a tail. Phages in terms of life cycle are lytic and lysogenic cycle. The therapeutic application of bacteriophages in children is different. For example, the first trial was in 1919 by d'Herelle, which was performed on 5 children suffering from diarrhea. Furthermore, bacteriophages were used in the treatment of meningitis in a neonate, UTIs, staphylococcal skin diseases,

atopic dermatitis, and infection of the upper respiratory tract in the course of asthma in children (12-15).

Therefore, phages were not only accepted for health but were also used as a prophylaxis of diarrhea in children as well as prescribed for this purpose in a neonates at the Wolski hospital in Warsaw (16-18).

Jasienski described phage therapy in forma subcutaneously or topically in cases of osteomyelitis and suppurative skin disease in children aged 1 - 3 months (19).

Defects in application of bacteriophages were recently detected such as manifestation of mutants resistant to phages, or transfer of bacterial toxin genes and antibiotic genes resistance by phages.

Unlike antibiotics and bacteriophages, lysins can be used to selectively specific pathogenic bacteria without disadjusting the normal flora. In fact, the low chance of bacterial resistance as well as no transfer of the bacterial toxin and antibiotic resistance genes with a view to kill bacteria colonizing mucous membranes, has spread the tendency to use of lysins for the treatment of systemic infections (20-22).

1.1. Endolysin Structure

First, endolysins are produced by bacteriophages and bacterial viruses. Then in the lytic cycle, they attack bacterial cells and cause lysis of bacteria (Figures 1 and 2).

The structure of endolysins is different from the aspect of their objective; Gram-positive and Gram-negative bacteria, generally ones that the Gram-positive bacterial infection are designed modular and are between 25 kDa to 40 kDa in size.

Enzymatical active domain (EADs)3 at the N-terminus is separated from functional domain that is termed cell wall binding domains (CBDs)4 at the C-terminus (23). The EADs3 catalyzes break down peptidoglycan of bacterial cell wall (Figure 3).

Endolysins, based on the specific bond of the peptidoglycan that are attacked by the EADs3, are classified into at least 5 different groups: N-acetyl-ˆ-D-muramidases (also termed lysozymes), lytic transglycosylases, N-acetyl-ˆ-D-glucosaminidases, N-acetylmuramoyl-L-alanine amidases, and endopeptidases (25-27).

CBDs4, by recognizing and binding to ligand molecules in parts of the peptidoglycan, confer specificity to endolysins for certain cell wall types (28). The specific activity of CBDs4 causes that endolysins not only kills the disease organism with no effect on the normal human bacterial, but also makes resistance to endolysins as a rare event, unlike antibiotics that have broad spectrum and kill many different from the normal human bacterial resistant do (29).

The dominant domain in the peptidoglycan has binded to GlcNAc residues in the sugar backbone of the peptidoglycan (29), however, common ligand to most staphylococcal strains is the glycine-rich interpeptide bridge that was reported for the cell wall targeting domain of lysostaphin (23, 30).

The lytic activity of endolysins can be detected through a variety of macroscopic phenomena such as: decrease in turbidity of a bacteria in the turbidity reduction assay (TRA), development of a clear zone within a semisolid matrix with bacteria in the zymogram or overlay assay, and decrease in the number of bacteria in suspension that is determined by serial dilution plating in the Minimum inhibitory concentration (MIC) assay (31).

A number of staphylococcal endolysins are characterized, including: LysK, phill, Twort, 187, P68, phiWMY, SAL-1, SAP-2, ClyS, and MV-L (11, 31-40).

However, the endolysin structures are not just limited to the module that is listed above. For example, in the streptococcal phage, XSA2 endolysin has centrally located CBDs4, with separating 2 terminal EADs3 (41), or in staphylococcal lysins 2, even 3 different catalytic domains are linked to a single binding domain, also the presence of 3 ECDs3 is described for the lysine Ply187 (42).

1.2. Bacterial Resistance to Endolysins

Unlike antibiotics that have expanded resistance genes within target pathogen and commensal organisms, the Genus- or species specificity of endolysins have presented (43).

Due to coevolution of endolysins to their species hosts led to highly binding and cleaving conserved targets in the cell wall, thus, resistance to endolysins will be a rare event (31).

Several studies from the Fischetti laboratory attempted to create MRSA strains that were resistant to the chimeric ClyS endolysin (44).

