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# Synergistic therapy involving curcumin, PYO- bacteriophage, and neem extract to reduce MRSA infection

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**Abstract**
Methicillin-resistant *Staphylococcus aureus* (MRSA) is a multidrug-resistant form of the gram-positive *S. aureus* bacterium. HA-MRSA is a major nosocomial pathogen as it is significantly present in healthcare facilities. CA-MRSA infections are those acquired from the community, such as through contact with an infected individual or a carrying fomite. MRSA makes up a portion of normal microbiota in the upper respiratory tract, but can be life-threatening if exposed to previously unexplored parts of the human body. MRSA infection can result in red, warm, swollen, and painful abscesses, which are often mistaken for insect bites, and is accompanied by fever and cellulitis; it can result in life-threatening complications if it enters the bloodstream, since it can result in endocarditis, sepsis, and other such conditions. MRSA is the leading cause of bacterial, respiratory, and skin infections around the globe and numerous patients die of MRSA infection-related complications, making it a paramount issue to solve. In the research provided, the related work is comprised of similar research being done and builds on it to incorporate the novel method being proposed. The proposed method aims to reduce the MRSA infection by utilizing a synergistic therapy involving curcumin, the PYO-bacteriophage cocktail, and neem extract. The future work deals with the need for more experimentation with the proposed method and the consideration of alternative methods that may be more effective, depending upon the conduction of studies upon humans after animal testing.

**Keywords:** methicillin-resistant *Staphylococcus aureus* (MRSA), curcumin, PYO-bacteriophage cocktail, bacteria, nosocomial pathogen, synergistic therapy

## Introduction

As MRSA is gaining more resistance against various antibiotics through methods such as horizontal gene transfer, more research is being done on its potent structural biology. MRSA, like all gram-positive bacterial species, has a thick peptidoglycan-based cell wall with amino acid bridges that connect the layers together.. The cell wall is targeted by β-lactam antibiotics such as cephalosporins and penicillins, which target the cell wall biosynthesis pathway to inhibit the overall growth of the multi-drug resistant bacterial species. MRSA is now resistant to penicillin, methicillin, amoxicillin, oxacillin, and certain cephalosporins, as well as many other β-lactam antibiotics. Phage therapy has been considered in recent years as MRSA began to become resistant to vancomycin, daptomycin, and other immensely potent antibiotics. Bacteriophages are the most abundant organisms in the biosphere and are bacteria-specific; the PYO-bacteriophage cocktail phages, which include several from the *Myoviridae* family, can significantly affect the MRSA biofilms but would be significantly more effective if combined with another therapy. Natural therapies have been proposed due to their efficacy in terms of treatment; curcumin, which is extracted from the rhizome of the turmeric plant, is one such product. Curcumin has been a widely proposed method to reduce MRSA infection due to its antibacterial effects. The curcumin is assumed to bind to the cell wall of MRSA and disrupt its cohesiveness. In addition, curcumin has also been seen to reduce low- affinity penicillin binding protein 2a (PBP2a) secretion in MRSA. To continue, curcumin has been seen to impact cells wall and cell membranes through membrane damage and cell lysis. While almost all types of curcumin exhibit antibacterial activity, curcumin analogs, including nanocurcumin, dimethyl curcumin, diacetyl curcumin, etc. are more potent. Utilizing these analogs could potentially have a faster and more effective impact in reducing MRSA infection.

Also, *in vitro* experimentation has demonstrated that curcumin's effects are more potent when combined with other antibacterial therapies, which suggests that a synergized method may be more effective ((Mun, Su-Hyun, *et al*. Curcumin Reverse Methicillin Resistance in Staphylococcus Aureus. MDPI. 2014.)). Another natural therapeutic plant product that is being considered is the extract of the neem tree.The neem tree has been a source of vital ayurvedic medicine for thousands of years. Neem extract has various antihelminthic and antibacterial properties, as it can, in the case of MRSA, inhibit the formation of large aggregates, which can allow for a more potent treatment if synergized with other therapies since the overall efficacy can be increased. The research presented in the paper proposes the appropriately combined usage of curcumin, PYO-bacteriophage, and neem extract to potentially reduce MRSA infection, due to the enhanced effect of each individual method when introduced in a synergized therapy.

