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INHIBITION OF METHICILLIN-RESISTANT STAPHYLOCOCCUS AUREUS (MRSA) BIOFILM: THE ESSENTIAL ROLE AND POTENTIAL USAGE OF BACTERIOCINS

INHIBISI BIOFILM METHICILLIN-RESISTANT STAPHYLOCOCCUS AUREUS (MRSA): PERAN DAN POTENSI PENGGUNAAN BAKTERIOSIN

Literature Review
Studi Literatur

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A B S T R A C T

Background: *The potential of Methicillin-Resistant Staphylococcus aureus (MRSA) to develop biofilms and its resistance to antibiotics become major worldwide issue. Complementary anti-microbial strategies have been used recently, in particular for the treatment of MRSA biofilm-associated resistance.* ***Purpose:*** *To review the potential, essential role, and mechanism of bacteriocin that can inhibit MRSA biofilms. The review was conducted by searching and analyzing published articles from Elsevier, ProQuest and PubMed database.* ***Review:*** *Globally, the incidence of MRSA in 85 countries based on WHO surveillance reaches more than 20%. Biofilm, as one of the virulence factors of MRSA, can result in the failure of antibiotic therapy. According to reports, bacteriocins, such as peptides synthesized by Gram-negative and Gram-positive bacteria, have antimicrobial activity that has the potential to inhibit antibiotic-resistant pathogens and biofilms formed by MRSA.* ***Result:*** *The bacteriostatic and bactericidal activity of bacteriocins against MRSA has been shown through research across several countries on the usage of bacteriocins, which was isolated from different types of bacteria against MRSA biofilms. Bacteriocins contribute to the inhibition of MRSA biofilms by inhibiting the synthesis of cell walls, leading to pores in the cytoplasmic membranes of bacterial cells, interrupting the synthesis of extracellular membranes, disrupting cell membranes, and reducing the number of planktonic cells within MRSA biofilms.* ***Conclusion:*** *Bacteriocins have an effective mechanism for treating MRSA biofilms with low toxicity and risk of resistance, hence they are safe to be developed as complementary components to antibiotics in an effort to treat MRSA biofilms.*

A R T I C L E   I N F O

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A B S T R A K

Latar belakang: Potensi *Methicillin-Resistant Staphylococcus aureus* (MRSA) dalam membentuk biofilm dan resistansinya terhadap antibiotik menjadi masalah utama di seluruh dunia. Strategi anti-mikroba komplementer baru-baru ini telah digunakan, khususnya untuk pengobatan resistansi terkait biofilm MRSA. **Tujuan:** Menelaah potensi, peran penting, dan mekanisme bakteriosin dalam menghambat biofilm MRSA. Telaah dilakukan dengan pencarian dan analisis artikel yang diterbitkan pada *database Elsevier*, *ProQuest,* dan *PubMed*. **Telaah pustaka:** Secara global, insiden MRSA dari 85 negara berdasarkan surveilans WHO mencapai lebih dari 20%. Biofilm sebagai salah satu faktor virulensi dari MRSA dapat mengakibatkan kegagalan terapi antibiotik. Berdasarkan penelitian, bakteriosin sebagai peptida yang disintesis oleh bakteri Gram-negatif maupun Gram-positif, memiliki aktivitas anti-mikroba yang berpotensi menghambat patogen resistan antibiotik serta biofilm yang dibentuk MRSA. **Hasil:** Aktivitas bakteriostatik dan bakterisidal bakteriosin terhadap MRSA telah dilaporkan dari beberapa negara melalui penelitian penggunaan bakteriosin yang diisolasi dari berbagai jenis bakteri terhadap biofilm MRSA. Bakteriosin berkontribusi terhadap penghambatan biofilm MRSA dengan menghambat sintesis dinding sel, menyebabkan pori-pori pada membran sitoplasma sel bakteri, mengganggu sintesis membran ekstraseluler, mengganggu membran sel, dan mengurangi jumlah sel planktonik dalam biofilm MRSA. **Kesimpulan:** Bakteriosin memiliki mekanisme efektif untuk menangani biofim MRSA dengan toksisitas dan risiko resistansi yang rendah, sehingga aman untuk dikembangkan sebagai komponen komplementer antibiotik dalam upaya menangani biofilm MRSA.

Kata kunci:
Bakteriosin, Biofilm, Resistansi, MRSA

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INTRODUCTION

On the WHO antibiotic development program, *Staphylococcus aureus* is one of pathogens considered priority list bacteria (Velázquez-Suárez *et al*., 2021; WHO, 2017). These bacteria pose a major risk to international health since it is resistant to multiple antibiotics (Liu *et al*., 2022; Nour El-Din *et al*., 2020; Okuda *et al*., 2013; WHO, 2017). *S. aureus* which is resistant to multiple antibiotics and causes nosocomial infection was known as *Methicillin-Resistant Staphylococcus aureus* (MRSA). MRSA is responsible for both hospital-acquired infections *Hospital Associated-Methicillin Resistant Staphylococcus aureus* (HA-MRSA) and *Community Acquired Infections-Methicillin Resistant Staphylococcus aureus* (CA-MRSA). HA-MRSA is an infection that is transmitted while a patient is in a hospital, as opposed to CA-MRSA, which can be transmitted through contact in the community, outside of the medical setting (Al Atya *et al*., 2016; Kourtis *et al*., 2020; Kranjec *et al*., 2020; Liu *et al*., 2022).

