Current Antibiotics and Future Herbal Extract Methods to Treat Methicillin-Resistant *Staphylococcus aureus* (MRSA): Focusing on Inhibition of Penicillin-Binding Protein 2a (PBP2a)

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Abstract

Methicillin-Resistant *Staphylococcus aureus* (MRSA) has developed resistance towards a number of antibiotics. This resistance creates a challenge when trying to treat MRSA with a number of antibiotics. This is mainly caused by the penicillin-binding proteins 2a (PBP2a). PBP2a have significantly less affinity for beta-lactam antibiotics compared to the other penicillin-binding proteins (PBPs) expressed by non-resistant strains. New treatments involving a combination of antibiotics and herbal extracts are being developed and used to inactivate PBP2a, allowing the previous ineffective antibiotics to be more effective.

Current Antibiotics and Future Herbal Extract Methods to Treat Methicillin-Resistant *Staphylococcus aureus* (MRSA): Focusing on Inhibition of Penicillin-Binding Protein 2a (PBP2a)

Staphylococcus aureus is a Gram-positive bacterium discovered in the 1880s. *S. aureus* is a pathogenic bacterium that causes infections as minor as those that affect the skin to severe post-operative wound complications. During the early 1940s, before the use of penicillin to treat *S. aureus* infections, these infections had a mortality rate as high as 80%, but as soon as penicillin started being used as a treatment option in 1942, a first resistant strain was isolated in a hospital. In 1962, a similar situation happened, where after the introduction of penicillin derivatives as an alternative to penicillin for treatment for *S. aureus* infections, resistant strains were isolated (Public Health in the 21st Century: MRSA (Methicillin Resistant Staphylococcus aureus) Infections and Treatment, 2010). These resistant strains were observed in the hospitals as well as in the community, which led to the coining of the terms Hospital Acquired-MRSA (HA-MRSA) and Community Acquired-MRSA (CA-MRSA).

Currently, *S. aureus* is one of the major pathogens in the world. *S. aureus* is a serious threat to the public health and is a major nosocomial infection since hospitals are concentrated with people with weakened immunity (Wishart, Loughrey, McClurg, Goldsmith, & et al., 2007; Kelley, Jousselin, Barras, Lelong, & Renzoni, 2015; Otero et al., 2013). The high usage of implanted prosthetic biomaterials contribute to some of the cases involving MRSA outbreaks in hospitals since such devices could be breeding grounds for MRSA colonies (Pozzi et al., 2012). Compared to other clinically important bacteria, MRSA distinguishes itself from the rest because the bacterium possesses a

variety of virulence factors including the production of biofilms; MRSA is resistance to a broad spectrum of antimicrobial drugs and can be found in both hospital and community settings at significantly high occurrences (Public Health in the 21st Century: MRSA (Methicillin Resistant Staphylococcus aureus) Infections and Treatment, 2010).

Penicillin-Binding Protein (PBP)

The bacterial cell wall is an important part of the overall structure of the cell. The cell wall is mainly composed of peptidoglycan that help the bacterium to resist intracellular and extracellular pressures while maintaining the rigidity of the cell (Sauvage, Kerff, Terrak, Ayala, & Charlier, 2008). This also helps give the bacterial cell a more defined shape compared to animal cells that lack a cell wall. Likewise, penicillin-binding proteins (PBP) are responsible for the polymerization and cross-linkage of peptidoglycans to form the bacterium cell wall (Sauvage et al., 2008). The last D-alanine of stem pentapeptides is one of the main regions on the peptidoglycans used by PBPs during cross-linkage. These cross-linking regions have structural similarities to penicillin (and its derivatives) that allow some PBPs form acyl-enzymes with the penicillin, inhibiting any cross-linking capabilities (Sauvage et al., 2008).

There are many different types of PBPs coded by different genes. Bacteria can have as many as 6 different PBPs. PBPs are divided into two main groups: the high molecular mass (HMM) PBPs, and the low molecular mass (LMM) PBPs (Sauvage et al., 2008). HMM PBPs deal with polymerization of peptidoglycans and insertion of peptidoglycans into the cell walls of pre-existing cells. LMM PBPs mainly deal with cell remodeling activities such as cell separation, peptidoglycan maturation, and recycling (Sauvage et al., 2008).

Penicillin-Binding Protein 2a (PBP2a)

Penicillin-binding protein 2a (PBP2a) is an alternative structural protein synthesized by MRSA to take over the functions of normal PBPs when subjected to stress in the environment, such as through the addition of antibiotics. PBP2a has an extremely lower affinity for antibiotics than the normal PBPs, which creates a serious problem when developing new antibiotics against MRSA (Pinho, de Lencastre, & Tomasz, 2001; Pinho, Filipe, de Lencastre, & Tomasz, 2001). PBP2a consists of an N-terminal transmembrane anchor, a C-terminal transpeptidase domain, and a bilobal nonpenicillin-binding domain (Figure 1). The protein is approximately 130 by 60 by 58 Å, where Å= 10^{-10} m (D. Lim & Natalie, 2002). The *mec*A gene is responsible for encoding PBP2a (Alexander, 2003; Ender, Berger-Bachi, & McCallum, 2009; Haghighat, Siadat, Sorkhabadi, Sepahi, & Mahdavi, 2013; Kim et al., 2013; D. Lim & Natalie, 2002). The acquired *mec*A gene is only 2.1 kilobases (kb) in length and is located on the *S. aureus* chromosome in the mobile genetic element that is known as *staphylococcal cassette chromosome mec* (*SCCmec*) (Pozzi et al., 2012). Although this element is mobile and capable of being transferred from one bacterium to another, *SCCmec* still inserts itself at specific and consistent positions on the *S. aureus* chromosome. (Katayama, Robinson, Enright, & Chambers, 2005; Public Health in the 21st Century: MRSA (Methicillin Resistant Staphylococcus aureus) Infections and Treatment, 2010).

There are currently seven identified variants of the *SCCmec* that differ in their size but serve the same function of conferring antibiotics resistance to MRSA (Katayama et al., 2005). *SCCmec* type 1 contains 34.3 kb; type IV contains 20.9-24.3kb; type V contains 28 kb; type VI has 20.9 kb; and type VII has 35.9 kb. These variants confer