## Objectives

- To develop a novel synergy to reduce MRSA infection by synthesizing relatively recent research.
- To analyze the interaction of curcumin, PYO-bacteriophage cocktail, and neem extract.

- To explore the mechanisms of the individual methods presented which include the introduction of curcumin, PYO-bacteriophage cocktail, and neem extract.

**Related Works**

A deeper knowledge of MRSA subset types and strains, as well as the mechanisms and effects of the PYO bacteriophage cocktail, curcumin and its analogs, and neem extract serves to formulate a better understanding of the proposed synergistic therapy. MRSA's multidrug resistance factor stems from the *mec*A biomarker gene, which confers resistance against methicillin and certain β-lactam antibiotics. A mechanism first reported in 1928, bacterial transformation utilizes horizontal gene transfer and can result in the uptake of the *mec*A gene by a MSSA or previously docile strain. Bacterial conjugation can allow for the dissemination of the *mec*A gene through the one-sided transferring of plasmids. *mec*A encodes for PBP2a, which differs from other penicillin-binding proteins in that its active site does not bind to methicillin or other specific β-lactam antibiotics [2]. PBP2a can allow for the continued catalyzation of the transpeptidation reaction required for peptidoglycan's cross-linking, allowing for cell-wall synthesis regardless of the presence of certain β-lactam antibiotics, which are actually meant to inhibit the synthesis of the peptidoglycan layer. More research regarding CA-MRSA has demonstrated that in terms of the SCCmec genotypes, it is conferred with types IV and V, which typically have small elements and lack resistance genes other than *mec*A, while HA-MRSA typically contains types I-III, which are large elements and bestow additional resistance genes [3]. Virulence can, at times, decrease because carrying larger plasmids like SCC*mec*-III can serve as a hindrance to the bacterium. HA-MRSA tends to excel in hospital settings, with decreased virulence but increased antibiotic resistance, which is not maladaptive because it targets immunocompromised individuals, who commonly frequent hospitals. In order to offset the increased virulence expression required to infect healthy hosts, CA-MRSA tends to carry SCCmec elements that do not serve as impediments. As MRSA's growing resistance to potent antibiotics such as vancomycin continues to instill anxiety among scientists all over the world, a new, harrowing diagnosis has recently been provided: By 2050, drug-resistant disease could become the leading cause of death, surpassing cancer and other dangerous diseases [4]. This has led to more experimentation with phage therapy, though it is yet to be completely approved in many parts of the world, including the United States. S. aureus strains, including MRSA, have lost a major genetic barrier against lytic phage infection, making it more vulnerable to phage therapy. The PYO-bacteriophage cocktail contains various phage species that can target multiple different bacterial hosts, including MRSA. The specific bacteriophage families that contain the species that are effective against MRSA include *Podoviridae* and *Myoviridiae*. The presence of Group I introns interrupting protein encoding genes has been found in various phages that belong to the *Myoviridae* infecting *Staphylococcus* species. Romulus and Remus serve as an example of these phages; they contain Group I introns in the genes encoding for helicase, DNA polymerase, ribonucleotide large subunit, and endolysin. *Podoviridae* phages are more host-specific and require a precise wall teichoic acid (WTA) glycosylation