***Review Article***

**Pyocin As An Alternative For Antibiotic Resistance**

**ABSTRACT**

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| Antimicrobial resistance (AMR) poses a major threat to health worldwide. Undermining decades of progress in medical science, pyocin, a bacteriocin synthesized by *Pseudomonas aeruginosa*, has caught the attention of academics as a viable alternative to conventional antibiotics. In particular, the usefulness of pyocin in combating multidrug-resistant organisms (MDROs), such as methicillin-resistant *Staphylococcus aureus* (MRSA) and Pseudomonas aeruginosa, is underlined by its properties. This is especially important for pyocin strains that target antibiotic-resistant bacteria. Pyocins are released through bacterial cell lysis and function via various antimicrobial mechanisms. These include depolarization of the bacterial cell membrane or DNA cleavage, making them promising candidates. These properties make pyocins promising candidates for managing antibiotic-resistant infections. Nonetheless, challenges remain in large-scale production, stability, and clinical application. This review elucidates the therapeutic potential of pyocins, examines their mechanisms of action, and identifies avenues for future research. |

*Keywords: Pyocins, Antimicrobial resistance (AMR), Pseudomonas aeruginosa, Bacteriocins, Multidrug-resistant organisms (MDROs)*

1. **INTRODUCTION**

Antimicrobial resistance (AMR) poses a significant threat to global health, undermining over seven decades of medical advancements. The World Health Organization listed antibiotic resistance among the top 10 global threats to public health; endangering the progress registered into the treatment and care provided through the public health systems [75], Currently, the issue has grown out of proportion over the years due to inappropriate use and abuse of antibiotics both in human medicine, crop production and animal husbandry which contributed to the development of highly resistant bacterial strains. Bacterial infections resistant to routine treatments have led to increased morbidity, higher mortality, and escalating healthcare costs annually. Estimates from 2019 state that drug-resistant infections contributed to approximately 5 million deaths. Of that estimate, though, 1.27 million deaths were reported as being due to antibiotic resistance [4].

The emergence of multidrug-resistant organisms (MDROs), such as methicillin-resistant Staphylococcus aureus (MRSA) and Pseudomonas aeruginosa, has further complicated treatment options [29]. These pathogens are multiresistant to drugs and are mostly of nosocomial origin, further adding to the increasing burden on healthcare practitioners. In the United States alone, there are at least 2.8 million cases per year concerning antibiotic-resistant infections, resulting in the death of over 35,000 people annually [19]. Moreover, there is greater concern about the usage in agriculture, contributing to 73% of total antibiotics used globally , leading to foodborne bacteria resistance [71].

Additionally, antimicrobial resistance has serious socio-economic implications. The World Bank projects that by 2050, gross domestic product will decline almost 3.8% and push more than 26.2 million people below the extreme poverty line due to infections from resistant drugs [74]. The findings in this report offer a dire need for alternative methods of treatment since the usual method of antibiotics has proven ineffective against resistant microorganisms.

Pyocins, in the fight against AMR, has gained widespread popularity to act as substitutes for regular antibiotics. These protein-based bacteriocins of *Pseudomonas* origin are superior to other bactericidal activities against most gram-negative bacteria; hence, they act as the ideal choice to address AMR with minimum disturbance to normal flora, which is one major limitation otherwise common to most broad-spectrum antibiotics [61,70].

Essentially, these pyocins exist in the form of three major categories: S-type, R-type, and F-type, which operate distinctly. The S-types work as nucleases, cleaving bacterial DNA or RNA, while the R-type and F-type pyocins have structures similar to the tails of bacteriophages that disrupt the cell membrane and lead to cell death [56]. Pyocin’s mode of action, especially against clinically relevant pathogens like *P. aeruginosa*, has stirred interest in the use of pyocins as potential therapeutics in bacterial infections that have become resistant to various classes of antimicrobial agents [53, 74]

Thus, the peculiar feature of pyocins that separates it from conventional antibiotics exists in its ability of overcoming common bacterial defense mechanisms such as efflux pumps and β-lactamases. This unique feature makes them much more effective against strains that are antibiotic-resistant, providing a new dimension in the war against increasing AMR. The results from recent studies showed the efficacy of pyocins both in vitro and in vivo, with indications of its ability to reduce bacterial loads in animal infection models [42,21]. Furthermore, research on pyocin engineering has been found to expand their use therapeutically outside the confinements of only *Pseudomonas* species. The recent progress in synthetic biology has made pyocin variants amenable to working efficiently against a broader spectrum of pathogens, including *Escherichia coli* and *Klebsiella pneumoniae*, to further raise the clinical possibility [35].