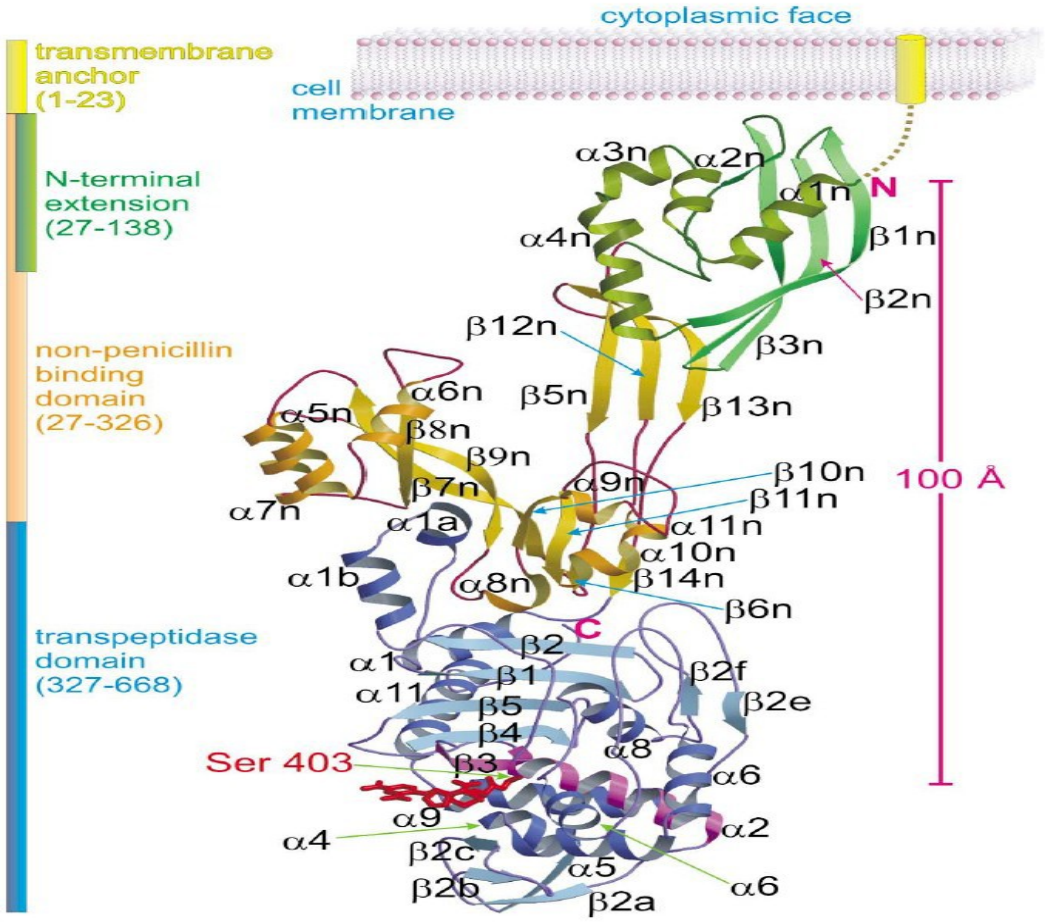

Figure 1: Structure of PBP2a. Reprinted by permission from Macmillan Publishers Ltd: [NATURE STRUCTURAL & MOLECULAR BIOLOGY] (D. Lim & Natalie, 2002), copyright (2002).

resistance against β-lactam antibiotics only. The type II (53.0 kb) and III (66.9 kb) confer resistance against multiple classes of antibiotics (Public Health in the 21st Century: MRSA (Methicillin Resistant Staphylococcus aureus) Infections and Treatment, 2010). However, the *mec*A gene is not always active. The *mec*A gene is only active when normal PBPs fail to function or are inhibited by antibiotics. The *SCCmec* also contains other genes, *mecR1* and *mecI* that serve as regulatory components to the *mec*A gene (Ender et al., 2009; McKinney, Sharma, Craig, & Archer, 2001). These regulatory genes have promoter regions that overlap with that of *mec*A. The *mecR1* encodes a protein that

senses the stress in the bacterial environment and activates the *mec*A gene. The *mecI* gene does the opposite and is responsible for repressing the transcription of *mec*A when the bacterium does not need PBP2a (Ender et al., 2009; McKinney et al., 2001). Therefore, in order for PBP2a to be synthesized, *mecR1* needs to be activated; otherwise, *mecI* will suppress the gene. Some bacteria lack both *mecR1* and *mecI*. This DNA segment is either truncated or absent all together. In these bacteria, the regulation of *mec*A is controlled by the *blaR1*/*blaI* genes. These genes work in a similar fashion to *mecR1/mecI*. The *blaR1*/*blaI* genes regulate the transcription of *blaZ*, which codes for β-lactamases, enzymes that degrade β-lactam antibiotics such as penicillin (Ender et al., 2009; McKinney et al., 2001). This is way that bacteria like MRSA confer resistance against antibiotics. In the absence of both *mecR1/mecI* and *blaR1/blaI*, the *mec*A will be always turned on, and so the majority of the cell wall proteins synthesized will be PBP2a, and not the normal PBP.

When the bacterium senses the surrounding environment contains toxic levels of antibiotics, its transmembrane sensors, mecR1/blaR1, undergo a conformational change, which is followed by autoproteolytic cleavage of the n-terminal of the cytoplasmic domain of the transmembrane sensors. This activates the cytoplasmic peptidases, which degrade the repressor proteins of the *blaZ/mecA* genes, and begins the transcription of their respective proteins (Ender et al., 2009).

The minimum inhibitory concentrations (MIC) of antibiotics are MRSA-strain specific. The MICs of antibiotics of two strains of MRSA can be close to one another or have a large difference. MICs for an antibiotic like oxacillin can start as low as 1 μg/ml to as high as values greater than 500 μg/ml (Ender et al., 2009). At this point the MICs are

not clinically practical and the strain is therefore considered resistant to antibiotics. Experiments have also shown that there are other genomic factors, collectively known as *fem/aux* factors, that work together with the *mec*A gene that confers antibiotic resistance to MRSA. These include genes that are involved in the synthesis of the precursors needed in the formation bacterial cell wall such as teichoic acid synthesis (Ender et al., 2009).

The cell wall of MRSA and other Gram-positive bacteria is made up of peptidoglycan macromolecules that are cross-linked to each other. These molecules provide the necessary strength and stability, which helps in maintaining the shape of the bacteria, and protect the bacteria from osmotic influences of the environment (Public Health in the 21st Century: MRSA (Methicillin Resistant Staphylococcus aureus) Infections and Treatment, 2010). Peptidoglycans are the building blocks of bacterial cell walls and are made up of alternating chains of N-acetylglucosamine and N-acetylmuramic acid residues connected by peptide bridges (Public Health in the 21st Century: MRSA (Methicillin Resistant Staphylococcus aureus) Infections and Treatment, 2010).

The synthesis of peptidoglycans involves three stages. The first stage takes place in the cytoplasm and involves taking a UDP-linked N-acetylmuramic acid molecule and sequentially adding amino acids. During the second stage, the sugar residue is transferred to bactoprenol, a lipid carrier. The lipid carrier transports the sugar residue across the cytoplasmic membrane. At this point N-acetylglucosamine residues will be added. In the third stage, which takes place on the outer surface of the plasma membrane, transglycosyation occurs, where polysaccharide chains are added to the existing chain

(Public Health in the 21st Century: MRSA (Methicillin Resistant Staphylococcus aureus) Infections and Treatment, 2010).

Treatments

Common Treatments

Bacterial infections are mainly treated by antibiotics. Bacterial species can differ in terms of their overall structure and metabolic process, which creates a need to develop different kinds of antibiotics to counter specific strains of bacteria. There are at least 11 different classes of antibiotics, each with its own sub-classes and unique members (Table 1). These classes include aminoglycosides, β-lactams, glycopeptides, lipopeptide, macrolide, oxazolidinone, quinolone, and tetracycline (Bush, 2012). Each class has a specific mechanism of action. The β-lactams, glycopeptide, lipopeptide, and colistin (polymixin E) target the cell membranes of the bacteria. Aminoglycosides, macrolides, ketolides, tetracycline, oxazolidinone, and streptogramins block protein synthesis. Quinolone and sulfa drugs mainly affect DNA and replication (Hauser, 2013). But the list from table 1 is not all inconclusive. There are currently many other antibiotics available for clinical use, and many others that are in the clinical trial phase.