pattern for infection. But when utilized as a combined therapy in the PYO bacteriophage cocktail, the effect will be more pronounced due to the species associated with *Myoviridae* and *Siphoviridae*. Natural components have been used for thousands of years as antibacterial, antihelminthic, and antiviral agents; curcumin, a curcuminoid of turmeric, is one of these components. Curcumin's antibacterial effects on MRSA arise due to a large number of specific interactions between curcumin and MRSA. One interaction that has been thought of as the main interaction is the interaction between curcumin and FtsZ, a prokaryotic homologue of eukaryotic cytoskeletal protein tubulin. While it has not been directly experimented with in MRSA, it has been studied in *Bacillus subtilis.* In *Bacillus subtilis*, curcumin interacts with FtsZ *in vitro* and essentially inhibits FtsZ protofilament assembly from being formed and increases the GTPase activity of FtsZ. The authors, Sin-Yeang Teow *et al*., believe that curcumin has similar mechanisms against MRSA [5]. Protofilaments play major roles in bacterial cytokinesis; therefore, without the protofilaments, bacterial cytokinesis will be inhibited [6]. Failure of bacterial cytokinesis will result in an overall failure of bacterial cell division, thus decreasing the spread of MRSA. MRSA's cell wall is known to be made of numerous layers of glycan and amino acid bridges. In an experiment measuring the binding of curcumin with peptidoglycan (PGN), it was shown that with increasing concentrations of PGN, the binding of curcumin to the PGN was confirmed, thus proving the researchers hypothesis that curcumin binds to the cell wall of MRSA, which further disrupts cell wall cohesiveness. Also, MRSA produces the virulence factor α-hemolysin (Hla), a 33.2 kDa hemolytic toxin which is pore-forming. Hla attacks mammalian cells by binding to the target cell's membrane, oligomerizing itself into a 232.4 kDa membrane-inserted heptamer, then finally penetrating itself into the cell's membrane. This eventually results in cell lysis, damage, and death. In a prior study by the same researchers, it has been reported that Hla's interaction with some natural flavonoid compounds, such as curcumin, could neutralize particular activity of Hla, specifically the hemolytic activity. This could occur due to the curcumin inhibiting the conformation change of binding cavities. The researchers also found that curcumin at low concentrations could potentially inhibit Hla-mediated hemolysis. The researchers identified that curcumin binds to novel active sites of Hla, which could result in inhibition of the Hla self-assembly process. If the Hla self-assembly process is inhibited, mammalian cell lysis will not be able to occur, thus protecting mammalian cells [7]. In addition, in a western bloc analysis, curcumin has also been seen to reduce low- affinity penicillin binding protein 2a (PBP2a) secretion in MRSA, indicating that curcumin may interrupt protein synthesis by damaging RNA [1]. Figure 1 summarizes the techniques used by curcumin to inhibit MRSA infection. Curcumin also has many analogs that are considered more potent. Some include nanocurcumin, diacetylcurcumin, etc. In an experiment by Ramin Negahdari *et al*., 3 groups were prepared: the nanocurcumin group, the chlorhexidine group (positive control), and the distilled water group (negative control). Each group was applied to internal cavities of implants with particular/different torques. Each implant was then submerged in the bacterial suspensions (the bacterial suspensions were created by cultivating *E.coli, S. aureus,* and *E. faecalis*). The researchers then compared the mean