However, a lot has to be done for the realization of pyocins full therapeutic potential. Large-scale production, stability, and efficient delivery in the clinics are some of the issues yet to be resolved for general use. Nevertheless, present investigation on ways to surmount these challenges is quite promising, to a level soon that could see pyocins fall into mainstream therapies for treating resistant infections [18].

**1.1. AIM OF THE REVIEW**

This review is aimed at discussing the therapeutic potential of using pyocins as alternatives to traditional antibiotics. It specifically aims to explore the mechanism of interaction of pyocins with bacterial cells and summarize new insights on pyocin research, including issues related to their development for clinical application. The review would also point out gaps in knowledge and suggest possible areas for further investigation in the future.

1. **MECHANISM OF ACTION PYOCINS**

*Pseudomonas aeruginosa* lipopolysaccharide (LPS) functions as both a receptor for R-type pyocin and a key factor in immune evasion and virulence [27]LPS consists of three main regions: the O-specific antigen (OSA), lipid A, and the core oligosaccharide [76]. The *P. aeruginosa* core frame is relatively preserved across its strains and acts as a stage for binding different OSA side chains: common polysaccharide antigen (CPA) and OSA are located in *P.aeruginosa* LPS [28]. However, heterogeneity and their appearance are determined by variants of the same strain and different isolates [70]. LPS is vital in preserving the outer membrane integrity and facilitates the work in bacterial existence, making it extremely hard for altering bacterial fitness [32]. LPS is made up of lipids that are conserved, which is a fragment holding the exterior membrane and central oligosaccharide section[59].This main area functions as the connection point between the OSA and CPA side chains, (in addition to R-pyocins) adding to the total variability of P. aeruginosa LPS [27].OSA structures can vary in length, sugar content, and linking patterns, which adds to antigenic diversity and immune evasion techniques used by the bacteria throughout infection [67]. The presence and makeup of OSA and CPA side chains in P. aeruginosa LPS may differ considerably among strains; thus, the wide repertoire of OSA constructions can impact the bacterium's relationships with not only other microbes involved in infection but also with the host's defenses, permitting immune surveillance escape and enabling chronic infection development in CF patients [24]. Furthermore, several P. aeruginosa isolates were observed to decrease or altogether delete OSA expression during persistent CF infections, resulting in improved adaptation and survival in the host natural setting [28].

P. aeruginosa faces a challenging host environment during cystic fibrosis (CF) infections, including immune responses and drug exposure. In this scenario, several varieties of P. aeruginosa were discovered to downregulate or lose OSA expression[49]. This behavior is hypothesized to give a survival benefit by decreasing detection and clearance by the host immune system[43]. By regulating OSA expression, these isolates can survive and adapt more efficiently within the CF lung environment, causing protracted infections that are difficult to cure. The reduction or deletion of OSA expression by specific isolates during persistent CF infections emphasizes the bacterium's flexibility and endurance in its host environment [30]. Studying both the functional and structural characteristics of P. aeruginosa, LPS is critical for creating tailored therapy methods to successfully battle these tough infections, especially when addressing LPS-binding antimicrobials such as bacteriophage and R-type bacteriocins [36].

**2.1. TYPES OF PYOCINS**

*Pseudomonas aeruginosa* produces three distinct types of bacteriocins, known as pyocins [16, 44]. Pyocins consist of two main protein components [33]. Pyocins are composed of three types: R, S, and F type [41]. The R-type pyocins are hereditarily and architecturally interconnected to the muscular end of P2 bacteriophages but has an absent phage head structure [7], this prevents the transfer of genes encoding virulence factors and antibiotic resistance [26]. Additional, R-type pyocins are categorized by three variants R1, R2, andR5 [36]. LPS structure is the target location for R-type pyocin [53] the cell envelope is the path used in insertion of core structure, leading to depolarization of the cell wall, and resulting in cytoplasmic lysing [62]. R- pyocins are extremely efficient against a variety of P. aeruginosa species [10] as well as against other species [48].

