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BREAKING THE RESISTANCE: BACTERIOPHAGE "NOVEL" PISF-AB082 AS AN ALTERNATIVE TREATMENT FOR MULTIDRUG RESISTANT A. BAUMANNII

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Abstrak

Acinetobacter baumannii is a gram-negative, non-motile bacterium that causes infections, particularly in wounds and burns. MDR A. baumannii strains are resistant to multiple types of antimicrobials, making them difficult to treat. Phage therapy is a potential strategy to combat MDR A. baumannii infections, as bacteriophages can specifically target and kill bacterial cells without harming human cells. This study aims to review the efficacy of bacteriophages as an alternative treatment for MDR A. baumannii and explore their synthesis methods. The article presents a method for isolating and purifying bacteriophages from waste samples using MDR A. baumannii as an indicator. Bacteriophage therapy has no detectable side effects and can reduce the impact on human gut microflora compared to antibiotics. However, more clinical research is needed to establish the safety and effectiveness of using bacteriophages as an antibiotic-resistant therapy. In conclusion, bacteriophages have the potential to become a therapeutic agent to control nosocomial infections caused by MDR A. baumannii. The specificity of bacteriophages for the infected pathogen reduces their impact on human gut microflora compared to antibiotics. However, more research is required to establish the safety and effectiveness of bacteriophages as an antibiotic-resistant therapy, including studies on potential side effects and administration methods. Overall, this study highlights the potential of bacteriophages as an alternative to antibiotics and the need for further research in this area.

Kata Kunci: Acinetobacter baumannii, Bacteriophages, Antibiotic resistance, Phage therapy, Nosocomial infections

INTRODUCTION

Acinetobacter baumannii (A. baumannii) is a bacterium that causes various infections, especially burns and wound infections (Mirzaei et al., 2020). The bacterium is non-motile, gram-negative, aerobic, and is listed as one of the six most dangerous pathogens, the ESKAPE pathogens (Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumonia, Acinetobacter baumannii, Pseudomonas aeruginosa, and Enterobacter spp.) (Rice, Cohen, Long, & Jurjevich, 2020). ESKAPE pathogens are resistant to antibiotics and are involved in most nosocomial infections (Navidinia, Mobarak, & Malekzadeh, 2019). Some strains of A. baumannii have been found to be resistant to almost all known antibiotics. Multidrug-resistant (MDR) A. baumannii refers to strains that are resistant to at least three of the five types of antimicrobials, including beta-lactamase inhibitors, carbapenems, cephalosporins, fluoroquinolones, and aminoglycosides. In Indonesia, MDR A. baumannii has been found in various hospitals. A study at Adam Malik Hospital in 2012 provided data on the prevalence of A.

baumannii, which was 644 out of 3,693, with 147 of them resistant to first-line antibiotics. A study at Dr. Soetomo Hospital found more MDR strains in 2016 (Sutandhio et al., 2019). The bacterium's ability to form biofilm makes it resistant because it can protect itself from drug diffusion. Therefore, alternative treatment for this infection is urgently needed.

Bacteriophage therapy is an alternative treatment for MDR bacterial infections. A bacteriophage (phage) is a virus that infects and lyses its bacterial host. Phage therapy is a century-old therapeutic method that has been applied to treat bacterial infections (Papon, Haque, & Mulani, 2019). With the increasing antimicrobial resistance, the focus on phage therapy has been renewed (Steenburgh & Nakai, 2020). The use of phages for therapy has shown many advantages, including host specificity (not affecting normal flora and eukaryotic cells), rapid replication within bacteria, and killing of host cells (Parker et al., 2021). In addition to lytic phages in the treatment of bacterial infections, phage-derived antimicrobial agents such as endolysin have been shown to be potent antimicrobials for bacterial infections in vitro and in animal models (Zhuo et al., 2022). Thus, the isolation of phages is a potential strategy to combat MDR A. baumannii (Li et al., 2016). Before clinical use and application, phage therapy needs to be examined for safety and effectiveness.

This study aims to review the efficacy of the "novel" bacteriophage as an alternative treatment for MDR A. baumannii and to explore the synthesis method of bacteriophages as an alternative therapy for MDR A. baumannii.

