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

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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 antimicrobial 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.

ABSTRAK


Kata kunci: Bakteriosin, Biofilm, Resistance, 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, antibiotic 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-methylanthionine, 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 Ia), 2) Two-peptide bacteriocins (group IIb), 3) Cyclic bacteriocins (group IIc), 4) Nonpediocin single linear peptides (group IIId). 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

<table>
<thead>
<tr>
<th>No.</th>
<th>Type of bacteriocins</th>
<th>Bacteriocin producing bacteria</th>
<th>Origin of MRSA isolation</th>
<th>Country</th>
<th>Result</th>
<th>Bacteriocins inhibition</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Garvicin KS</td>
<td><em>Lactococcus garviae</em> KS 1546</td>
<td>ATCC 33591, USA300, MRSA, parental strain ATCC 33591, Xen 31</td>
<td>Norway</td>
<td>Utilized in the creation of hybrid hydrogels for the chronic skin wounds treatment, inhibits pre-formed of <em>S. aureus</em> biofilms in vitro.</td>
<td>MIC50 &gt;5</td>
<td>Kranjec et al., 2020; Thapa et al., 2021</td>
</tr>
<tr>
<td>2</td>
<td>Micrococcin P1</td>
<td><em>Lactococcus garviae</em></td>
<td>USA300 and ATCC 33591</td>
<td>Norway</td>
<td>Micrococcin P1 combined with Garvicin KS caused the MRSA strain to be sensitive to penicillin G.</td>
<td>MIC50 &gt;1.0x 10^-1</td>
<td>Kranjec et al., 2020</td>
</tr>
<tr>
<td>3</td>
<td>Nisin</td>
<td><em>Lactococcus lactis</em></td>
<td>Clinical, from DFI patients</td>
<td>Lisbon</td>
<td>High efficacy in preventing the formation of MRSA biofilms was obtained in the treatment using biogels containing nisin</td>
<td>OD value 0.2 - 0.3 on the wavelength 660 nm found at the level 22.5 µg/ml, it</td>
<td>Santos et al., 2019</td>
</tr>
<tr>
<td>4</td>
<td>Plantaricin GZ1-27</td>
<td><em>Lactococcus plantarum</em></td>
<td>MRSA ATCC 43300</td>
<td>China</td>
<td>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.</td>
<td>After 48 hours, MRSA biofilm decrease was between 40.2 and 55.3%.</td>
<td>Du et al., 2020</td>
</tr>
<tr>
<td>5</td>
<td>Bacin A2</td>
<td><em>Bacillus</em> sp. TL12</td>
<td>MRSA ATCC 43300</td>
<td>China</td>
<td>The characteristic of this substance is non-toxic. It can reduce MRSA biofilm that already presents, and disrupts cell membranes</td>
<td>Inhibition of biofilm formation at &gt;0.5x MIC, reduction of biofilm already-formed biofilm at &gt;4x MIC</td>
<td>Liu et al., 2022</td>
</tr>
<tr>
<td>6</td>
<td>Lysostaphin</td>
<td><em>E. coli</em> BL 21(DE3)/pET15b</td>
<td>MDR <em>S. aureus</em> strain USA300 and Newman, SA 113</td>
<td>Egypt, India</td>
<td>This substance results in the disintegration of the MRSA cell wall via endopeptidase activity.</td>
<td>The 0.05% LST gel reduced MRSA biofilm development by up to 5.5 times</td>
<td>Nithya et al., 2018; Nour El-Din et al., 2020</td>
</tr>
<tr>
<td>7</td>
<td>Enterocin AS - 48</td>
<td><em>Enterococcus faecalis</em> UGRA10</td>
<td>Clinical isolate</td>
<td>Spain</td>
<td>The mechanism of this substance is focused on cell membrane disruption, usually combined with biocides to increase the ability of reduced MRSA biofilm</td>
<td>32 mg/l Enterocin AS-48 for 48 hours is particularly disrupts MRSA biofilm</td>
<td>Caballero Gómez et al., 2013; Velázquez-Suárez et al., 2021</td>
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<tr>
<td>No.</td>
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<td>8</td>
<td>Lantibiotic CMB001</td>
<td><em>Paenibacillus</em> sp.</td>
<td><em>S. aureus</em> ATCC 29213</td>
<td>United Kingdom</td>
<td>The effectiveness of the Lantibiotic CMB001 to break the MRSA cell wall is equivalent to that of vancomycin</td>
<td>Based on the result of in vivo study using rat model, the top dose is 30 mg/kg to inhibit MRSA biofilm</td>
<td>Karczewski <em>et al.</em>, 2021</td>
</tr>
<tr>
<td>9</td>
<td>Nisin A</td>
<td><em>Lactococcus lactis</em></td>
<td><em>S. aureus</em> MR23</td>
<td>Japan</td>
<td>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</td>
<td>The formation of pores with a diameter of 2-2.5 nm strongly inhibits MRSA biofilms.</td>
<td>Okuda <em>et al.</em>, 2013</td>
</tr>
<tr>
<td>10</td>
<td>Lacticin Q</td>
<td><em>Lactococcus lactis</em></td>
<td><em>S. aureus</em> MR23</td>
<td>Japan</td>
<td>Large toroidal pores were formed by this substance, allowing the escape of the bactericidal protein molecules</td>
<td>MRSA biofilms were significantly decreased by the development of pores with a diameter of 4.6 to 6.6 nm</td>
<td>Okuda <em>et al.</em>, 2013</td>
</tr>
<tr>
<td>13</td>
<td>Enterocins DD28 and DD93</td>
<td><em>Enterococcus faecalis</em> 28 and 93</td>
<td>MRSA S-1 strain</td>
<td>France</td>
<td>This substance can reduce MRSA S-1 biofilm</td>
<td>The reduction of MRSA S-1 biofilm occurred after 24 hours (6.58 ± 0.17 of biofilm detachment)</td>
<td>Al Atya <em>et al.</em>, 2016</td>
</tr>
<tr>
<td>13</td>
<td>Nisin</td>
<td><em>Lactococcus lactis</em></td>
<td>MRSA Xen 31</td>
<td>South Africa</td>
<td>Nisin develops pores within the target membrane</td>
<td>During nisin and 2-or 3-dihydroxybenzoic acid (DHBA) were combined, MRSA biofilms decreased by 88% after 24 hours in vitro</td>
<td>Ahire and Dicks, 2015</td>
</tr>
<tr>
<td>14</td>
<td>Enterocin DD14</td>
<td><em>Enterococcus faecalis</em></td>
<td>MRSA S-1 strain</td>
<td>France</td>
<td>Enterocin DD14 inhibits cell wall synthesis</td>
<td>Enterocin DD14 inhibits MRSA S-1 biofilm up to 30% (in vitro)</td>
<td>Belguesmia <em>et al.</em>, 2021</td>
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</table>
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).

**Suppress 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 *Staphylococcus*. 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 *Trypanosomatidae*, 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 combatting antibiotic resistance, especially in MRSA biofilms, further research and development are needed.

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