growth rates of the three bacteria in different groups. Figure 2 presents the data regarding *S. aureus*, showing that nanocurcumin had a great impact in reducing mean growth rates of *S. aureus* [8]. Diacetylcurcumin is another curcumin analog that has enhanced antibacterial activity. Diacetylcurcumin has shown to have inhibitory activity on MRSA strains at much lower concentrations. Diacetylcurcumin has been presumed to have major anti-film activity against MRSA. Also, diacetylcurcumin has the ability to reduce bacterial adhesion to human cells. Bacterial adhesion is an important process as it enables the bacteria to attach to other cells and eventually colonize the host [9]. Bacterial adhesion is a major contributor to bacterial pathogenesis [10].

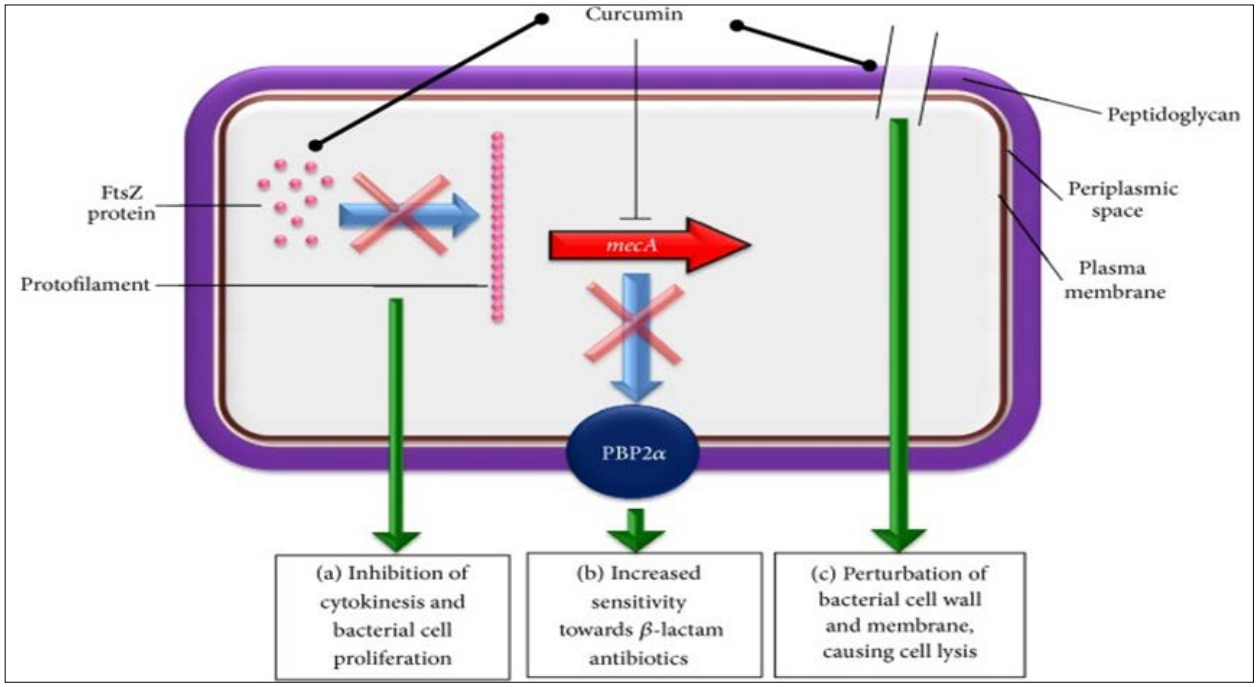


**Fig 1:** Summary of techniques utilized by curcumin to inhibit MRSA infection. Image from Sin-Yeang Teow *et al*., Hindawi Journal of Tropical Medicine]

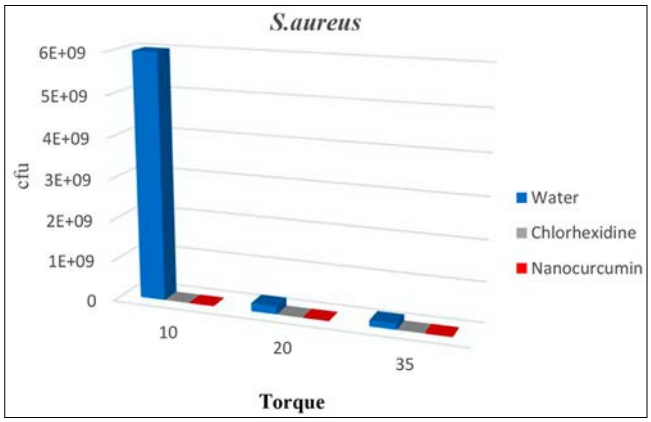


**Fig 2:** Mean growth rates of *S.aureus* in different groups. Image from Ramin Negahdari *et al*., *Wiley Online Library*

Since diacetylcurcumin is able to reduce bacterial adhesion, it can limit the spread of the MRSA infection. The oil of the neem leaf has been proven to have antihelminthic, antiviral, and antibacterial properties as well, and research has shown that it can optimally be used to negatively affect MRSA biofilms if utilized appropriately, as through a synergistic therapy. Various performance analysis results have demonstrated that neem leaf extract, possibly ethanolic, was utilized against MRSA, it has a inhibitory effect on biofilm and planktonic aggregation [11]. However, it fails, alone, to completely eradicate the growth of *S. aureus*; this has also been seen in the usage of neem oil to treat infections caused by *Klebsiella* and *Salmonella* spp.