RESEARCH METHODS

The method used in writing this scientific paper is a literature review conducted by searching various online databases and search engines. The online databases used were PubMed, while the search engines used were Google and Google Scholar. The search was conducted using the keywords antibiotic resistant, phage therapy, and Acinetobacter baumannii. The literature used for this paper consisted of publications from 2006 to 2022 that were relevant to the topic being reviewed

RESULTS AND DISCUSSION

Results

Achinobacter Baumanii: The Story So Far

Acinetobacter baumannii is a pleomorphic, non-motile, aerobic, gram-negative bacillus and opportunistic pathogen. A. baumannii has a high incidence in immunocompromised individuals, especially those who have been hospitalized for more than 90 days. Multidrug-resistant (MDR) pathogen phenomenon is a serious concern related to nosocomial and community-acquired infections. WHO states that antimicrobial resistance is one of the three most important health problems faced by humans (Bassetti & Filipović, 2022). The most common MDR pathogens are included in "ESKAPE," an acronym for Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa, and Enterobacter spp. A. baumannii has wide resistance to most first-line antibiotics. A. baumannii is a cause for concern in the desert conflict zone in Iraq and is referred to as "Iraqibacter."

A. baumannii targets moist tissues such as mucous membranes or exposed skin areas, either due to accidents or injuries. Infected skin initially appears like "peau d'orange" (like the skin of an orange). In the affected skin area, hemorrhagic blisters are visible with necrotic processes followed by bacteremia. If left untreated, the infection can

become septicemia and lead to death (Taskeen et al., 2022). However, co-pathogens such as Klebsiella pneumoniae, Candida albicans, and Enterococcus faecalis, contribute to aiding A. baumannii in entering the bloodstream. A. baumannii is rarely found in normal skin flora. Acinetobacter is found in body lice of homeless people, indicating another potential reservoir for the pathogen. The main risk group is soldiers deployed to conflict zones, particularly in Iraq where it infects wounded soldiers. Soft tissue infections are the main colonization site in the axillary region, groin, and toes. Patients with weakened immunity in hospitals undergoing dialysis and antimicrobial therapy are at risk of A. baumannii infections in the respiratory tract, blood, pleural fluid, and urinary tract as the colonization site. Pneumonia can be a threat to patients requiring ventilation because A. baumannii can form biofilms on the surface of endotracheal tubes and colonize the lower respiratory tract.

OmpA, one of the "Outer membrane proteins" (OMPs) group, is known as a potential infection factor of the pathogen. OmpA from A. baumannii attaches to the host epithelium and mitochondria, causing dysfunction and leading to mitochondria swelling, release of cytochrome c and heme, and inducing apoptosis. OmpA also plays a role in resistance to complement and biofilm formation for persistent growth in unfavorable environments. This allows the bacteria to survive outside and inside the host.

Biofilm formation can occur on abiotic surfaces such as glass and intensive care unit equipment, as well as biotic surfaces such as epithelial cells. Biofilm formation occurs due to the availability of nutrients, pili, and outer membrane proteins. Pili and BAP (biofilm-associated protein) contribute to biofilm production and maturation. A. baumannii attaches to specific surfaces, and when pili attach, microcolonies form, followed by the development of the biofilm structure. BAP can mature biofilms on biotic and abiotic surfaces (Colquhoun & Rather, 2020). Other proteins, such as phospholipase D and C, contribute to A. baumannii virulence. Phospholipase D is important for resistance to human serum, evasion of epithelial cells, and pathogenesis. Phospholipase C increases toxicity to epithelial cells (Camarena & Marra, 2021). Along with OmpA, fimbriae are also expressed on the bacterial surface for adhesion to the host epithelium.

The presence of multi- and pandrug-resistant Acinetobacter explains the organism's ability to quickly adapt to changes in the environment (Peleg et al., 2008). Acinetobacter strain AYE contains an 86-kb region known as a "resistance island" that contains 45 resistance genes. Acinetobacter can change its genomic structure in response to antibiotics, as seen in UGD where broad-spectrum antibiotics are used. A. baumannii's possession of AmpC cephalosporinase makes it resistant to cephalosporins. A. baumannii's OXA-51 enzyme can hydrolyze penicillin. Resistance to carbapenems is mediated by oxacillinase encoded by the blaOXA-23 gene.