Vancomycin. Vancomycin is one of the most important antibiotics used to treat Gram-positive infections like MRSA. Vancomycin is a tricyclic glycopeptide with a large and a complex structure that works mainly by inhibiting the formation of the bacterial cell wall (Figure 2; McNamara & Steckelberg, 2005). Vancomycin halts the production of peptidoglycans, which form the basis of the cell wall of bacteria and binds to the D-alanyl- D-alanine terminal of the cell wall subunits (McNamara & Steckelberg, 2005). Other drugs such as penicillins and cephalosporins can also inhibit the production of

Table 1: *Classes and sub-classes of different antibiotics*

Classes of antibacterial agents approved for clinical use.

Class	Subclass	Original member of the class or subclass	Date of original class identification	Recent compounds in development post-2000
Aminoglycoside		Streptomycin	1943	Plazomicin
β-Lactam	Penicillin	Benzylpenicillin (Penicillin G)	1928	None
	Cephalosporin	(Cephalosporin C)	(1948)	Ceftobiprole, ceftaroline[a], ceftolozane
		Cephalothin	1962	
	Carbapenem	Imipenem	1976	Doripenem[b]
	Monobactam	Aztreonam	1981	BAL30072, MC-1
	β-Lactamase inhibitor (clavam)	Clavulanic acid	1976	Non-β-lactams: avibactam, MK-7655
Glycopeptide		Vancomycin	1952	Dalbavancin, oritavancin, telavancin[a]
Lipopeptide		Daptomycin	1985	None
Macrolide		Erythromycin	1949	None
	Ketolide	Telithromycin	1997	Cethromycin, solithromycin
Oxazolidinone		Linezolid	1995	Radezolid, tedizolid
Quinolone	Fluoroquinolone	Nalidixic acid	1962	Delafloxacin, JNJ-Q2, nemonoxacin
Tetracycline	Tetracycline	Chlortetracycline	1945	TP-434
	Glycylcycline	Tigecycline	1998	Omadacycline

[a] Approved by the FDA.
[b] Approved by the FDA and EMA.

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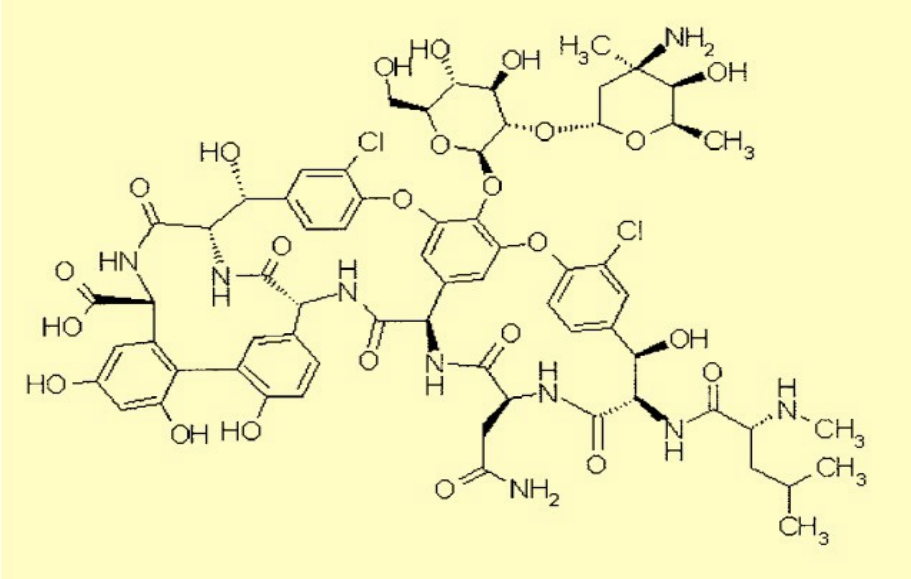

Figure 2: The structure of Vancomycin. From the National Library of Medicine, accessed November 30, 2016, at http://www.nlm.nih.gov/pubs/techbull/ma00/ma00_chemid_fig8.html

bacterial cell wall. These drugs usually disrupt the process of cell wall formation wall in similar stages and sometimes result in cross-resistance of antibiotics, where the resistance against one type of drug can allow an organism to be resistant to a different type of drug. Vancomycin is not usually faced with problems of cross-resistance since many of the other antibiotics affect the cell wall formation process at a much different stage. Vancomycin can be used to treat the majority of Gram-positive bacteria; however, vancomycin is almost useless against Gram-negative bacteria. The cell wall composition of Gram-negative bacteria is slightly different from that of Gram-positive bacteria like *Staphylococci* species (McNamara & Steckelberg, 2005). The cell walls of Gram-negative bacteria are thinner and have slightly different amino acid linkers than Gram-positive bacteria.

Vancomycin doses are usually administered intravenously to treat systemic or orthopedic infections. This is because the absorption of vancomycin in the gastrointestinal tract is low, and most of the drug will pass through unchanged. Oral doses are mainly used for infections that are in the gastrointestinal tract such as colitis caused by Gram-positive bacteria such as *Clostridium difficile*. Once in the blood stream, vancomycin is mainly excreted through renal glomerular filtration. The liver metabolizes only a small fraction of the drug (McNamara & Steckelberg, 2005).

Although drug-resistance continues to be a problem with many antibiotics, vancomycin is still considered the first-line of attack against invasive MRSA infections. Vancomycin was developed over 50 years ago, and is still one of the strongest drugs used against these infections (Public Health in the 21st Century: MRSA (Methicillin Resistant Staphylococcus aureus) Infections and Treatment, 2010). Over the years vancomycin has

been used for various conditions including endocarditis, pneumonia, and wound infections. The cure rates for these conditions are 63%, 75%, and 90%, respectively (Public Health in the 21st Century: MRSA (Methicillin Resistant Staphylococcus aureus) Infections and Treatment, 2010). But vancomycin still faces challenges in some aspects such as the treatment of lung infections because of the poor penetration rate. Also, some strains of bacteria including MRSA have been reported to develop resistance against vancomycin.

Vancomycin faces additional criticism from some of its adverse reactions that some patients develop from its use. There have been reports for nephrotoxicity when treatments are combined with aminoglycoside agents. Another adverse reaction seen in patients taking vancomycin is "Red man syndrome", which is caused by rapid infusion of vancomycin leading to a reddish itchy rash on the upper trunk, neck and face as a result of histamine release (McNamara & Steckelberg, 2005).