Today, *Methicillin-Resistant Staphylococcus aureus* (MRSA) represents an important risk to human health due to both its antibiotic resistance and its ability to form a biofilm (Al-Seraih *et al*., 2017; Du *et al*., 2020; Kranjec *et al*., 2020). In over 80% of cases, chronic infections are caused by the biofilm of MRSA. In comparison to planktonic cells, bacteria that form biofilms are 10 - 10.000 times more resistant to antibiotics (Al Atya *et al*., 2016; Kranjec *et al*., 2020; Liu *et al*., 2022).

The WHO indicates that if there is no innovation in new antibiotics, the world's ability to combat diseases caused by antibiotic resistance may be reduced. Complementary antimicrobial substance therapy has gained in acceptance at present, particularly in the management of biofilm-associated resistance. This approach combines a number of antibiotic classes that may both prevent and influence different phases of biofilm formation (Kranjec *et al*., 2020).

One of the complementary antimicrobial substances that could be used in combating biofilms is bacteriocin (Kranjec *et al*., 2020). The production of bacteriocins is a method of controlling other bacteria in the environment, which then impacts the dynamics of the world's population of bacteria. Several regions, such as Canada, The United States, Europe, have investigated bacteriocins as an inhibitors of food degradation (Du *et al*., 2020). The development of bacteriocins is important to combat resistant bacterial infections, especially MRSA, that are capable of forming biofilms. The use of bacteriocins for therapy is still uncommon. There is an example of research related to the use of bacteriocin to combat MRSA in diabetic foot infection wounds that is still in the development phase, both in vitro and in vivo (Nour El-Din *et al*., 2020; Santos *et al*., 2019; Thapa *et al*., 2021).

The objective of this article was to review the potential, essential role, and mechanism of bacteriocin that can inhibit MRSA biofilms. The review was conducted by searching and analyzing published articles from several publication databases. In order to treat infections and reduce the harmful consequences of MRSA biofilms, understanding the mechanism and potential application of the bacteriocins is expected to be valuable.

LITERATURE STUDY

This literature study was conducted through scientific journals searching in several databases. Searching terms used in this literature study included (bacteriocin) and ('biofilm *Methicillin-resistant Staphylococcus aureus*") and (activity or against or inhibit) in Elsevier (SCOPUS), ProQuest, and National Library Medicine (PubMed, PubMed for handheld/ Pubmed via PICO). The eligibility criteria of the journals used are indexed research journals in English that were published between 2013 - 2022. The inconsistent reference to the writing objectives was excluded. Other articles, short communications, and book chapters that meet comparable descriptions and relate to the objectives of this literature study objectives were used as additional search results.

Methicillin-Resistant Staphylococcus aureus **(MRSA)**

The first *Staphylococci* were identified from human pus in 1880 by Alexander Ogston in Scotland. The term *Staphylococci* originates from the Greek words "staphyle" (grape) and "kokkos" (berry). It was assigned to these isolated bacteria because they appeared like bunches of grapes when viewed under a microscope. In 1886, Anton J. Rosenbach from German identified two Staphylococcus strains in pure culture. One of the strains isolated from this pure culture was assigned as *S. aureus*. The colonies of *S. aureus* are yellow/gold pigmented (in Latin, aureus means "golden") (Fetsch, 2018).

On the skin, nose, and mucous membranes, *S. aureus* is the most common commensal bacteria. *S. aureus* has the ability to form colonies as large as 25 – 30% on the skin and mucosa of both humans and animals (Al Atya *et al*., 2016; Kranjec *et al*., 2020;Liu *et al*., 2022; Santos *et al*., 2019; Velázquez-Suárez *et al*., 2021). As an opportunistic bacterium, *S. aureus* is associated with a wide range of diseases, from minor skin infections to serious ones. It occurs when the host immune system is compromised (Fetsch, 2018; Field *et al*., 2015; Kranjec *et al*., 2020; Nour El-Din *et al*., 2020).

Not only in the human and animal sectors, *S. aureus* also causes illness in the industrial and food sectors, for instance mastitis in dairy animals and bumblefoot in chickens (Al Atya *et al*., 2016; Kranjec *et al*., 2020; Liu *et al*., 2022; Santos *et al*., 2019; Velázquez-Suárez *et al*., 2021). Although *S. aureus* is a planktonic cell, it can also form biofilms. The biofilm of this bacterium can protect the cell from the host cell's immune response as well as that of antimicrobials and disinfectants (Fetsch, 2018; Field *et al*., 2015; Kranjec *et al*., 2020; Nour El-Din *et al*., 2020).