1.3. Safety

Endolysins are biodegradable and approximately have a short half-life of 15 - 20 minutes (36, 45). The main concern of systemic administration of lysins in humans or animals is the release of pro- inflammatory substances due to cellular debris associated with bacterial lysis such as teichoic acids, lipoteichoic acids, which can potentially lead to serious complications such as septic shock and multiple organ failure (46).

Herein, we sought to review the effectiveness of endolysins of bacteriophage for controlling methicillin-resistant *Staphylococcus aureus* infections in children.

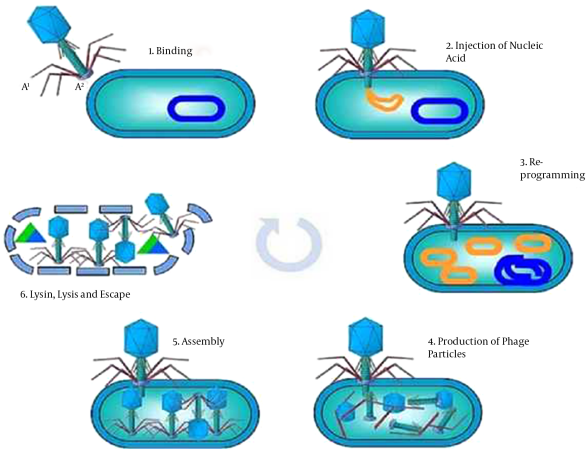

Figure 1. Lytic Cycle of Bacteriophage (http://www.yissum.co.il/technologies/project/7-2015-3131)

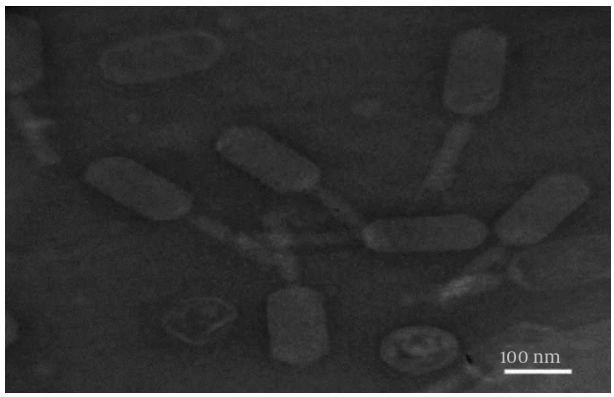

Figure 2. Electron Micrographs of the Phage A Negatively Stained With 2% Uranyl Acetate (pH = 4 - 4.5). Voltage 150 kV, Scale = 60 nm (69).

2. Evidence Acquisition

We performed a review by searching studies indexed in international databases including PubMed, Scopus, Web of Science, Science direct, and Google Scholar published from 2000 until 2016. The searched keywords were included review, endolysins, MRSA1, and Child, Infection. The qualitative results derived from the reviewed article were discussed here.

3. Results

3.1. Applications of Endolysins

3.1.1. Sepsis

Lysins have a rapid and short half-life that makes no sufficient time to observe a therapeutic effect (47). In a study by Daniel et al. in 2010, mice were intraperitoneally infected with type MRSA1 strain MW2 and treated 3 hrs later with an intraperitoneally administered single dose of (2 mg/mL) ClyS. The result has shown that the rate of survival in treated mice with ClyS was significantly higher (88%), whereas all of the control mice died (48). However, in a study by Paul et al. in 2011, IP administration of endolysin-deficient phage P954 as 2 doses (immediately and after 2 hours) fully protected the mice against lethality (49). Although, Endolysins completely act proprietary for each species, some endolysins have a wide host range, as shown in a study by Yang et al. in 2015. They showed a unique "chimeolysin", ClyR, with robust activity, and an extended-spectrum streptococcal host range, most streptococcal species, as well as representative enterococcal and staphylococcal species (50). Similar studies have been reported that LysK, from staphylococcal phage K, has been activated against 9 Staphylococcus species from both human and bovine sources, including MRSA. Similar results were also reported by Daniel B et al. (51-53). Gilmer et al. in 2016, reported bacteriophage lysin PlySs2 with broad lytic activity against MRSA1 and Streptococcus strains (54).

So far, a number of staphylococcal endolysins have been characterized, including: a study by Mathias Schmelcher et al. in 2014, has shown high potential of 9 PGHs, LysK, phi 11, Twort, 187, P68, phiWMY, SAL-1, SAP-2, ClyS, and MV-L, for treatment of *S. aureus* infections. The results showed that 80a, phi 11, LysK, lysostaphin, 2638A, WMY, and vancomycin protected 100% of the animals from death, and Twort- or phiSH2-treated mice had less protection from systemic infection (50% - 60% survivors), and P68 (administered in 20%) (55).