Acinetobacter-caused community-acquired pneumonia has been reported in Australia and Asia. The source of infection is from the throat, which occurs in people with excessive alcohol consumption. This is characterized by a severe and sudden onset, with secondary bloodstream infections and a relatively high mortality rate (Leung et al., 2006). Acinetobacter is a common cause of bloodstream infections acquired from UGD. Acinetobacter is also a burn pathogen that is difficult to treat in patients with severe burns. The average time from trauma to diagnosis with Acinetobacter infection is 15 days. All patients have a "peau d'orange" appearance, with severe infections causing blisters on the skin surface. Acinetobacter post-neurosurgical meningitis is becoming more common with many other gram-negative bacteria that are also problematic in postoperative care. External ventilation tube placement becomes a site of opportunistic infection.

A. baumannii has the ability to become resistant to antibiotics, such as degradation enzymes against beta-lactams, enzymatic modification against aminoglycosides, alteration of binding sites on quinolones, and other mechanisms to modify the outer membrane proteins (Tiruneh, Siman-Tov, Givon, Trauma Group, & Peleg, 2020). This makes it a highly antibiotic-resistant pathogen, and difficult to determine the appropriate drug. Carbapenems are used as the drug of choice for serious Acinetobacter infections. Although useful in the short term, this method jeopardizes the future efficacy of the drug as an effective antimicrobial.

Bacteriophage therapy has gained attraction due to the high specificity and rapid action of phages. Bacteriophages have been used as an alternative treatment to combat antibiotic resistance. Research by Yang et al resulted in the isolation of bacteriophages that showed efficacy against A. baumannii, making it a promising new therapy.

Isolation and Characterization of a Novel Phage, pIsf-AB02, with Lytic Activity Against Multi-Drug Resistant Acinetobacter baumannii

From the explanation of the phage method previously, there are several limitations in its clinical application. However, in a study by Kusradze et al., a strain of MDR A. baumannii, MDR-AB02, isolated from a patient's pneumonia catheter was found to be resistant to more than three groups of antibiotics (Sisakhtpour et al., 2022). This bacterium was used to screen bacteriophages in hospital waste. The isolated phages were labeled pIsf-AB02 as a "novel" phage with a clear, round, 2-3 mm plaque in two agar layers, indicating lytic phage properties. Most MDR A. baumannii isolates were sensitive to pIsf-AB02. The phage belongs to the order Caudovirales and the family Myoviridae. pIsf-AB02 remained stable with different temperatures, pH, and chloroform. Phage stability was also tested by incubating it for 24 hours at 37 °C. The phage's potency remained unchanged and remained stable against chloroform and normal pH. Resistance to high temperatures with a broader host range makes the phage usable in clinical practice to eradicate A. baumannii. Genome analysis showed that pIsf-AB02 had a double-stranded DNA with nine structural protein bands, where the main band was estimated as endolysin (Sisakhtpour et al., 2022).

pIsf-AB02, also known as "Novel phage" or "New phage," can infect and lyse 56.3% of A. baumannii isolates. The results showed that the phage was specific to A. baumannii and did not affect Klebsiella, Pseudomonas, or E. coli, which were previously included in the lytic spectrum to determine the range of bacteriophage hosts. To ensure the safety of phage therapy, HeLa cells were used to determine the phage's protective efficacy level when infected with the concentration of bacteria called MDR-AB02. The results showed that the phage protected HeLa cells from A. baumannii infection and could also eliminate A. baumannii (Sisakhtpour et al., 2022).

Most dsDNA phages can lyse cells using the holin-endolysin system as an antimicrobial agent that can break covalent bonds in peptidoglycan. However, endolysin from gram-negative bacteria can interfere with other bacterial cells. The pIsf-AB02 genome needs to be considered to ensure the safety of phage therapy. Phage will produce a synergistic effect when combined with antibiotics. The formation of bacterial biofilms was reported to decrease with combination phage-antibiotic therapy (Sisakhtpour et al., 2022).