S. aureus is a highly adaptable bacterium that is easily adapted to acquiring antibiotic resistance (Field *et al*., 2015; Kranjec *et al*., 2020; Nour El-Din *et al*., 2020). MRSA is *S. aureus* which has a *Minimum Inhibitory Concentration* (MIC) of up to ≥4 g/ml for oxacillin (Siddiqui and Koirala, 2022). Most β-lactams (penicillin, cephalosporins, and carbapenem) have no ability to eliminate MRSA (Du *et al*., 2020). This bacterium is also resistant to another group of antibiotics; for instance, fluoroquinolones, macrolides, and aminoglycosides (Liu *et al*., 2022). *Staphylococcus* resistance is a result of several factors, including:

Synthesis of β-lactamase

The active site of β-lactam antibiotics can be broken down by β-lactamase that is produced by Staphylococcus. It is resulting in the antibiotics being ineffective. β-lactam resistance-associated plasmid transmission increases the resistance of Staphylococcus (Brooks *et al*., 2013).

Methicillin and oxacillin chromosomal resistance

This resistance mechanism depends on the sequence of chromosomal genes called *Staphylococcal Cassette Chromosome mec* (SCCmec) (Brooks *et al*., 2013; Kranjec *et al*., 2020; Willey *et al*., 2010). There are four types of SCCmec: type I, II, III, and IV. SCCmec types I - III are associated with HAI, whereas SCCmec type IV is frequently obtained in populations (communities). The mecA gene mainly encodes for a low-affinity PBP leading to resistance (Brooks *et al*., 2013; Willey *et al*., 2010).

Increased synthesis of cell walls and modified cell wall composition

Antibiotics such as penicillin that target the bacterial cell wall may trigger the modified bacterial cell wall composition, and increase the synthesis of the cell wall. We can find this mechanism in the variant of *S. aureus* that is susceptible to vancomycin. Usually, this bacterium is obtained from patients receiving long-term vancomycin therapy for complicated infections (Brooks *et al*., 2013).

Genetic transmission

In addition to genes originating from internal chromosomes, resistance may be also caused by the transmission of resistance genes to other genera (Brooks *et al*., 2013; Kranjec *et al*., 2020). This mechanism is associated with the enterococci VanA and mecA genes, which result in *Vancomycin-Resistant S. aureus* (VRSA) (Brooks *et al*., 2013).

Antibiotic tolerance

This mechanism involves the inefficiency of autolytic enzymes against the cell wall. In addition, reversible or irreversible antibiotic tolerance in *S. aureus* can be triggered by other factors, including concurrent antibiotic exposure, human serum, or particular compounds (Brooks *et al*., 2013).

Epidemiology

Methicillin-Resistant Staphylococcus aureus (MRSA) was initially identified in 1961. Since then, it has significantly increased in human and animal health worldwide. Globally, the incidence of MRSA in 85 countries based on WHO surveillance reaches more than 20% (Du *et al*., 2020; Velázquez-Suárez *et al*., 2021). In the US, MRSA infections ranged from 7 - 60%. In the pre-antibiotic era, the mortality rate due to *Staphylococcus* infections in the respiratory system reached 80 – 90% (Siddiqui and Koirala, 2022). Based on the latest report posted at https://www.cdc.gov/mmwr, there were 19.832 deaths in 2017 (Kourtis *et al*., 2020).

Hospitalized *Diabetic Foot Infection* (DFI) patients are more likely to acquire biofilms of MRSA (15 – 30%) than non-hospitalized patients (Santos *et al*., 2019). The mortality rate due to MRSA infection ranges from 30 to 37%. As compared to other *Gram-positive* and *Gram-negative* bacterial infections, it has the highest number of cases (Siddiqui and Koirala, 2022).

In the hospital, the frequent use of intravenous catheters leads to the prevalence of MRSA biofilm infections. Other risk factors for MRSA infection in the hospital include hemodialysis, prolonged stays in the hospital, open wound, and intensive care. Patients with MRSA infections in hospitals can spread resistant bacteria to healthcare professionals who come into contact with them (Siddiqui and Koirala, 2022).

Although MRSA resistance was initially identified in hospital cases, it can be transmitted to the environment. As a result of the resistant strain transmission, there are now significantly greater numbers of CA-MRSA in the community (Al Atya *et al*., 2016; Kourtis *et al*., 2020; Kranjec *et al*., 2020; Siddiqui and Koirala, 2022). MRSA also presents in various kinds of milk and raw meat, including beef, poultry, and pork (Du *et al*., 2020).