The terminating mechanism of R-pyocin is through a single-hit cell disruption; a single pyocin molecule has the ability to cause the degeneration of a cell no matter its amount of adhesions to its surface [41]. The strong matches and its specificity in inducing mortality has attracted interest in the use for developing them as effective therapeutic for antibiotic alternatives [39]. On the other hand, S- pyocins are small proteins, they are soluble, and have quick response to proteases [16]. S-type pyocins are large-scale multi-domain polypeptides having an adaptive resistance protein this inhibits the catalytic region of the functional pyocin[65]. Until now, S-type pyocins have three types that have been studied in detail like: S1, S2, and AP41 though S-type pyocin has six types in general S4, S5, and S3 [33]. AP41,S1, and S2 are in control of killing activity, breaking down chromosomal DNA due to Dnase activity, and are made up of large proteins, Mrof 84,00, 65,000, and 74,000, respectively [26]. The small proteins are responsible for immunity function, protection in a similar molar ratio of the producing strain DNase action of large proteins [16]. These three S-pyocin genes (S1, S2, and AP41) are noted to be arranged in an identical fashion as seen through sequencing and cloning [8].

Lastly, F-type pyocins resemble a phage tail, however, it’s flexible and non-contractile [15]. The first pyocin to be documented is Pyocin 28 a variation of F-pyocin and 430F [53], F1, F2, F3 as well. F1,F2, and F3 are all similar in serological properties and structure, nevertheless they all have different receptors that have specificities [50]. F-type and R-type pyocins are faulty phages acquired from unrelated types of phage [53].

**2.2. HOW PYOCINS TARGET BACTERIA**

Pyocins provide future replacement therapeutic methods to treat both chronic bacterial infections and multidrug-resistant [35]. The protein and antimicrobial peptides may vary extensively between Gram-negative and Gram-positive bacteria [5]. It is noticeable that pyocins are associated with pathogenesis and are responsible for increasing colonization, displacing bacteria flora, and thus leading to infection [57]. Primarily, these pyocins are extremely targeted antibiotics that attack purely bacteria affiliated to the same manufacturer that gets positioned throughout competition for supplies with competing varieties [57]. Their selectivity renders them interesting as medicines that allow targeted approaches [9]. Undoubtedly, the major problem with current antibiotics is the dysbiosis activated by broad-range assassination of bacteria [20]. Bacteriocin's restricted fatal wavelength indicates bacteria accountable for the infection must be identified before treatment, while its benefit is targeting a species, or strain of bacteria. Additionally, maintaining a wholesome healthy microflora [9,19,79]. Finally, their ability to kill in a narrow spectrum decreases elective tension for resistance to microorganism spectators [3].

**2.3. COMPARISON WITH TRADITIONAL ANTIBIOTICS**

In recent times, treating infections caused by *P.aeruginosa* is a hard nut to crack in the health care sector due to the increased incidence of infection resulting from multidrug resistance (MDR) strains. Over 10% of healthcare-associated infections are caused by various pathogens [38]. The growth and propagation of MDR pose a danger to conventional antibiotics and result in less protection [77, 78]. Phage therapy has been proposed as a future treatment for bacterial infections. However, the rapid emergence and spread of multidrug-resistant (MDR) strains have increased interest in pyocins as a potential alternative [40, 47].

Additionally, there are shortages of new brands of drugs to tackle antibiotic resistance, and the conventional brands are no longer effective against bacterial infection while production cost and risk of production are high to develop such products [13]. It is estimated that by 2050 functioning antibiotics are at hand for infection treatments, when no new drugs are developed [51]. Currently, infections simulated by Gram-negative bacteria are paramount, as seen with Gram-negative pathogens like; (*Acinetobacter baumannii*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*) having obstruction from impermeable exterior barrier and preventing the passage of various kinds of antibiotics [61,78].

Hence, alternative methods to resist antibiotic-resistance pathogen and combat antibiotic-resistant infectious agents are vital [31]. There are guaranteed lead proposed by antimicrobial peptides from different sources, like bacteriocins[12, 80]

**CURRENT RESEARCH ON PYOCIN**

**3.1. THERAPEUTIC POTENTIAL**

In recent years, new insights about *Pseudomonas aeruginosa* virulence factors have uncovered potential targets for treating *P. aeruginosa* infections. These targets consist of structural components like pili and flagella, which play a crucial role in bacterial adhesion and movement, as well as lipopolysaccharides (LPS), a major surface-associated virulence factor. Moreover, targets such as the type III secretion system, which allows *P. aeruginosa* to transport harmful toxins into host cells, and the quorum sensing system have been identified [2]. Pyocins, once considered only bacterial defense proteins, are now recognized for their potential in medical applications. Pyocins are highly diverse antimicrobial peptides produced by bacteria. They possess strong antibacterial effects, particularly against bacteria from the same taxonomic group. *P.aeruginosa*, whether clinical or environmental, produces an array of pyocins that have inhibitory effect against other multidrug-resistant strains of *P.aeruginosa*. They have been viewed as a viable therapeutic medicinal and antibacterial agent for antibiotic-resistant pathogens with a distinctive mode of action that kills antibiotic-resistant bacteria by destroying their biofilms, making them attractive candidates for alternative therapeutic antimicrobials [63, 81].