Quinupristin and dalfopristin. Quinupristin and dalfopristin are antibiotics that are members of the class streptogramins. These antibiotics, which are administered intravenously, became available for use in the United States in 1999 (Hauser, 2013). Individually, the drugs have moderate antibacterial activity but are much more effective when used together. These antibiotics inhibit protein synthesis in bacteria by binding to the large (50S) subunit of the bacterial ribosome. Dalfopristin causes a conformational change in the ribosome, which allows the binding of quinupristin (Hauser, 2013). Even though quinupristin and dalfopristin have shown great potency against MRSA, there have also been numerous cases of resistance against these antibiotics. Resistance against quinupristin and dalfopristin have been due to conformational modification of the

ribosome, which prevents the binding of streptogramins; as a result, there have been reported cross-resistance with drugs such as macrolides and clindamycin because of having similar binding regions (Hauser, 2013). Enzymatic degradation of the drugs and efflux pumps are other factors that could lead to drug resistance (Hauser, 2013).

Daptomycin. Another option that can be used to treat MRSA infections is daptomycin. Daptomycin is a cyclic lipopeptide consisting of 13 amino acids with a decanoyl side chain that has been used to treat complicated skin and soft tissue infections (cSSTIs) (Figure 3) (Public Health in the 21st Century: MRSA (Methicillin Resistant Staphylococcus aureus) Infections and Treatment, 2010). Daptomycin is derived from *Streptomyces roseosporus* as a fermentation product. Studies have shown that daptomycin is as effective as vancomycin in treating MRSA related bacteremia and endocarditis. Daptomycin is also as effective against Methicillin-Sensitive *Staphylococcus aureus* (MSSA). Its high potency and rapid bactericidal activity, especially against *S. aureus*, suggests daptomycin can be a better alternative in patients at risk of serious complications of infection such as those on dialysis who are suffering from blood stream infections caused by strains of *S. aureus* (Public Health in the 21st Century: MRSA (Methicillin Resistant Staphylococcus aureus) Infections and Treatment, 2010). There is a low risk associated with the development of spontaneous mutational resistance against daptomycin. This compounded with its ability to not develop cross-resistance with other known classes of antibiotics provides an excellent alternative to treating *S. aureus* related infections, including both resistant and non-resistant groups since there have been cases where patients develop severe complications as a result of vancomycin

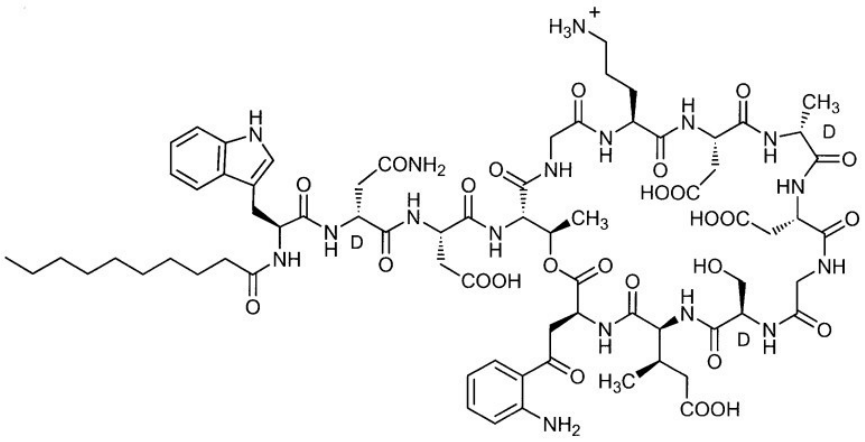

Figure 3: Daptomycin Structure. Reprinted from Chemistry & Biology, Vol 11, Jason Micklefield, Daptomycin Structure and Mechanism of Action Revealed, 887-888, Copyright (2004), with permission from Elsevier.

doses when the bacteria is MSSA and not MRSA (Public Health in the 21st Century: MRSA (Methicillin Resistant Staphylococcus aureus) Infections and Treatment, 2010).

Linezolid. A synthetic oxazolidone that binds to the bacterial 23S ribosomal RNA of the 50S ribosomal subunit, linezolid, inhibits protein synthesis (Figure 4). Linezolid has shown success against *S. aureus* infections as well as other Gram-positive bacteria. The efficacy of linezolid has been shown to be similar to vancomycin. Compared to vancomycin, there have been fewer reports of resistance, which may be due to its lower usage. Failed treatments when using linezolid are usually caused by deep infections and failures to drain abscesses (Public Health in the 21st Century: MRSA (Methicillin Resistant Staphylococcus aureus) Infections and Treatment, 2010).

Proposed Alternative Treatment Methods

***Duabanga grandiflora* on MRSA biofilms.** Many factors allow bacterial cells to become resistant to antibiotics. One such factor is the ability of the bacterial cells to grow

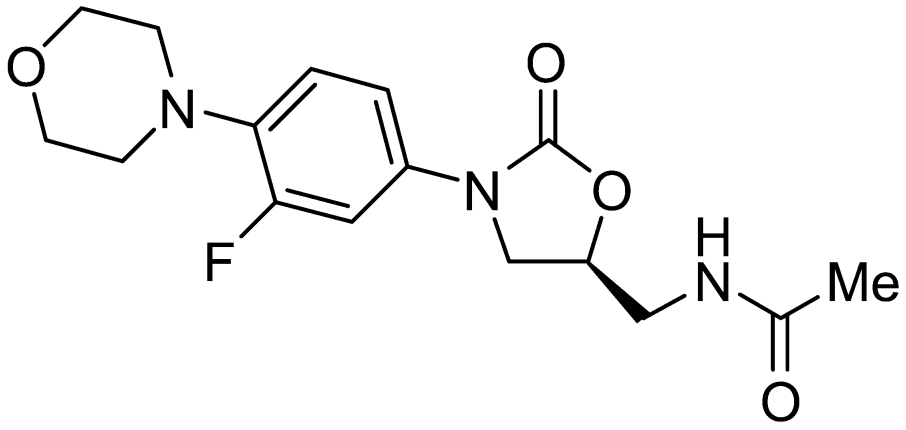

Figure 4: Structure of linezolid. Reprinted from Tuberculosis, Vol 88, Linezolid, 122-125, Copyright (2008), with permission from Elsevier.

in biofilms. Biofilms significantly increase the resistance of bacteria to antibiotics by protecting bacteria from any changes in their immediate environment including the introduction of any potential antimicrobial agents. Studies have shown that biofilms created by immobile organisms, such as MRSA, are physiologically different from those of mobile organisms like planktons, which also allow for a greater survival rate within the infected hosts. Also, these two groups of organisms have different modes of growth (Santiago, Kuan-Hon, Hwei-San, & Kang Nee, 2015). This difference contributes to their resistance towards antibiotics. Therefore, treatments for infections involving biofilm-producing bacteria like MRSA are much more difficult to create, which could lead to chronic device-related infections (infections associated with surgically implanted medical devices) (Santiago et al., 2015).

MRSA's ability to form biofilms is one of the features that have allowed this microbe's increased occurrence as a nosocomial infection whose treatments are made difficult by other virulence factors such as the PBP2a protein. Studies have shown that PBP2a is involved in the formation of biofilms in MRSA (Santiago et al., 2015). One hypothesis suggests that PBP2a facilitates interactions between cells in the development

of biofilms (Santiago et al., 2015). Therefore, plausible approaches in developing new treatments against MRSA infections is through the development of drugs that disrupt any of the steps involved in biofilm production, or interfere with the expression of PBP2a proteins which would eventually affect biofilm production.