Clinical manifestation

In hospitals, MRSA is frequently identified in patients with implant infections, patients on ventilators, and cases of surgical site infections (Field *et al*., 2015, Velázquez-Suárez *et al*., 2021). Although it is frequently found in hospitals, MRSA infection can occur in communal settings. In communal settings, MRSA poses a risk of food contamination and outbreaks of food poisoning (Du *et al*., 2020). Depending on location and severity, different clinical manifestations of MRSA infection may develop. Skin and soft tissue infections represent only two examples of the diseases brought on by CA-MRSA. The skin area of MRSA infection is often painful, swollen, and has red patches of skin that may resemble large pimples or spider bites. It can also contain pus and other fluids (Willey *et al*., 2010).

The spread of MRSA through the bloodstream can potentially result in pneumonia, endocarditis, and various secondary illnesses that occur in the osteoarticular and lung (Field *et al*., 2015; Kranjec *et al*., 2020; Liu *et al*., 2022; Siddiqui and Koirala, 2022). As the primary cause of DFI, which leads to limb amputation, MRSA is frequently observed forming biofilms (Santos *et al*., 2019).

***Methicillin-Resistant Staphylococcus aureus* (MRSA) biofilm**

Methicillin-Resistant Staphylococcus aureus (MRSA) is forming biofilm, an important characteristic that contributes to the infection. The planktonic MRSA cell will grow to the sedentary form. The sedentary MRSA form consists of the growth of bacterial clusters and biomolecules (proteins, lipids, and polysaccharides) that are integrated into the extracellular matrix (Kranjec *et al*., 2020; Liu *et al*., 2022; Santos *et al*., 2019). This extracellular matrix irreversibly adheres to a surface (Santos *et al*., 2019). Extracellular matrix exopolysaccharide synthesis is carried out by the icaADBC gene (Al Atya *et al*., 2016).

Biofilm becomes a protective barrier for MRSA cells, supporting the persistence and survival of this bacterium. Not only does it protect from antibiotics, biofilm also makes MRSA cell resistant to host immune responses and other extreme conditions (Curtis *et al*., 2018; Kranjec *et al*., 2020; Liu *et al*., 2022; Okuda *et al*., 2013). In addition, MRSA biofilm can spread to the environment and enhance resistance through horizontal gene transfer (Fetsch, 2018). Biofilms act as a physical barrier that inhibits the diffusion of antibiotics into cells. (Belguesmia *et al*., 2021). MRSA biofilm contributes to the persistent infection. It is quite challenging to treat (Kranjec *et al*., 2020).

In the clinical setting, biofilm causes infective antibiotic therapy (Field *et al*., 2015; Santos *et al*., 2019). It is associated with the wide range of spread of MRSA biofilm, which can adhere to biotic and abiotic surfaces, including several types of medical equipment. It can enhance survival and proliferate on extreme biotic and abiotic surfaces (Du *et al*., 2020). Several steps in the complex and multiple stages of MRSA biofilm formation are as follows:

Surface attachment

At this stage, planktonic MRSA cells primarily attached to biotic or abiotic surfaces. This step is the first important step in biofilm formation. The synthesis of adhesive matrix molecules (MSCRAMMs) facilitates this step. This molecule is capable of adhering to a number of host extracellular matrices, such as fibrinogen (fib), laminin (eno), elastin (ebpS), fibronectin A (fnbA), fibronectin B (fnbB), collagen (cna), ligand clumping factors A (clfA), and ligand clumping factors B (clfB) (Belguesmia *et al*., 2021; Moormeier and Bayles, 2017). The attachment is reversible and carried out via the Van der Waals bond, resulting in weak interaction (Fetsch, 2018; Moormeier and Bayles, 2017).

Irreversible adsorption to the biotic or abiotic surfaces

Several components contribute to this stage, not only adhesive proteins but also bacterial structures, including fimbriae and flagella. MRSA will utilize hydrophilic or hydrophobic interactions, acid-base interactions, and electrostatic interactions to strengthen the attachment on biotic and abiotic surfaces (Fetsch, 2018).

Bacterial proliferation and synthesis of *Extracellular Polymeric Substance* (EPS) matrix

After the planktonic MRSA cells strengthen the attachment, they will grow and start to multiply, then forming microcolony. The microcolony consists of multiple layers of cells and EPS. The cells will be connected through an EPS matrix. The complex structure of EPS consists of extracellular nucleic acids, proteins, lipids, and polysaccharides. The EPS matrix can cover the MRSA cells inside against external factors such as biologicals (protozoa, host immune defense), physicals (temperature, ultraviolet radiation), and chemicals (heavy metals, chemical reagents). It also maintains the stability of biofilm (Fetsch, 2018; Moormeier and Bayles, 2017). Due to the fact that MRSA cells in biofilm are immobile, the EPS matrix supplies nutrients for the cells by enhancing a nutrient-rich environment. The hydrolytic enzymes in the EPS matrix may degrade complex substances so that the cells living inside the biofilm can use the degraded substances as a source of energy (Fetsch, 2018).