Isolation and Purification of Bacteriophages from Waste Sample Using MDR A. baumannii as an Indicator

To isolate phages, a waste sample is collected and MDR A. baumannii is used as an indicator for bacteriophage screening. Briefly, 50 ml of waste is centrifuged and filtered through a 0.45 μm pore size membrane and mixed with an equal volume of 2x nutrient broth containing 1 ml of exponential phase MDR A. baumannii (OD600 = 0.6) at 35°C overnight with shaking at 160 rpm. The culture is then centrifuged for 10 minutes and filtered through a membrane filter to remove any remaining bacteria. 200 μl of the filtrate is mixed with 100 μl of MDR A. baumannii (OD600=0.6) and 2.5 ml of soft nutrient agar. The mixture is then layered onto solidified nutrient agar and incubated for 24 hours at 37°C. Clear plaques are picked and the double agar layer method is used to obtain pure phages. The purification process is repeated until single plaques are visible. The phage titer is determined using the double agar layer method (Sisakhtpour et al., 2022).

Each purified plaque is cultured in 5 ml of nutrient broth containing MDR-AB02 (OD600=0.6) and incubated at 37°C for 24 hours. The suspension is transferred to 500 ml of nutrient broth and shaken overnight at 35°C. Chloroform is added and left at room temperature for 15 minutes to kill bacteria. Solid NaCl is added to the culture, which is then incubated in an ice bath for one hour. To remove any remaining cells, centrifugation is performed with the addition of PEG6000 and dissolved at room temperature. The solution is incubated for 1 hour in the ice to precipitate phage particles. After centrifugation for 10 minutes at 4°C, the pellet is then suspended in 5 ml of SM buffer. The same volume of chloroform is added to separate phage particles from PEG6000. After centrifugation for 10 minutes, the supernatant is filtered through a 0.22 μm pore size membrane and stored at 4°C (Sisakhtpour et al., 2022).

To observe the morphology of the phages using transmission electron microscopy (TEM), a drop of phage solution is placed onto a copper grid surface with negative staining containing 25% phosphotungstic acid. The grid surface is then examined using a microscope (Sisakhtpour et al., 2022).

The Benefit and Its Potentials

Bacteriophages are capable of working against both Gram-positive and Gram-negative bacteria (Lood et al., 2015). Phages can be quickly isolated, and the development cost is cheaper than antibiotics because they are abundant in various environments. Bacteriophages are viruses that are restricted to bacteria and can be found in different environments and can be isolated from soil, waste, water, hospitals, hot springs, feces, human digestive tracts, and others. Phage therapy has no detectable side effects with high specificity for the infected pathogen, reducing the effect on human gut microflora compared to antibiotics (Lood et al., 2015). Phages can quickly spread throughout the body and organs such as the brain, prostate gland, and bones that cannot be reached with antibiotics.

The presence of phage hosts helps the success of treating infections caused by the host. There is no cross-resistance that can be developed against phages like antibiotics, where bacteria can easily become resistant. Bacteriophage therapy can provide effective treatment for MDR, XDR, and PDR bacteria. Phages attack and kill bacteria by lysing them without attacking human cells. Phages specifically attach to receptors on the bacterial cell wall and inject genetic material to lyse the cell (Ibrahim & Abdulazeez, 2021).

CONCLUSION

The review article discusses a novel bacteriophage called pIsf-AB082, which has shown stability and compatibility at different pH and temperature levels. The bacteriophage also demonstrated in vitro endolysin activity and has the potential to become a therapeutic agent to control nosocomial infections caused by MDR A. baumannii. However, this review is based on a literature study, and more clinical research is needed to ensure the safety of administering bacteriophages to infected patients. Therefore, the authors recommend further research to evaluate the efficacy and effectiveness of using bacteriophages, as there is still limited research on this topic. The authors also suggest conducting more in-depth studies on the potential side effects of using bacteriophages as an antibiotic-resistant therapy. Furthermore, since there is no literature available on the administration route for bacteriophages in humans, research is needed to identify the best method for administering bacteriophages to humans. In conclusion, while bacteriophages have shown promise as an alternative to antibiotics, more research is needed to establish their safety and effectiveness.

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