Duabanga is a genus in the plant kingdom originally placed in the *Sonneratiaceae* family. Presently, *Duabanga* is classified under the subfamily Duabangoideae of the family *Lythraceae*. *Duabanga* is indigenous to Southeast Asia (Malik et al., 2016). *Duabanga* consists of three main species, *Duabanga grandiflora*, *Duabanga moluccana*, and *Duabanga taylorii Jayaweera*. In some parts of Southeast Asia including Malaysia, the three species have other names used to identify them, *Berembang Bukit*, *Megawasih*, and *Pedada Bukit*, respectively (Malik et al., 2016).

D. grandiflora trees, the subject of this section, which grow up to 30m high, are commonly found along valleys and streams in tropical evergreen forests. These trees, and especially its leaves, have been shown to possess medicinal properties. The leaves help stimulate the production of type II collagen, which helps heal inflammation and slow the aging process of the skin. The leaf extracts are also used to treat skin conditions including eczema and atopic dermatitis (Malik et al., 2016; Santiago et al., 2015). Patients with such skin conditions are generally predisposed to *Staphylococcus aureus* infections. Extracts and purified fractions obtained from *D. grandiflora* have been shown to inhibit bacterial growth. In addition to growth inhibition, studies have shown that these extracts and fractions re-sensitized MRSA towards ampicillin and other antibiotics that were previously ineffective against MRSA (Santiago et al., 2015). These extracts contain high

concentration of phytochemicals such as tannins, phenolic compounds, flavonoids and steroids. These phytochemicals also possess antiviral properties against human and animal viruses.

In one study, Santiago et al. used a semi-purified fraction isolated from *D. grandiflora* by sequential extraction using hexane, ethyl acetate, and ethanol. This fraction named F-10 significantly inhibited the production of biofilms in bacterial cultures including MRSA; hence, the results were an indication of promising anti-MRSA activity (Santiago et al., 2015). As expected, F-10 contained tannins, flavonoids, and steroids, among other things. Flavonoids disrupt the activity of sortase, a bacterial enzyme known to influence the adhesive property of bacterial cell walls (Santiago et al., 2015). This suggests that the presence of flavonoids in F-10 may explain its ability to inhibit the production of biofilms by MRSA. This study also revealed that MRSA cultures had a significantly lower expression of PBP2a in their biofilms when treated with F-10 (Santiago et al., 2015). Disruption of biofilms and lower expression of PBP2a due to *D. grandiflora* extract treatments may make MRSA less virulent and more susceptible to antibiotics such as ampicillin that were previously ineffective.

***Acalypha wilkesiana* on PBP2a.** A genus in the plant kingdom that is comprised of about 450 different species of plants (S. W. Lim et al., 2011), *Acalypha wilkesiana* is a shrub belonging to the Euphorbiaceae family, sometimes referred to as the spurge family (S. W. Lim et al., 2011; Odoh et al., 2014; Olukunle, Adenubi, Biobaku, & Sogebi, 2015). This species is mainly found in Fiji and nearby islands in the South Pacific. Over the years, its occurrence has spread to other parts of the world, especially the tropics of Africa, America, and Asia. As a result, this species of plants has gathered a wide variety

of names that people commonly use to refer to the plant. Some of these common names are Joseph's coat, referring to the different colors observed on its leaves, red leaf, fire dragon, beefsteak plant and match-me-if-you-can. In Northern Nigeria, the Hausa people named the plant "Jiwene" and "Jinwinini," while the Yoruba people of Southern Nigeria named the tree "aworoso" (S. W. Lim et al., 2011; Odoh et al., 2014; Olukunle et al., 2015). In western parts of Nigeria, the aqueous leaf extract of *A. wilkesiana* has been used as a short-term remedy for neonatal jaundice, whereas in the south, the extract is mostly used for the treatment of skin infections in children (Odoh et al., 2014). *A. wilkesiana* has been reported to show antimicrobial properties against a variety of bacteria, including *Staphylococcus aureus, Yersinia enterocolitica, Escherichia coli, Salmonella typhi, Pseudomonas aeruginosa,* and *Klebsiella aerogenes*. The effects of *A. wilkesiana* on *S. aureus* led to further studies on antibiotic resistant strains of bacteria such as MRSA. Extracts from the plant have also been used in the management of hypertension, diabetes, gastrointestinal disorders, pain management and has potential cytotoxic effects against cancerous cells.

PBP2a is one of the key factors that confer antibiotics resistance to MRSA. Therefore, inactivating or decreasing the production of PBP2a will significantly help in the fight against MRSA. Santiago et al. observed that purified extracts of *A. wilkesiana* when used in conjunction with ampicillin, a non-effective treatment against MRSA, had synergistic properties, which resulted in increased susceptibility of MRSA to ampicillin. The minimum inhibitory concentration (MIC) of ampicillin needed to elicit antibacterial activity against MRSA is not practical and cannot be used in a hospital setting since ampicillin at such concentration would be toxic to patients, and therefore have no clinical

application. However, when extracts from *A. wilkesiana* are used in combination with ampicillin, the MIC of ampicillin drops significantly lower. This value is also lower than the MIC of ampicillin needed to inhibit the growth of sensitive strains of *Staphylococcus aureus* (Santiago et al., 2014). Using western blot analysis of the MRSA strains showed low expression levels the PBP2a protein in those cultures treated with the purified extracts from *A. wilkesiana*. Although extracts from the plant decreased the expression of PBP2a proteins in MRSA, such extracts did not show any bactericidal properties (Santiago et al., 2014), meaning, these extracts can make MRSA less resistant, but not kill the bacteria. This creates the possibility of combining ampicillin and the extracts to overcome ampicillin resistance, but at a much lower concentration than would normally be needed.

The study conducted by Santiago et al. demonstrated the antimicrobial activity of the extract of *A. wilkesiana*. Furthermore, the extract showed a synergistic relationship between the extract, which was named 9EA-FC-B, and ampicillin in the inhibition of PBP2a production, which also allowed the MRSA cultures to be inhibited by ampicillin. The growth of MRSA was distinctly suppressed when both, the extract and ampicillin, were used compared to MRSA cultures that were treated alone with either compound. This effect was mainly observed during the exponential phase of the bacteria kinetic growth curve (Santiago et al., 2014). The exponential phase is the greatest increase in growth of bacteria observed in the presence of necessary nutrients. There was minimal MRSA growth during this stage when 9EA-FC-B and ampicillin were introduced in their environment. Changes were also observed during the lag phase of MRSA growth. This is the stage where the bacteria begin to acclimate or adapt to changes in the environment.

This stage was observed to take longer in MRSA cultures treated with 9EA-FC-B and ampicillin (Santiago et al., 2014). Previous studies have shown compounds such as corilagin and tellimagrandin I posses similar antimicrobial activities (Santiago et al., 2014). Corilagin and tellimagrandin I are weak carbonic anhydrase inhibitors and are present in numerous plants including *Punica granatum*, *Myrtaceae*, and *Elaegnaceae*. Corilagin has also been previously isolated from *A. wilkesiana*, which suggests that the active ingredient in the 9EA-FC-B extract maybe corilagin (Santiago et al., 2014).