Biofilm maturation

At this stage, biofilm continues to grow, becomes more complex and thick, and increases the rate of EPS production. It is also found to alter the metabolic activity of microcolony and inhibit particular genes of biofilm formation. Because of the altered metabolism, biofilm will effectively use nutrients as well as adapt to the environment (Fetsch, 2018). Mature biofilms consist of different populations of bacteria. The different cell populations with distinct phenotypes will enhance the resistance to antibiotics and the tolerance level of biofilm (Ray *et al*., 2021).

Dispersion

This is a final stage of biofilm maturation. During the biofilm dispersion, sedentary cells move in large numbers and become planktonic cells, which enable growth and the formation of other biofilm in other environments. Biofilm dispersion occurs because of internal and external factors. The internal factors that can result in dispersion are the presence of hydrolytic enzymes, which can degrade the EPS matrix and reduce biofilm substances. External factors influence biofilm dispersion, including physical triggers (fluid flow pressure), chemical treatment (chlorhexidine, chloride, urea), signaling molecules, antibiofilm peptides, and the unavailability of nutrients (Fetsch, 2018). The following are aspects that affect biofilm formation (Fetsch, 2018): 1) Substrate characteristics: initial bacterial cell attachment impacted by the surface charge of the substrate, texture and hydrophobicity, 2) Environmental factors: the biofilm formation depends on temperature, oxygen level, pH, nutrient availability and presence of antibiotics, 3) Intrinsic component of the cell: genetic characteristics of the strain and expression of the ica gene in MRSA, which are responsible for synthesizing *Polysaccharide Intercellular Adhesin* (PIA).

Bacteriocin

Bacteriocins produced by both *Gram-positive* and *Gram-negative* bacteria act as peptidic toxins to inhibit other clinically relevant bacterial strains (Du *et al*., 2020; Field *et al*., 2015; Kranjec *et al*., 2020; Okuda *et al*., 2013). Bacteriocins are synthesized in ribosomes with different functions and structures (Liu *et al*., 2022; Nour El-Din *et al*., 2020; Velázquez-Suárez *et al*., 2021). Bacteriocin is also known as an *Antimicrobial Peptide* (AMP) that can control susceptible and resistant bacteria. In general, antimicrobial peptides can attach to and disrupt the bacterial cell membrane without negatively impacting eucaryotic cells. It occurs because of the cationic amphiphilic characteristic of the AMP (Field *et al*., 2015).

Bacteriocins also have a selective effect on eucaryotic cells. It specifically targets anionic bacterial membranes, whereas the major components of eucaryotic membranes are neutral lipids. The first approach between the peptide and the cell surface is driven by electrostatic interactions between the positively charged amino acids of the AMP and the negatively charged bacterial cell membrane (Field *et al*., 2015). Depending on the type of peptide, bacteriocins have a different species-specific or genus-specific antibacterial spectrum (narrow or broad spectrum). Certain bacteriocins with a narrow spectrum can inhibit the growth of other bacteria; others with a broad spectrum can result in bacterial cell death (Liu *et al*., 2022; Nour El-Din *et al*., 2020).

In contrast to the antibiotic-producing bacteria, the specific mechanism of bacteriocins is not inhibited by other antimicrobial substances. Regarding their biological and chemical characteristics over a wide pH and temperature range, bacteriocins remain stable. Since this characteristic increases the beneficial attributes of bacteriocins, it is appropriate to refer to their use in the treatment of infection (Liu *et al*., 2022).

RESULT

Inhibition of *Methicillin-Resistant Staphylococcus aureus* (MRSA) biofilms by bacteriocins

The report articles included in this review come from several countries in Asia, Europe, and Africa. Bacteriocin-producing bacteria used to inhibit MRSA in the study included, can be divided into *Gram-positive* (*Lactococcus*, *Bacillus*, *Paenibacillus*) and *Gram-negative* (*E. coli*, *Enterococcus*). *Lactococcus*, a genus of *Gram-positive* bacteria, is the most dominant microorganism used as a bacteriocin-producing bacteria inhibit MRSA in vitro.

Table 1 describes the different effects of several types of bacteriocins on MRSA biofilm. In order to inhibit biofilms, bacteriocins should mainly inhibit bacterial adhesion, prevent the growth of biofilms, prevent mature biofilms from spreading, and kill cells to reduce mature biofilms (Velázquez-Suárez *et al*., 2021).