**3.2. PYOCIN AS THERAPIES**

Bacteriocins from Gram-negative bacteria, particularly pyocins from *P. aeruginosa*, are ideal for medicinal development for several reasons. They offer pre packaged antibiotics with high potency (as low as pM affinity) and potential for protein engineering. The modular structure of pyocins enables the development of chimeric proteins with R-, T-, and C-domains from different pyocins, enhancing their therapeutic potential [82,83]. This technique was utilized with pyocins S2 (T- and C-domains) and S5 (central R-domain) to identify the R-domain of Pyocin S5. It was also proven using Pyocin S1 and AP41 55 [2]. Pyocin production influences resistance to antibiotics and genotoxic agents with previous research findings stating that transposon insertion mutations in the *P*. *aeruginosa* pyocin biosynthetic locus boost ciprofloxacin resistance with Phosphate Regulon Transcriptional Regulator (PrtR) homeostasis contributes to *Pseudomonas aeruginosa* pathogenicity and ciprofloxacin resistance. These investigations proposed that a wild-type strain's susceptibility is due in part to genotoxic stress, which induces pyocin synthesis and cell lysis proteins [63,84].

**3.3. CLINICAL TRIALS AND APPLICATION**

Because pyocins has a modular makeup and preserve toxic activity, it is possible to create active pyocin/colicin chimeras. This is becoming more and more significant because several chronic diseases, including rheumatoid arthritis, diabetes, obesity, and inflammatory bowel disease, have been linked to microbial imbalances in the natural gut flora.

S-type pyocin has a great deal of promise as a therapeutic agent against infections and pathogenic strains [23,80]. This might improve treatment efficacy and lessen the financial load on healthcare systems. Pyocin’s ability to inhibit *P. aeruginosa* activity was examined in relation to *Galleria mellonella* caterpillar, an invertebrate host. By inoculating larvae with a lethal dosage (104 CFU) of *P. aeruginosa* strain YHP14, Smith *et al* (2015) evaluated the efficacy of recombinant S2 pyocin in shielding *G. mellonella* larvae against a fatal P. aeruginosa infection. Subsequently, the larvae received an injection of 27 mg/kg of S2-pyocin. According to the study, S2-pyocin shielded the *Galleria mellonella* larvae from a deadly *P. aeruginosa* infection, resulting in a 100% survival rate among pyocin-treated larvae as opposed to 0%. However, the larvae did not exhibit any toxicity from S2-pyocin. In a related investigation, [45] looked at the *G. mellonella* caterpillar and the effectiveness of pyocins PaeM4, S5, L2, and L3. A fatal dosage (500 CFU) of *P. aeruginosa* strain A19-infected larvae were injected with 10 µg of PaeM4-, S5-, L2-, or L3-pyocins. Furthermore, the larvae were protected from the infection by the S5-pyocin, which demonstrated the highest efficacy among the tested pyocins, despite differences in each pyocin's protective effects against a fatal *P. aeruginosa* infection. By comparison, Pyocins L2 and PaeM4 were able to save 90% and 75% of the larvae from the infection. L3 did not provide any protection to the larvae against infection by *P*. *aeruginosa*. Moreover, The S-type pyocin's physicochemical resistance to temperature swings, pH variations, and organic solvents highlights its potential for a wide range of uses in the food, hygiene, and pharmaceutical industries.

**4. RESEARCH GAP AND FUTURE RESEARCH**

Bacteriocins produced by bacteria, such as pyocins, may eventually be less expensive than standard antibiotics [9,83]. Recently, the manufacture of bacteriocins in plants has been accomplished at costs that may support commercialization. However, the interactions of pathogenic and probiotic species in an in vivo microbiome are not fully understood to predict their performance [54,81]. A main challenge to the effective treatment of many chronic infections, such as *P. aeruginosa* infection in the lungs (cystic fibrosis), is the incapacity of antibiotics to destroy bacteria in a biofilm [1]. Pyocin has proven to be a better alternative but despite the plethora of advantages it offers, there are still limitations.