***Pongamia pinnata* effect on MRSA.** *Pongamia pinnata* is a legume that belongs in the subfamily Papilionoideae (Scott et al., 2008; Yadav et al., 2011). *P. pinnata* is commonly found in the Indian subcontinent and South-east Asia. Presently, *P. pinnata* can be found in other parts of the world such as Australia, New Zealand, China, USA, Philippines, and the humid tropical regions of the world. The tree derives its name from the Tamil language of India (Scott et al., 2008). Other names associated with *P. pinnata* are "Ponga," "Dalkaramacha," "Pongam," "Punku," "Karanj," "Papar," and "Kanji." *P. pinnata* is a medium-sized evergreen tree that grows easily on alluvial and coastal environment in areas found at sea level to 1200m above sea level, and one of the most admired city trees in India due to its glowing lime-green colors (Scott et al., 2008).

P. pinnata contains various phytochemical compounds. The most common ones are flavonoids and fixed oils. The seeds of *P. pinnata* also contain two sterols, three sterol derivatives, and one disaccharide, as well as eight fatty acids, of which three were saturated. The leaves and stem of the tree are the major sites for flavonoids and chalcone derivatives (Scott et al., 2008).

A number of herbal remedies involve parts of the plant, *P. pinnata*. There have been several documented reports that show that root extracts of the plant had significant prophylactic abilities against aspirin-induced ulceration, although little success was observed against ethanol-induced ulcerations. The root extracts helped to augment the mucosal cells, mucosal cell glycoproteins, cell proliferation, and prevent lipid peroxidation in the stomach walls (Scott et al., 2008).

P. pinnata has also shown promise in antibacterial activities. One notable impact is its effect against MRSA. A study by Inala et al. showed crude extracts from *P. pinnata* seeds were tested on clinically significant pathogens such as *Klebsiella pneumonia, Proteus vulgaris, Pseudomonas aeruginosa, Serratia marcescens, Micrococcus luteus, and* non-resistant strains of *Staphylococcus aureus*. Positive results from this study prompted more tests to be done on MRSA (Inala et al., 2015). A phytochemical analysis of the plant seeds showed the presence of alkaloids, tannins, saponins, steroids, glycosides and flavonoids (Inala et al., 2015). Inala et al. isolated the flavonoid components using adsorption column chromatography and used an agar well diffusion assay to test the efficacy of the extracted flavonoids against MRSA strains that were still susceptible to vancomycin, and the results were compared to MRSA strains that were resistant to vancomycin (Inala et al., 2015). The results were similar, in which the compounds displayed similar antibacterial capabilities against both vancomycin-resistant and vancomycin-sensitive MRSA strains. Therefore, extracts from *P. pinnata* have promising potential substitutes for antibiotics against MRSA that are no longer effective.

***Curcuma longa* against MRSA.** A perennial tree that belongs to the Zingiberaceae family (Reddy et al., 2012), *Curcuma longa* usually grows up to a height

of 1m and can be found distributed in the tropical and subtropical regions of the world but mainly cultivated in India and China. In India, commonly known as “Haldi,” Malaysia, and Indonesia, there have been numerous studies on the plant due to the economic importance in Malaysia and Indonesia. Other vernacular names associated with *C. longa* are “Haridra” from the Telugu language, “Manjal” from the Malayalam and Tamil language, and “Arisina” from the Kannada language (Reddy et al., 2012). All these language groups are indigenous to India. *C. longa* is considered a sterile plant because of its inability to produce any seeds. *C. longa* is used for both medicinal and food preparation. The rhizomes of the plant are commonly used as household remedies in Nepal (Reddy et al., 2012). There have been several claims in India that the plant can be used to fight against biliary disorders, anorexia, coryza (an inflammation of the mucous membrane of the nose, often caused by colds or hay fever), cough, diabetic wounds, hepatic disorders, rheumatism and sinusitis. The plant can also be boiled, dried and grounded to make turmeric, a bright yellow spice (Reddy et al., 2012).

Curcumin or diferuloymethane is a phenolic diketone (Sharma, Gescher, & Steward, 2005). Curcumin has a molecular weight of 368.37 and melts at 183 degrees Celsius. There are three isoforms of curcumin that have been identified in *C. longa*: Curcumin I, Curcumin II, and Curcumin III (Figure 5). In acidic and neutral environments as well as in cell membranes, the bis-keto form of curcumin predominates (Figure 6). The enolate predominates in basic environments where curcumin acts as an electron donor (Sharma et al., 2005).

Curcumin has a low rate of absorption. Pharmacokinetic studies in animals have shown that about 40 to 85% of curcumin administered orally passed through the

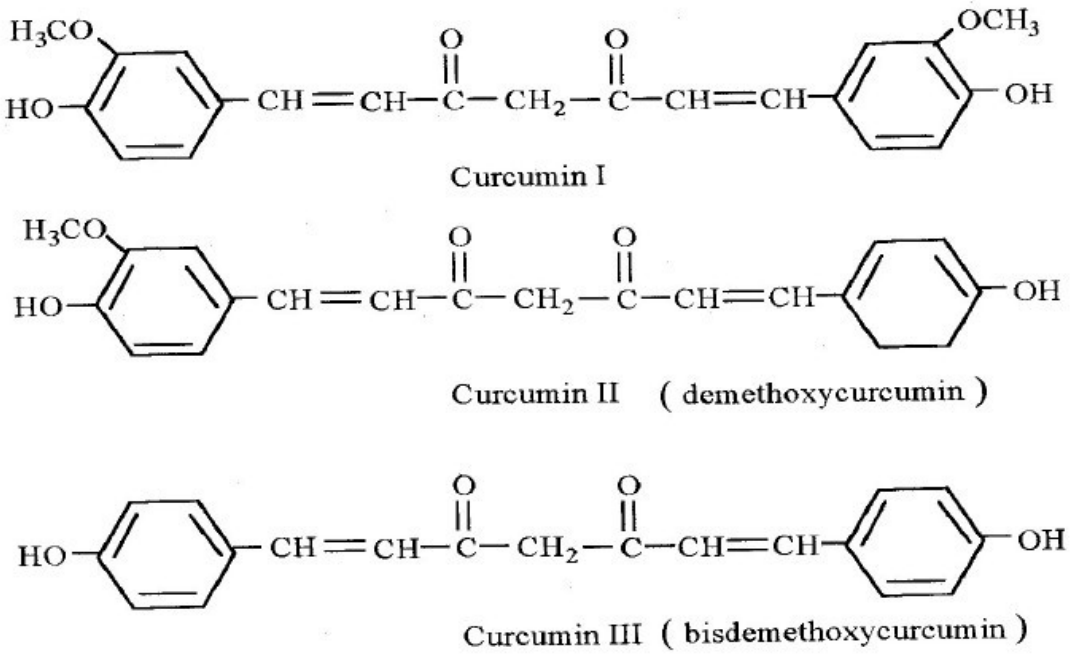

Figure 5: Isoforms of curcumin (Reddy et al., 2012).