DISCUSSION

In accordance with the research shown in Table 1, nine studies used *Gram-positive* bacteria as bacteriocin-producing bacteria, and another four studies used *Gram-negative* bacteria to inhibit MRSA biofilm. All of the studies defined significant MRSA inhibition, with the highest MRSA biofilm reduction (88%) found by Ahire and Dicks after 24 hours of in vitro incubation (Ahire and Dicks, 2015). In this study, the nisin used was mixed with DHBA, and then incorporated into nanofibers to improve the inhibition activities of reduced planktonic cells and MRSA biofilm. Regarding the types of bacteriocins used, based on the studies included, we found that there are two major categories of bacteriocins used to inhibit MRSA:

Class I bacteriocins: lanthionine

Uncommon amino acids like dehydrobutyrine, 3-methyllanthionine, lanthionine, and dehydroalanine define unique class I bacteriocins, usually known as lantibiotics. Since the lanthionine residue is composed of two alanine residues linked by thioethers, antibiotics frequently have a cyclic structure (Karczewski *et al*., 2021; Okuda *et al*., 2013). This uncommon amino acid is the result of a post-translational modification that remains extremely stable in challenging environments (Okuda *et al*., 2013).

Class II bacteriocins: non-lanthionine

This group of peptides is characterized by its short length, resistance to heat, and absence of distinctive amino acids. Class II bacteriocins are divided into four classes as follows (Okuda *et al*., 2013): 1) Pediocin-like bacteriocins (group IIa), 2) Two-peptide bacteriocins (group IIb), 3) Cyclic bacteriocins (group IIc), 4) Nonpediocin single linear peptides (group IId). Nisin, a non-lanthionine bacteriocin, was the most frequently used bacteriocin to inhibit MRSA, according to the thirteen studies that were included in this literature review. MRSA biofilms are reduced through the following mechanisms: (1) Inhibiting the formation of bacterial cell walls, (2) educing the cell envelope, and (3) Generating pores in the cytoplasmic membrane (Al-Seraih *et al*., 2017; Belguesmia *et al*., 2021).

Antibiotics are capable of combating MRSA by performing either bactericidal or bacteriostatic activity. Bacteriostatic action occurs by inhibiting the synthesis of bacterial cell walls through lipid II masking. However, the bactericidal activity is performed by pore formation the bacterial membrane to kill the bacteria (Karczewski *et al*., 2021; Okuda *et al*., 2013). The pore formation in the bacterial cytoplasmic membrane of bacterial cells reduces membrane permeabilization, which then results in the loss of internal chemicals and cell death (Al-Seraih *et al*., 2017).

Table 1. The effects of several bacteriocin types in inhibiting *Methicillin-Resistant Staphylococcus aureus* (MRSA) biofilms

No.	Type of bacteriocins	Bacteriocin producing bacteria	Origin of MRSA isolation	Country	Result	Bacteriocins inhibition	Reference
1	Garvicin KS	*Lactococcus garviae* KS 1546	ATCC 33591, USA300, MRSA, parental strain ATCC 33591, Xen 31	Norway	Utilized in the creation of hybrid hydrogels for the chronic skin wounds treatment, inhibits preformed of *S. aureus* biofilms in vitro.	MIC50 >5	Kranjec *et al*., 2020; Thapa *et al*., 2021
2	Micrococcin P1	*Lactococcus garviae*	USA300 and ATCC 33591	Norway	Micrococcin P1 combined with Garvicin KS caused the MRSA strain to be sensitive to penicillin G.	MIC50 >1.0x 10 -1	Kranjec *et al*., 2020
3	Nisin	*Lactococcus lactis*	Clinical, from DFI patients	Lisbon	High efficacy in preventing the formation of MRSA biofilms was obtained in the treatment using biogels containing nisin	OD value 0.2 - 0.3 on the wavelength 660 nm found at the level 22.5 µg/ml, it	Santos *et al*., 2019
4	Plantaricin GZ1-27	*Lactococcus plantarum*	MRSA ATCC 43300	China	After 48 hours of incubation, Plantaricin GZ-27 application showed the highest impact. MRSA biofilm mass reduced while adhesin polysaccharide and surface protein synthesis were inhibited.	After 48 hours, MRSA biofilm decrease was between 40.2 and 55.3%.	Du *et al*., 2020
5	Bacin A2	*Bacillus* sp. TL12	MRSA ATCC 43300	China	The characteristic of this substance is non-toxic. It can reduce MRSA biofilm that already presents, and disrupts cell membranes	Inhibition of biofilm formation at >0.5x MIC, reduction of biofilm already-formed biofilm at >4x MIC	Liu *et al*., 2022
6	Lysostaphin	*E. coli* BL 21(DE3)/ pET15b	MDR *S. aureus* strain USA300 and Newman, SA 113	Egypt, India	This substance results in the disintegration of the MRSA cell wall via endopeptidase activity.	The 0.05% LST gel reduced MRSA biofilm development by up to 5.5 times	Nithya *et al*., 2018; Nour El-Din *et al*., 2020
7	Enterocin AS - 48	*Enterococcus faecalis* UGRA10	Clinical isolate	Spain	The mechanism of this substance is focused on cell membrane disruption, usually combined with biocides to increase the ability of reduced MRSA biofilm	32 mg/l Enterocin AS-48 for 48 hours is particularly disrupts MRSA biofilm	Caballero Gómez *et al*., 2013; Velázquez-Suárez *et al*., 2021