In a study by Jingmin Gu et al. in 2011, has shown that

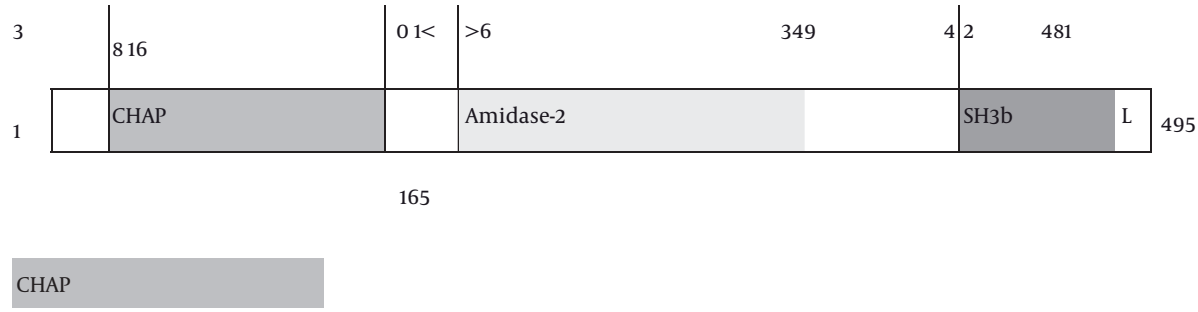

Figure 3. Enzymatical domains of endolysins K (Keary R, Sanz-Gaitero M, J van Raaij M, Mahony J, Fenton M, McAuliffe O, et al. Characterization of a Bacteriophage-Derived Murein Peptidase for Elimination of Antibiotic- Resistant *Staphylococcus aureus*. Current Protein and Peptide Science. 2016;17(2):183-90.) (24).

the best time and dose of endolysin were 1h after post infection by injected intraperitoneally of GH15 lysin (56).

A synergistic effect of Endolysins was also reported by Schuch et al. in 2016, in an investigation of combination therapy with lysin CF-301 and daptomycin, vancomycin, oxacillin antibiotics for treating MRSA1 (29).

3.2. Elimination of Biofilm

Staphylococcal infections are the most important cause of antibiotic resistant healthcare- associated infection, which may result in prolonged hospital stay or the use of medical devices that a critical hallmark of a chronic staphylococcal infection is the ability of bacteria to grow as a biofilm. In this study, Phi11 eliminated *S. aureus* biofilm formation on medical devices such as catheters (36).

In a study by Juna et al. in 2013, they exhibited rapid and effective bactericidal activity of SAL200 against encapsulated and biofilm-forming *S. aureus* as well as against planktonic *S. aureus* cells (57).

Schuch et al. in 2016, compared CF-301 with antibiotics for ability to eradicate MRSA1 biofilms that grown for 24 hours in polystyrene dishes. The results showed that CF-301 removed all visual biomass by 2 hours, whereas the antibiotics failed to remove biomass after 4 hours of treatment (58).

Endolysins are not only able to eliminate of biofilms but can also prevent formation of biofilm.

In a study by Fenton et al. in 2013, applied CHAPK has shown the potential of CHAPK as a decontaminating agent in the food and healthcare sectors for prevention and treatment of biofilm-associated staphylococcal infections (59).

Power of phages and their endolysins are different. In 2010, Son et al. compared thestaphylococcal biofilms removal activity of a bacteriophage SAP-2 and a derived endolysin from it. The results indicated that endolysin SAL-2 showed lytic activity against all strains of the Staphylococcus genus, whereas bacteriophage SAP-2 had antibacterial activity against only some *S. aureus* strains (44).

Some endolysins has shown to be effective on broad-spectrum biofilm. For example, in a study, LysH5 was determined to remove staphylococcal biofilms and kill sessile cells on 6 *S. aureus* and 3 S. epidermidis strains (60).

3.3. Future Perspective of Endolysin

According to an increasing threat imposed by multi resistant, this trend has expected to search for novel antimicrobials. The numbers of researches have described isolation and characterization of new endolysins. The potential applications of these enzymes in the fields of medicine, food safety, agriculture, and biotechnology will be intensified in the near future (10, 61, 62).

Currently, the dogma of endolysins that are effective only against Gram-positive bacteria failed, and researches have focused based on endolysins for the control of Gram-negative and intracellular pathogens; furthermore, endolysin can suggest for prevention of bacterial infections (63, 64).