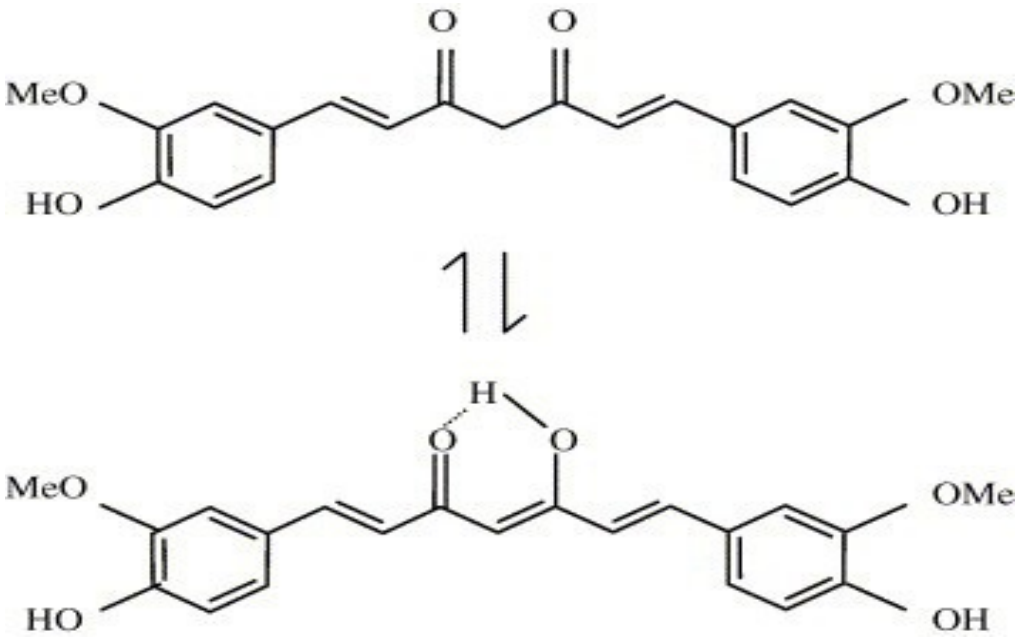

Figure 6: Tautomerism of curcumin. The top one predominates in acidic/neutral conditions, whereas the bottom one predominates in basic conditions. Reprinted from European Journal of Cancer, Vol 41, R.A. Sharma, A.J. Gescher, W.P. Steward, Curcumin: The story so far, 1955-1968, Copyright (2005), with permission from Elsevier.

gastrointestinal tract and remained unchanged. Most of the absorbed curcumin is rapidly metabolized into several metabolites (Figure 7) in the intestinal mucosa and the liver (Sharma et al., 2005). As a result, curcumin is usually formulated with bromelain to aid in absorption (Sharma et al., 2005). Bromelain is a class of sulfhydryl-containing proteolytic enzymes that is mainly extracted from a pineapple plant stem. Bromelain is mostly used as an oral supplement to help in digestion, and sometimes used topically as an anti-inflammatory agent (Baumann, 2008).

Curcumin is a pleiotropic molecule and can interact with a number of different molecular targets, some of which are involved in inflammation. Curcumin helps in down-regulating enzymes such as cyclooxygenase-2 (COX-2), lipoxygenase, inducible nitric oxide synthase (iNOS), monocyte chemoattractant protein (MCP), migration inhibitory proteins, mitogen-activated protein kinases (MAPK), and Janus kinases (Reddy et al., 2012; Sharma et al., 2005). These enzymes are either involved in the production of prostaglandins that mediate inflammation or control signals that elicit inflammatory reactions. This also helps in inhibiting the production of inflammation-mediating cytokines such as tumor necrosis factor-alpha (TNF-α), interleukin (IL) 1, 2, 6, 8, and 12. The anti-inflammatory activity of curcumin has also helped in alleviating a number of serious conditions. Conditions such as osteoarthritis, acute pancreatitis, and uveitis involve inflammatory responses (Reddy et al., 2012; Sharma et al., 2005). Treatment studies against these conditions using curcumin have shown great promise, and the results are a stepping-stone in finding other non-conventional treatments.

Studies have shown that *C. longa* poses numerous medicinal potential. Among these are anthelminthic and anti-cancer activities. In a model using Indian earthworms,

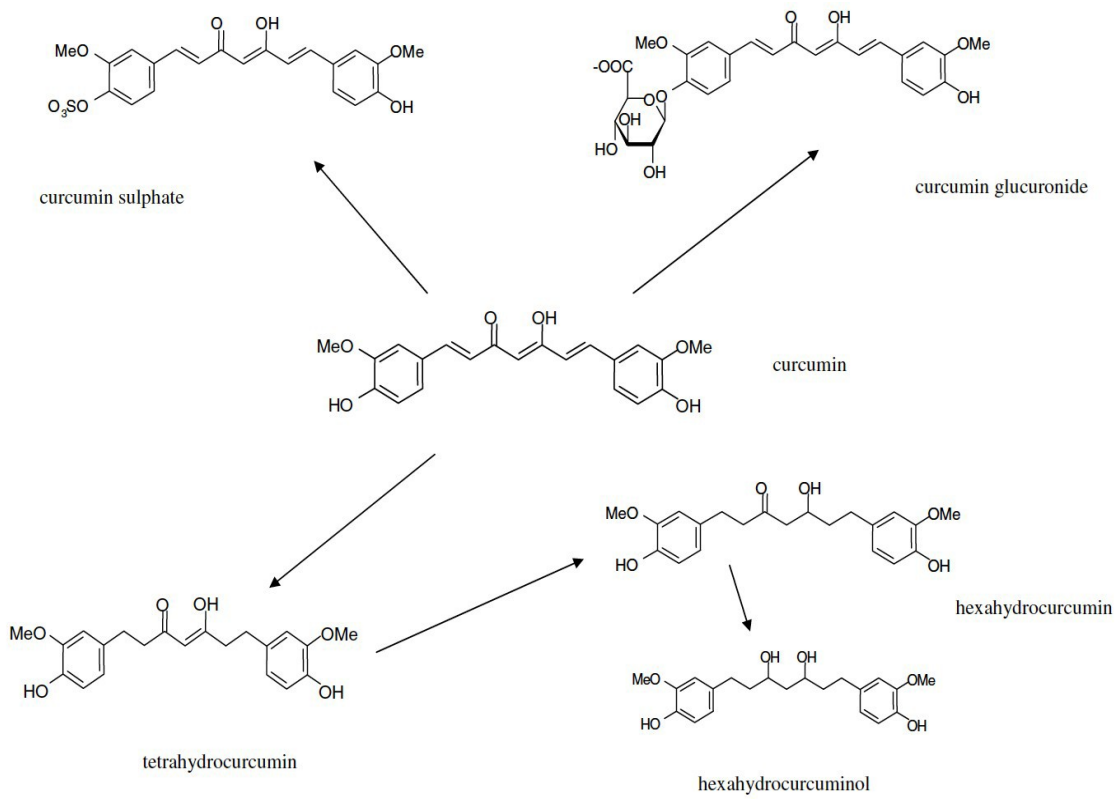

Figure 7: Major Metabolites of Curcumin in Rodents and Humans. Reprinted from European Journal of Cancer, Vol 41, R.A. Sharma, A.J. Gescher, W.P. Steward, Curcumin: The story so far, 1955-1968, Copyright (2005), with permission from Elsevier.