The continuation of Table 1

No.	Type of bacteriocins	Bacteriocin producing bacteria	Origin of MRSA isolation	Country	Result	Bacteriocins inhibition	Reference
8	Lantibiotic CMB001	*Paenibacillus* sp.	*S. aureus* ATCC 29213	United Kingdom	The effectiveness of the Lantibiotic CMB001 to break the MRSA cell wall is equivalent to that of vancomycin	Based on the result of in vivo study using rat model, the top dose is 30 mg/kg to inhibit MRSA biofilm	Karczewski *et al*., 2021
9	Nisin A	*Lactococcus lactis*	*S. aureus* MR23	Japan	This substance has bacteriostatic and bactericidal activity. The bacteriostatic activity occurs through a lipid masking mechanism, whereas the bactericidal activity focuses on developing pores in the MRSA membrane	The formation of pores with a diameter of 2-2.5 nm strongly inhibits MRSA biofilms.	Okuda *et al*., 2013
10	Lacticin Q	*Lactococcus lactis*	*S. aureus* MR23	Japan	Large toroidal pores were formed by this substance, allowing the escape of the bactericidal protein molecules	MRSA biofilms were significantly decreased by the development of pores with a diameter of 4.6 to 6.6 nm	Okuda *et al*., 2013
13	Enterocins DD28 and DD93	*Enterococcus faecalis* 28 and 93	MRSA S-1 strain	France	This substance can reduce MRSA S-1 biofilm	The reduction of MRSA S-1 biofilm occurred after 24 hours (6.58 ± 0.17 of biofilm detachment)	Al Atya *et al*., 2016
13	Nisin	*Lactococcus lactis*	MRSA Xen 31	South Africa	Nisin develops pores within the target membrane	During nisin and 2-or 3-dihydroxybenzoic acid (DHBA) were combined, MRSA biofilms decreased by 88% after 24 hours in vitro	Ahire and Dicks, 2015
14	Enterocin DD14	*Enterococcus faecalis*	MRSA S-1 strain	France	Enterocin DD14 inhibits cell wall synthesis	Enterocin DD14 inhibits MRSA S-1 biofilm up to 30% (in vitro)	Belguesmia *et al*., 2021

Among the bacteriocins that perform their roles according to the previously described mechanism is nisin. It is a member of class IIb bacteriocins. Nisin may associate with lipid II and decrease the synthesis of new cell walls by blocking the lipid cycle II (Al-Seraih *et al*., 2017). In addition, Enterocin is another substance that uses this mechanism (Belguesmia *et al*., 2021).

Suppresses regulatory factor activity and inhibits the synthesis of extracellular matrix

Plantaricin GZ-27 was used to prevent MRSA ATCC 43300 from forming biofilms. It also reduced MRSA biofilm from the polystyrene surface. At the beginning, the mass of the MRSA biofilm started increasing significantly after 48 hours of incubation without the application of Plantaricin GZ - 27. The *Optical Density* (OD) measured at 595 nm was 2.258 ± 0.071. However, using Plantaricin GZ - 27 reduced the majority of the MRSA biofilm by 40.2% at 12 MIC and 55.3% at 14 MIC. Generally, 48 hours after being treated with plantaricin GZ - 27, the biofilms showed stable conditions (Du *et al*., 2020).

The mechanism of action of plantaricin GZ - 27 is inhibition of surface protein and *Polysaccharide Intercellular Adhesin* (PIA) formation. Surface proteins contribute to the extracellular matrix. However, PIA regulates the formation of biofilms. The surface proteins suppressed were *Serine Aspartate Repeat Protein* (SdrC), *Iron-Responsive Surface* determinant (IsdB), protein A (SpA), and *Fibrinogen-Binding Surface Protein* (FnPBP). In the process of forming biofilms, all these surface protein types are involved in mass accumulation and attachment. In the step of biofilm maturation, the modification of extracellular serine protease is important (Du *et al*., 2020).

Bactericidal activity and cell membrane degradation

An experiment was carried out in China (Table 1) to determine the ability of Bacin A2 to inhibit the proliferation of MRSA ATCC 43300 as a planktonic cell. After prolonged incubation, no MRSA growth was observed, and Bacin A2 showed substantial inhibition at doses of 1 - 2 MIC and maximum inhibition at values of 6 – 8 MIC. According to the result of this report, the MRSA ATCC 43300 cell membrane has been broken down after three hours of observation. It occurred as the bactericidal impact of 2 - 4 MIC Bacin A2 on MRSA cell. The MRSA defect cell membrane was observed under a microscope, and compared to a smooth and attached control cell (Liu *et al*., 2022).