The fast evolution of the molecular engineering techniques is described create tailor, truncated or recombinant of endolysins, based on optimized antimicrobials for every application, and create powerful tools for the detection and control of pathogens (27, 65, 66).

Currently, a novel therapeutic approach based on the display of endolysins, are displayed on the surface of a specific phages, in order to generate lytic antibacterial nanoparticles. Phage display will lead to the selection of peptides and proteins with high affinity, specificity, and penetration to any target with decreasing expense (67).

Phage display will develop as a new tool for extension modern vaccines and can be used as an application of bacteriophages and particles of phages, for example, endolysin in vaccine design (68).

In the near future, it will be possible to treat local infections by endolysins, for example, *S. aureus* prosthesis infections that are very difficult to treat with the morbidity of these infections (63).

3.4. What Challenges Do We Face for Treatments with Endolysin?

We already know that endolysins that are used in patients should not be any real challenges, for example, the product of Staphefekt SA.100 (Micreos, Bilthoven, The Netherlands) is registered as a medical device for treatment of eczema, rosacea, and acne, that is designed as constructed endolysin, which only targets the *S. aureus* and MRSA (64).

Using Endolysins for systemic infections can be more challenging. For instance, due to the fact that endolysins are very large molecules, they may be not only stimulate the immune system response and cause prevention of activity endolysin, but can also not enter into the cells and are not useful in the case of intracellular infections. Therefore, they should be used as a recombinant protein, for example truncated endolysins or use by technique of phage display (64).

The ligands of endolysin staphylococcal strains are pentaglycine bridges that can be more challenging. It will anticipate that the through repeated use of endolysin may be creating resistance, due to the fact that the pentaglycine bridges are substrated for lysostaphinand. Previous studies showed that resistance to lysostaphin could be ascribed to modifications within the pentaglycine bridge (such as reduction to a single glycine residue) or incorporation of a serine residue (27).

Currently, there is an over-growing concern over the global spread of antibiotic resistance in children infections such as MRSA1 that is the most important cause of antibiotic resistant in healthcare-associated infections of pediatric and higher mortality rates.

Increasing frequency of MRSA1 infections among children and changing patterns in antimicrobial resistance have led to renewed interest in the use of lysins therapy to treat such infections (69-72).

Oral, subcutaneous or topical phage therapy were often used in children infections for treatment of osteomyelitis, myositis, suppurative wounds, respiratory infections, skin and subcutaneous tissues infections, furunculosis, gastrointestinal infections, and even in septicemia (12, 14, 73, 74).

Significantly, data indicates that an efficient phage therapy can be less expensive than antibiotic therapy. However, there is still a great lack of formal rout of administrations due to inactivation of administered phages by a neutralizing antibody and allergic reactions to them, appearance of mutants resistant to phages, capture and transfer of bacterial toxin genes, as well as antibiotic genes resistance by phages (75).

The preferences of lysins specifically kill the species of (or subspecies) bacteria without affecting the surrounding normal flora. Therefore, the occurrence of lysin-resistant bacteria unlikely phage and antibiotics, which are usually a broad spectrum, kill many different bacteria such as normal human bacterial flora.

In lysine therapy, unlike phage therapy, no transfer of bacterial toxin genes and antibiotic genes resistance is occurred. Lysins are used as recombinant enzymes that applied exogenously to Gram-positive bacteria that cause rapid lysis (76). Therapeutic lysin is systemic or intravenous thus far, and is observed to have no harmful, abnormal, or irritant side effects in preclinical trials in vivo. Lysins can elicit an immune response; this does not neutralize their activity or prevent their use as antibacterial in the treatment of systemic infections (77).

4. Conclusions

Currently, a novel therapeutic approach based on endolysins and creation of truncated or recombinant of endolysins, and phage has displayed lead to create of powerful lysins for control of pathogens, production of peptides and proteins with high affinity, specificity, and penetration to any target with decreasing expense.

Footnotes

Authors' Contribution: Study concept and design: Mohammad Sadegh Rezai, Pooria Gill, Golnar Rahimzadeh; acquisition of data: Golnar Rahimzadeh; Analysis and interpretation of data: Golnar Rahimzadeh; drafting of the manuscript: Golnar Rahimzadeh; critical revision of the manuscript for important intellectual content: Mohammad Sadegh Rezai and Golnar Rahimzadeh; study supervision: Mohammad Sadegh Rezai.

Conflict of Interest: None declared.

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