Pheretima posthuma, *C. longa* extracts were observed to paralyze and kill the earthworms. These observations led to the conclusion that *C. longa* extracts could potentially be used as an anthelminthic drug (Reddy et al., 2012). Also, rodents with mammary tumors induced by N-methyl-N-nitrosourea (MNU), an alkylating agent that interacts directly with DNA causing genomic mutations were tested with *C. longa* extracts before and after tumor induction in order to test the prophylactic and therapeutic potential of the plant (Reddy et al., 2012; Tsubura et al., 2011).

The results were analyzed on the basis of latency period of the tumor, tumor incidence, tumor burden, tumor volume, tumor growth inhibition, histology and hematological parameters. The results showed a more effective response in the

prophylactic treatment compared to the therapeutic treatment, with topical doses having a greater effect than oral doses (Reddy et al., 2012).

C. longa extracts have been observed to confer protection in the cardiovascular system. These extracts help in lowering cholesterol and triglyceride levels, which are associated with many cardiovascular disorders, and reduce the chances of peroxidation of low-density lipoprotein (LDL) (Reddy et al., 2012). Lipid peroxidation is one of the causes of oxidative stress in cells due to the production of free lipid radicals, damaging the cell membranes. Other cardiovascular effects of *C. longa* include inhibition of platelet aggregation, possibly through the inhibition of thromboxane synthesis (Reddy et al., 2012).

The aging process of cells is influenced by a number of factors, including lipid peroxidation that significantly damages cells, lipofuscin concentration and accumulation in neurons, and the activities of enzymes such as superoxide dismutase (SOD), glutathione peroxidase (GPx), and the Na^+, K^+ pump (Reddy et al., 2012). The influence of *C. longa* extracts on aging was tested on 6 and 24 month old rats. Long-term treatments with *C. longa* showed a significant drop in lipid peroxidation in the brain regions, as well as a drop in the lipofuscin contents. There was an observed increase in the activities of SOD, GPx, and the Na^+, K^+ pump in the brain regions as well (Reddy et al., 2012). These observations are consistent with that of a young growing mouse brain, indicating anti-aging prospects of *C. longa* extracts.

The antimicrobial activity associated with *C. longa* is an important feature. Curcumin is the main component of *C. longa* that has shown antimicrobial capabilities. Previous studies have observed positive results against pathogens, including *Escherichia*

coli, Bacillus subtilis, Yersinia enterocolitica, Saccharomyces cerevisiae, Bacillus cereus, Aspergillus niger, Penicillium notatum, and *S. aureus* (Su-Hyun et al., 2014). Such successes have led to further tests on MRSA.

Su-Hyun et al. performed an experiment to see whether curcumin could reverse MRSA's resistance against antibiotics such as oxacillin. Su-Hyun et al. used both MRSA and MSSA strains and tested for the presence of *mec*A gene, and treated both strains with various concentrations of oxacillin alone, curcumin alone, and a combination of both. The MSSA strains were inhibited by all treatments. MRSA was overall unaffected by the oxacillin treatment (Su-Hyun et al., 2014). This was due to the production of PBP2a, which helped overcome the stress caused by oxacillin. The PBP2a levels were as expected. When treated with curcumin alone, the expression of PBP2a dropped significantly, and even better results were observed when both oxacillin and curcumin were used on MRSA (Figure 8) (Su-Hyun et al., 2014). This indicated a synergistic relationship between curcumin and oxacillin, which is similar to the one between extracts from *A. wilkesiana* and oxacillin (Su-Hyun et al., 2014). Curcumin lowers the expression of PBP2a in MRSA, which allows oxacillin to render the normal PBP non-functional, disrupting the bacterial cell wall synthesis, and killing the cell in the process. Results from such experiments may result in creating alternative treatments against MRSA, and may eventually provide relief against bacterial resistance to antibiotics.

Over the years researchers have created a large number of antibiotics. Some of them are new classes of antibiotics, while others are derivatives of already existing ones. The need to create new and improved drugs is due to resistance associated with certain strains of bacteria, such as MRSA. However, overtime these strains develop resistance

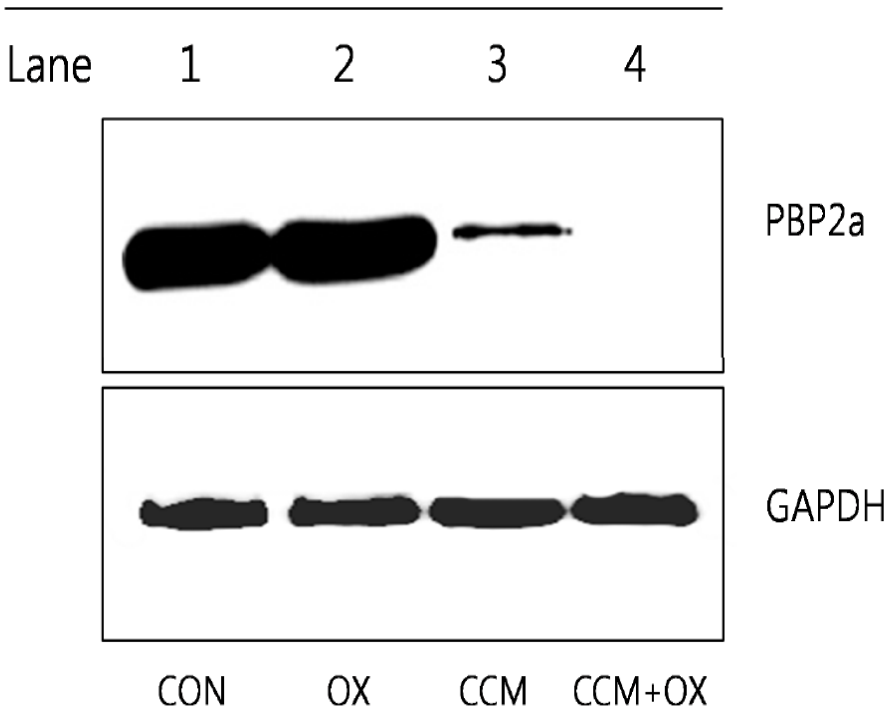

Figure 8: Western blotting showing PBP2a expression on different MRSA strains. CON represents control sample, OX represents strains treated with oxacillin, and CCM represents strains treated with curcumin. GAPDH was used as the control protein (Su-Hyun et al., 2014).

against newer medications, which leads further research in finding new drugs to counter-act MRSA's resistance. This also creates the need for continuous funding into research for new drugs. Patients in critical conditions that are infected with MRSA have a much lower chance of survival due to physicians having trouble finding the right treatment course to administer. These are some of the problems that make the fight against MRSA even that much harder.

The answer to these problems could lie with herbal extracts, such as curcumin. These extracts have shown to affect the resistivity of MRSA to common antibiotics. When the herbal extracts are augmented with antibiotics, the growth of bacteria is inhibited. This helps to avoid the need to constantly manufacture new antibiotics. The

millions of dollars spent in research to discover new antibiotics could be put to better use, such as funding research in cancer or HIV. Also, physicians can be confident that any treatment course administered will have a greater chance of success, which will give patients a much higher chance of survival.

Although the use of herbal extracts as part of the current available treatments is still in its infancy, it has shown great promise. The world is filled with diverse group of plants, which would create a cheaper alternative to modern cures.

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