Degradation of the *Methicillin-Resistant Staphylococcus aureus* (MRSA) cell wall by endopeptidase activity

Studies conducted in Egypt and India (Table 1) used LST to treat skin systemic infections caused by *Stapylococcus*. LST was first discovered in the 1960s and is commonly known as bacteriolysin. LST can disrupt the *Staphylococcus* bacterial cell wall (Nour El-Din *et al*., 2020).

***Methicillin-Resistant Staphylococcus aureus* (MRSA) biofilm and planktonic cells reduction**

Research conducted in Spain (Table 1) used a bacteriocin called Enterocin AS-48 to study the potential reducing effect of planktonic cells and the biofilm of MRSA. Enterocin AS - 48 is produced by *Enterococcus faecalis* UGRA10. The antimicrobial activity of Enterocin AS - 48 occurs through a common mechanism of action that targets the parasite T*rypanosomatidae*, *Gram-negative* and *Gram-positive* bacterial cell membranes, including MRSA. In this research, by providing up to 32 mg/l of Enterocin AS - 48 for 48 hours, MRSA cells and the surface matrix of MRSA biofilms can be reduced (Velázquez-Suárez *et al*., 2021).

Development of a bacteriocin-based *Methicillin-Resistant Staphylococcus aureus* (MRSA) infection treatment

The effectiveness of bacteriocins is improved by using them in combination with other antimicrobials or other active membrane agents. Several studies have combined the use of bacteriocins, conventional antibiotics, and acid compounds (Field *et al*., 2015; Liu *et al*., 2022; Santos *et al*., 2019). Currently, there is encouragement for the development of bacteriocin-based therapy. This covers the use of several treatment delivery methods to enhance the efficacy of bacteriocins and reduce the length of therapy to treat MRSA infections, in particular those caused by biofilms (Nithya *et al*., 2018; Nour El-Din *et al*., 2020; Santos *et al*, 2019). The following initiatives have been conducted to improve the use of bacteriocins:

Nisin biogel

Nisin biogel is a delivery system developed for the peptide nisin. It has been potentially tested in *Diabetic Foot Infection* (DFI) patients. Strong antibacterial effectiveness against the *Staphylococcus* biofilm that had grown on DFI was seen in DFI patients who received nisin biogel. In this research, several MRSA strains were also isolated to test the nisin biogel's antimicrobial activity in vitro. The combination of nisin biogel and chlorhexidine (as a complementary antiseptic agent) can potentially reduce the current use of antibiotics for DFI cases in clinical practice (Santos *et al.*, 2019).

***Lysostaphin Nano-Emulgel* (LNEG)**

For the treatment of skin infections brought on by MRSA, LNEG is an innovative formulation that combines the bacteriolytic enzyme lysostaphin into a nano-emulsion gel. It uses a small-size emulsion (<100 nm), which has a significant antimicrobial activity against MRSA both in vitro and in vivo. The nano-emulsion gel used in this research increases the stability and efficacy of lysostaphin. Based on the in vitro, LNEG degrades the MRSA cell wall, and then, based on in vivo testing, LNEG reduces the murine skin infection area and reduces the number of MRSA in the infection area (Nour El-Din *et al*., 2020).

Hybrid hydrogel for the treatment of chronic wounds

One of the hybrid hydrogels that is being developed is GarKS. The ingredients of GarKS are peptides, which have 32 - 34 amino acids. Based on the in vitro testing, GarKS has significant antimicrobial activity against Staphylococcus and other bacteria (*Bacillus*, *Listeria*, and *Enterococcus*). In addition, GarKS gel indicated an anti-MRSA biofilm effect in vivo after several treatments on infected rat wounds (Thapa *et al*., 2021).

Combination of nisin with DHBA nanofiber emulsion

A combination of nisin and DHBA nanofiber emulsions was used to treat diabetic wounds. It resulted in a reduction in the number of *Staphylococcus* cells after seven days of therapy. DHBA is a non-toxic substance derived from plants. Due to its high surface volume ratio and oxygen permeability, nanofiber was selected as a drug delivery component (Ahire and Dicks, 2015).

CONCLUSION

There is an opportunity to improve the treatment of MRSA biofilms with bacteriocins. Bacteriocins inhibit MRSA biofilms by inhibiting the synthesis of cell walls, leading to pores in the cytoplasmic membranes of bacterial cells, interrupting the synthesis of extracellular membranes, disrupting cell membranes, and reducing the number of planktonic cells within MRSA biofilms. Bacteriocins possess great low toxicity, low risk of resistance, and specific activity, which makes them safe to develop as agents against MRSA biofilms. In order to maximize this potential and provide novel bacteriocin variations that could be helpful in combating antibiotic resistance, especially in MRSA biofilms, further research and development are needed.

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