Nevertheless, with the proper dosage and exposure, as well as the creation of a synergistic therapy involving the PYO bacteriophage cocktail, curcumin in a more effective form, and neem oil, the neem oil can be a formidable treatment. For instance, it has been demonstrated that phage treatment can disrupt biofilms, allowing the neem oil to affect the biofilms further as the bacteriophages continue to perform their roles as lytic phages. This can be applied to MRSA since both agents have been proven to provide nearly effective treatment. Another example of a facet of the proposed synergistic therapy can be seen in that as the polyphenol curcumin affects the cell wall and cell membrane of MRSA, the PYO bacteriophages will continue to disrupt the biofilms, allowing curcumin to have straightforward access. Neem oil can concomitantly be paired with curcumin and can facilitate well with the bacteriophages as proven by various studies that have only synergized two of the four components mentioned.

## Proposed Work

In an experiment using *E.coli*, it was exhibited that curcumin enhances the effectiveness of T2 bacteriophages. With increasing volumes of curcumin, there were increased amounts of bacteriophages than the control. It was found that 40ug curcumin/mL LB broth had the most increase in bacteriophage yield with a yield of 313% of the control. Results of the experiment are shown in figure 3. The researchers explained how curcumin specifically enhanced metabolism of the replication of the T2 bacteriophage rather than the assembly of the bacteriophage [12], and increased adenylate cyclase and cAMP activity thus further leading to T2 bacteriophage enhancement.

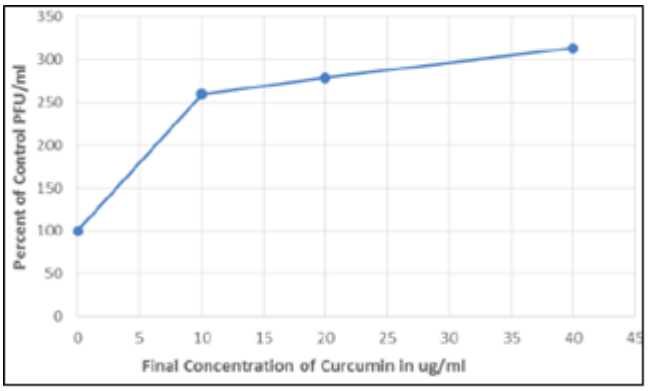


**Fig 3:** Curcumin's impact on the amount of T2 Bacteriophage. Image from Gerald Goldstein, *et al*., *ResearchGate*

A similar effect was seen when the PYO bacteriophage was introduced to MRSA in another experiment by different researchers. The researchers experimented with how the PYO bacteriophage and curcumin individually impact MRSA destruction and biofilm destruction. The researchers also experimented with how concurrent administration of the PYO bacteriophage and curcumin impacted these variables. On planktonic bacteria, curcumin was effective at killing MRSA at concentrations <0.2, 0.2, 0.8, and 1.0 µg/ml. On the other hand, when just phage therapy was utilized, there was barely an antibacterial effect. When concurrent application of curcumin and the PYO bacteriophage was recorded, the antibacterial effect against MRSA was still evident, but at much lower concentrations, pointing to the enhanced effect that curcumin and the PYO Bacteriophage has on reducing MRSA infection. When curcumin alone was utilized, biofilm proliferation was not affected and there was no impact of cell visibility. With combined therapy (curcuimin and PYO Bacteriophage) cell viability was greatly affected with about 50% of MRSA cells being destroyed [13]. Curcumin analogs, such as nanocurcumin, can also be used to enhance phage production and activity. Neem extract has been shown to have an impact on phage production as a whole, which indicates a similarity with the antibacterial properties of curcumin. The PYO bacteriophages will adhere to the receptors of the MRSA bacterium and will inject its genetic material into the cell, leading the host's genetic material to integrate into the bacterial genome. After production and assembly of the viral components occurs, the phages will lyse out of the cell, effectively proliferating and destroying the bacterium. As illustrated in figure 4, which evaluates the susceptibility of the MRSA biofilm through the usage of isothermal microcalorimetry, the biofilm indeed becomes more vulnerable due to the PYO bacteriophages because the heat flow is reduced overtime; larger biofilms will create more heat transfer, while smaller or receding biofilms will not be able to formulate much heat flow. This will lead to curcumin and the neem extract becoming more productive since they can use their properties, including anti-adhesion, cell wall and cell membrane lysis, and other such assets to effectively eradicate the MRSA infection over the course of approximately 1.5 weeks.