The most significant limitation is presented by the fact that there are only a few in-vivo studies on pyocins. While in-vitro studies indicated the effectiveness of pyocins as bactericidal agents towards *Pseudomonas aeruginosa* and other pathogens, in-vivo models are needed much for their better insight on the therapeutic performance [2]. The lack of extensive in-vivo experiments is an important consideration, as any successful clinical application requires full comprehension of the action of pyocins in a biological host. In addition, the narrow spectrum of action of many pyocins, acting on a relatively small number of strains of bacteria, limits their use as broad-spectrum agents [59,79].

The stability and large-scale production of pyocins remain challenges, as they are protein-based and prone to degradation during storage and administration [34]. Besides that, most of the production methods developed so far have not attained scalability, a major barrier in manufacturing when considering large quantities necessary for therapy [14, 80]. This is a huge constraint, because for any such molecule, scalable and economically viable methods of production have to be developed to advance pyocins from laboratory studies to clinical practice.

Although pyocin resistance has not been widely documented, its potential emergence with widespread use is a concern. To mitigate this issue, a possible solution is the creation of cocktails containing different pyocins that target diverse proteins. Therefore, the exploration of *P. aeruginosa* genomes for new pyocin genes is a crucial advancement in the development of this antibiotic class for therapeutic purposes [6, 81].

Despite the promising research, several gaps still exist on the clinical use of pyocins against antibiotic-resistant infections. Researchers have recognised a need to engineer currently known pyocins or identify newer variants that can act upon a larger variety of pathogenic bacteria, which includes multidrug-resistant strains like *Acinetobacter baumannii* and *Klebsiella pneumoniae* [46;75] Thus, while specificity is a desirable feature of pyocins in order to minimize off-target effects, it implies some limitation in the treatment of infections caused by multiple pathogens.

Another area which remains under-researched is that of delivery systems for pyocins. As large molecules, pyocins may have problems with penetration through biofilms or through intracellular pathogens in which most bacterial infections are found [17,82]. Biofilm formation is a key defense mechanism of pathogens like Pseudomonas aeruginosa, and there is limited data on pyocins' effectiveness in overcoming such barriers [37, 83]. Investigating delivery strategies such as encapsulation in nanoparticles or fusion with other molecules could help overcome these obstacles [22].

Moreover, insufficient research exists into the immunogenicity of pyocins. Being large, foreign proteins, pyocins might induce immune responses in the human body that could reduce their effectiveness or lead to adverse effects in treatment [63]. Although bactericidal action of pyocins has been extensively studied in vitro, only limited studies have investigated its long-term potential to act as an immunogen following repeated or high-dose treatments [48,84]. This represents a major knowledge gap because immunogenicity is a critical variable for their safe clinical application.

Ultimately, an important question regards the potential development of bacterial resistance to pyocins. Although susceptible strains exist, bacteria have the innate capacity for adaptation and have developed effective mechanisms against their action. Few studies have focused on their long-term activity or likelihood of causing resistance development [37,68]. This is important to consider since one of the major advantages of pyocins is their potential for the specific destruction of bacterial strains resistant to conventional antibiotics; any mechanisms of resistance could decrease their clinical relevance.

Although pyocins appear to be a promising class of antimicrobials, several research gaps are yet to be accomplished before their therapeutic potential is realized.

**5. CONCLUSION**

Antimicrobial resistance is one of the most pressing issues in healthcare, weakening immunity and increasing susceptibility to opportunistic infections. This emphasizes the urgent need for alternative treatments. Pyocins demonstrate their therapeutic potential by offering target-specific and efficacious mode of action compared to traditional antibiotics. However, with rising manufacturing costs and global economic constraints, new therapeutic discoveries, such as pyocins, must be evaluated to determine the prospects in addressing antibiotic resistance.

As pyocins represent the future of antibiotic resistance, more in vivo and in vitro research are needed to fully understand and optimize their therapeutic applications. Challenges such as large-scale production, wider spectrum variants, stability, scalable production techniques, novelty on the method of delivery, and studies on the immunogenicity and resistance of pyocins, are crucial in advancing pyocins from the laboratory to clinical practice. Pyocins have emerged as a promising alternative, particularly as the search for novel antibiotic replacements continues. Hence, addressing such challenges will establish pyocins as proper alternatives toward resistance.

**DISCLAIMER (ARTIFICIAL INTELLIGENCE**)

The author(s) hereby certify that no generative artificial intelligence (AI) tools such as Scalable Language Models (ChatGPT, COPILOT, etc.) or text-to-image generators were utilized in the authoring or editing of the paper.

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