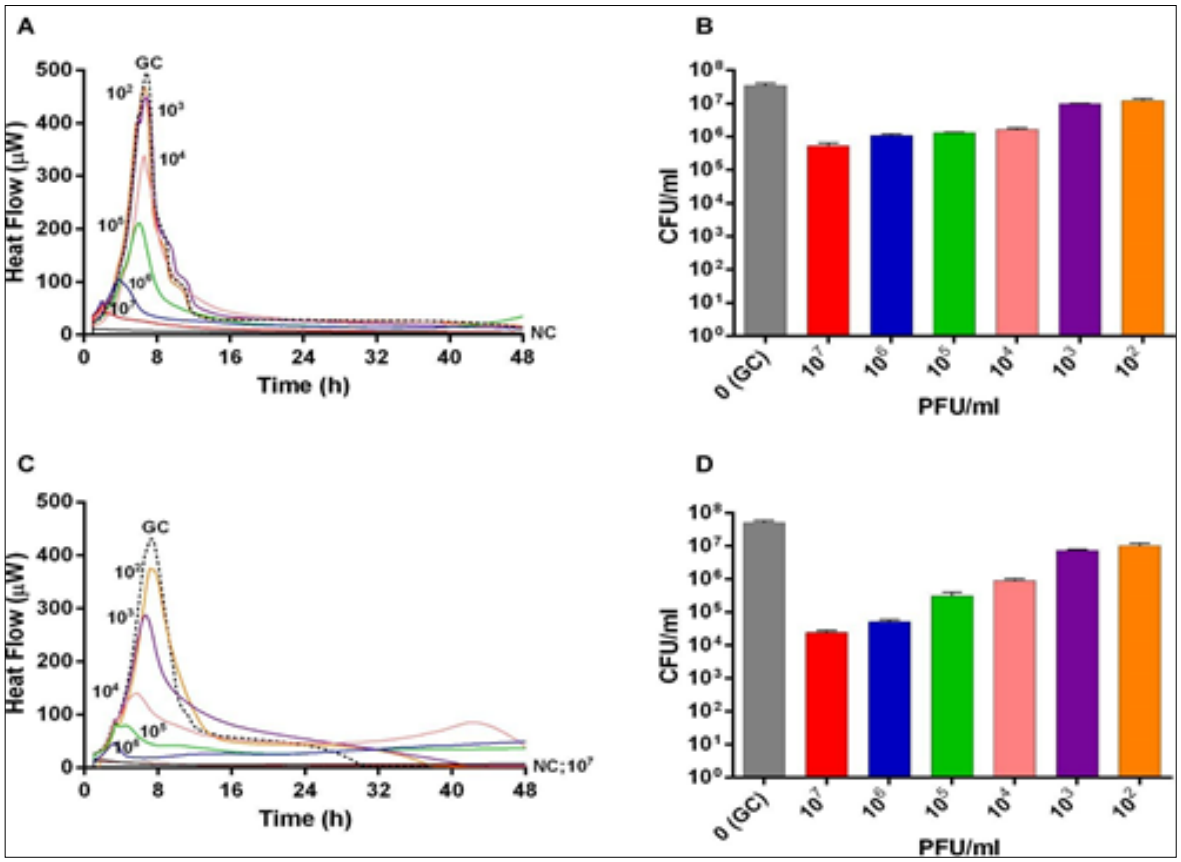


**Fig 4:** Evaluation of MRSA biofilm susceptibility to PYO at different time exposures by IMC. Image from Tamta Tkhilaishvili, *et al*., *Frontiers in Microbiology*]

PYO bacteriophages include those of the *Podoviridae*, *Myoviridae*, and *Siphoviridae* families; these families contain bacteriophages that are effective against *Proteus spp*., *P. aeruginosa*, *Streptococcus spp*., *Staphylococcus*

*spp*. and other bacterial species as well. Romulus and Remus, for instance, are *Myoviridae* phages that are generally effective against *S. aureus* strains. Moreover, recent research has shown that neem extract has anti-adhesion properties; an important facet of pathogenicity is adhesion through fimbriae, pili, or a capsule, as in the case of MRSA. If adhesion is decreased, there will be a marked decrease in the number of life-threatening cases. Additionally, neem extract can reduce the formation of large aggregates, which in turn decreases the presence of the MRSA biofilms present. When this occurs, the infection will be reduced. Neem extract concentrations ranging from 250 to 1000 µg/mL have been shown to effectively decrease the formation of large aggregates. Furthermore, in support of this component of the proposed method, neem oil, when used at different concentrations to test the minimum inhibitory concentration through the agar dilution method, has been shown to be completely effective at diluted concentrations of 40% and 50%, as indicated by figure 5. Testing these concentrations in synergy with the curcumin and PYO bacteriophages will lead to a better understanding of their effects, which will be beneficial to patients severely infected by MRSA.

| Neem oil (pure) (ml) | Agar (ml) | No of Strains inhibited (n=107) |
|---|---|---|
| 2 (10%) | 18 | 90 (84.11%) |
| 4 (20%) | 16 | 90 (84.11%) +14 (13.08%) |
| 6 (30%) | 14 | 90 (84.11%) +14 (13.08%) +3 (2.80%) |
| 8 (40%) | 12 | 107 (100%) |
| 10 (50%) | 10 | 107(100%) |

**Fig 5:** Minimum inhibitory concentration of neem oil by agar dilution method. Image from Sakshee Gupta, *Research Gate*]

Therefore, through the extensive required testing process, it can be determined that the PYO bacteriophage cocktail will have a minimizing effect on critical MRSA infections through the synergistic usage of neem extract. Curcumin and neem extract do not interact in a manner that may be detrimental to the properties of each other, since curcumin assumes the role of reducing the levels of PBP2a and dismantling the MRSA biofilms to a lesser extent than the neem extract, which has more potency in terms of affecting the large MRSA aggregates and biofilms and has significant anti-adhesion properties [14]. Through the usage of a synergistic therapy involving the PYO bacteriophages, curcumin, and neem extract, potentially life-threatening MRSA infections can be treated swiftly and effectively.

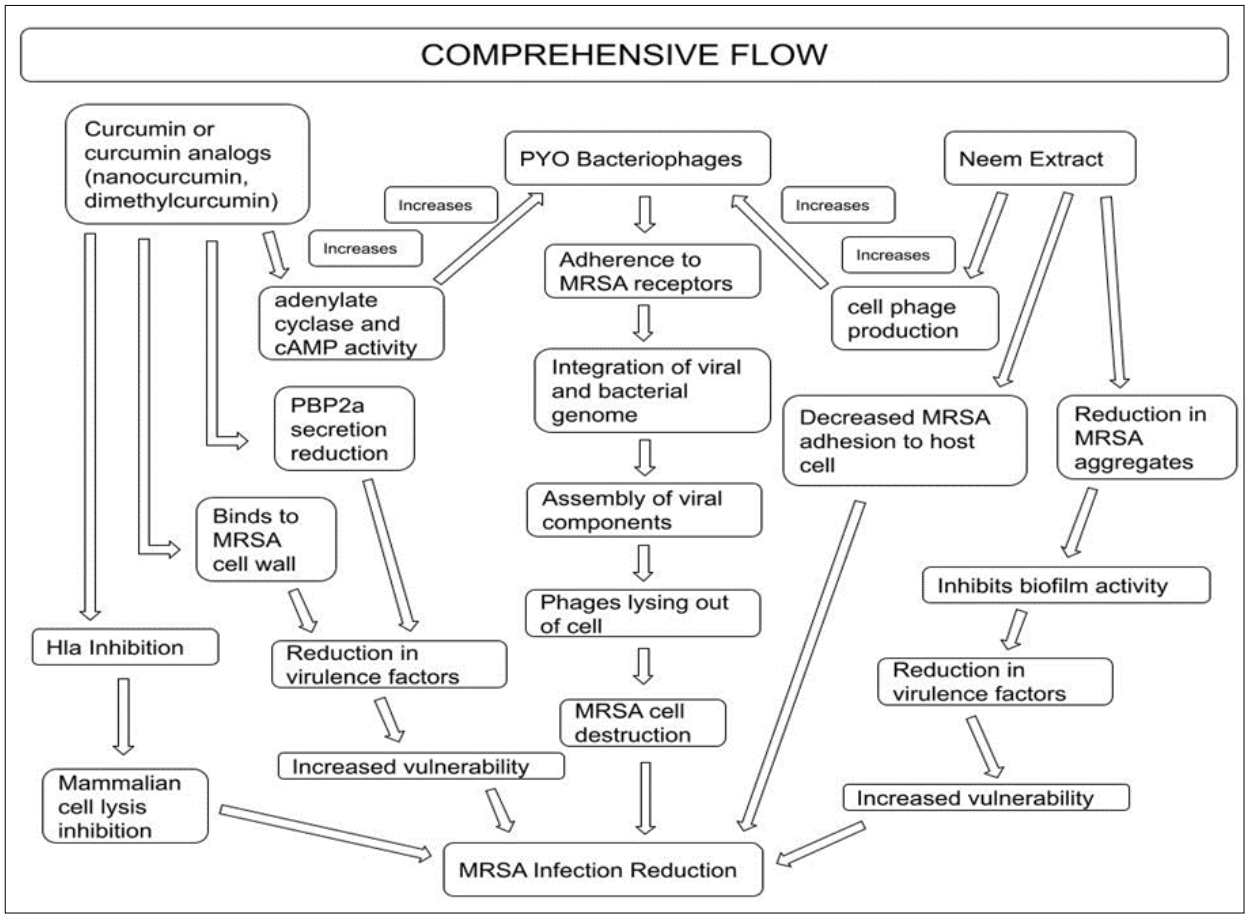


**Fig 6:** Comprehensive Flow of the Proposed System.]

## Conclusion

This paper proposes a synergistic therapy involving the usage of the PYO bacteriophage cocktail, curcumin, and neem extract that is effective against potentially fatal MRSA infections, which are becoming more common due to the unprecedented growth of antibiotic resistance in MRSA strains and other bacterial species. The proposed method was identified, explained, and specified as required for research and future implementation purposes. The therapy will involve the concentrations of neem extract and curcumin as suggested in the proposed work, as well as daily administration of the PYO bacteriophage cocktail at the site of the lesions associated with MRSA infection. Curcumin will increase phage production through adenylate cyclase and cAMP, inhibit Hla production and mammalian cell lysis, decrease PBP2a secretion, and bind to the MRSA

cell wall in order to instigate damage upon the capsule. While neem extract will also increase phage production and render the MRSA biofilms and aggregates vulnerable to the lytic PYO bacteriophages, its anti-adhesion properties will lead to a reduction in the possibility of critical infection.

**Conflicts of Interest**

As stated by the researchers, they have no conflicts of interest.

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