WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 5.990
Volume 4, Issue 8, 470-492. Review Article ISSN 2277– 7105

MRSA – A SUPERBUG

Surabhi Johari[1]*, Mridujuna Gogoi[2], Annesha Chakraborty[2], Rupanjali B. Sharma[3], Kanwar Narain[4], N. C. Barua[5]

[1]Centre for Studies in Biotechnology, Dibrugarh University, Assam.

[2]Centre for Bioinformatics Studies, Dibrugarh University, Assam.

[3]Department of Pharmaceutical Science, Dibrugarh University, Assam.

[4]Regional Medical Research Centre (ICMR), Post Box No. 105, Lahoal, Dibrugarh Assam.

[5]North Eastern Institute of Science and Technology (Formerly RRL), Jorhat, Assam.

Article Received on
05 June 2015,

Revised on 29 June 2015,
Accepted on 22 July 2015

***Correspondence for Author**
Surabhi Johari
Centre for Studies in Biotechnology, Dibrugarh University, Assam.

ABSTRACT

Staphylococcus aureus is a gram-positive bacterium that is responsible for many hospital-associated infections as well as skin infections in humans and is characterized by its thick peptidoglycan cell wall layer common to gram-positive bacteria. Recent cases of infections caused by community-acquired methicillin-resistant Staphylococcus aureus (MRSA) (CA-MRSA) strains in healthy individuals have raised concerns worldwide. Several computer-aided drug design approaches like molecular docking studies and QSAR studies can be used in drug discovery in several ways. Molecular docking was carried out on *Staphylococcus aureus* with Dehydrosqualene synthase inhibitors. The present review reflects the alternative inhibitors designed for combating MRSA infections.

KEYWORDS: MRSA, QSAR, Docking, S.aureus.

INTRODUCTION

The Gram-positive cocciis amajor nosocomial pathogens, particularly *Staphylococcus aureus*. The pandemic of 1940s to 1950s, when *S. aureus* phage type 94/96 was prime causative agent of most of the hospital acquired infections was the peak point of gram-positive cocci infections. It is the time when the penicillin-resistant *Staphylococci* emerged as the major nosocomial infections agents.[1-2] In the early 1940, the selection of resistant bacteria began on a global scale along with the introduction of the penicillin into clinical use.[3] It increased over the next 50 years as a large number of antibiotics with distinct mechanisms of actions

were introduced. The approach of pharmaceutical chemistry has to broaden the antimicrobial spectrum of each new antibiotic agent, and thus the introduction of enormous quantities of these potent drugs into the environment during therapy and in animal feed has challenged the entire prokaryotic world on our planet.[4]

Methicillin-resistant *Staphylococcus aureus* (MRSA) are a type of staphylococcus or "staph" bacteria that are resistant to many antibiotics, which include the penicillins (methicillin, dicloxacillin, nafcillin, oxacillin, etc.) and the cephalosporins. MRSA that is acquired in a hospital or health care setting is called healthcare-associated methicillin-resistant *Staphylococcus aureus* (HA-MRSA). MRSA has become more common in healthy people who are likely to have cuts or wounds and who are in close contact with one another. This type of MRSA is called community-associated methicillin-resistant *Staphylococcus aureus* (CA-MRSA).[5] It is not known why some healthy people develop CA-MRSA skin infections that are treatable while others infected with the same strain develop severe infections or die.[6]

MRSA was first identified in the year 1960s and was accepted as a major pathogen in the 1980s with the emergence of new strains of the epidemic potential. It is mostly resistant to multiple different classes of antibiotics. Epidemiological studies have suggested that nosocomial transmission in hospital admissions rather than social contact outside the hospital is more significant route of transmission for MRSA. MRSA positive patients spend considerably more days in hospital than MRSA negative patients. The use of antibiotics, surgery and indwelling intervenous access device have been identified as independent risk factors. MRSA is recognized to be one of the most prevalent nosocomial pathogens all over the world and are able to cause a wide range of hospital-linked infections.

In October, 1960, two single-colony of staphylococci were isolated and found to be resistant to "celbenin". Staphylococci resistant to celbenin can be obtained readily in vitro by reported subculture in the presence of compound. When celbenin was markted in September, 1960 no strains of penicillin-resistant *S. aureus* had been found which are resistant to celbenin. Celbenib-resistant strains of *Staphylococcus aureus* appears to be less than the strains encountered in the hospitals.[7] *S. aureus* also cause Chronic lung infection in cystic fibrosis (CF). *S. aureus* is most commonly found in younger patients.[8] There has been a general increase in MRSA infection in pediatric patients and the increased prevalence seen in CF may also reflect the widespread use of flucloxacillin for *S. aureus* infection.[9]

In October, 1960 and March 1964 nearly 45,000 cultures of Staph. Aureus were examined for methicillin resistance by screening test with paper discs containing the antibiotic. Borowski and his colleagues in 1964 reported that over 50% of the babies and mothers in a hospital become colonized with methicillin-resistant strains of *Staphylococcus aureus*. The observation raised a question wheather insusceptibility to methicillin might confer some other advantage on a staphylococcus. The accepted view has been that in the absence of methicillin, resistance is an irrevalent or "accidential" property of strains which posses the other characters of hospital staphylococci, to which there is a spread in hospital population may be attributed. It is well known that the strains of *Staphylococcus aureus* that have been rendered artificially resistant to methicillin *in vitro* also show an increased tolerance to benzyl penicillin. It was inferred that naturally occurring methicillin-resistant strains had the same property.[10]

Staphylococcus aureus is a versatile pathogen as it is known to cause a wide range of diseases in humans from minor skin infections to severe illness such as septicemia, toxic shock, endocarditic and pneumonia.[11] In 1961, the unusual behavior of the methicillin-resistant strains of *Staphylococcus aureus* were tested.[12] The reports found earlier indicated that methicillin-resistance S. aureus (MRSA) were heterogenous in their expression of resistant to β-lactam agents. It was further reported that varying the test conditions had major effects on the detection of resistance of *S. aureus.*[13-16] The origin of most methicillin resistance is the production of penicillin-binding protein, PBP2' or PBP2a,[17-18] mediated by the *mecA* gene.[19] *mecA* is an supplementary gene found in the methicillin-resistant staphylococci. There are also some extra genes that affect the expression of methicillin resistance in *S. aureus*,[20-21] but the genes were found in susceptible as well as resistant strains.[22]

MRSA at first reported in the United Kingdom in 1961 and by the mid-1970s it becomes prevalent in many countries.[23] Various strains of MRSA have been chosen epidemic strains; these are associated with a higher occurrence and have shown to spread within hospitals and with the countries.[24-28] MRSA now has become well-known outside the hospital environment and it appear in community populations with no identifiable risk factors.[29] To direct the spread of infections, cause of infectivity and the mechanisms of transmission should be recognized. Transmission of MRSA is in consideration to occur mostly from colonized or infected persons to another person's.[30-32] As the environment contributes to the MRSA transmission, transmission through food products has not been carefully investigated.

Because of the potential for MRSA to cause major morbidity and its inherent antibiotic resistance, management protocols aim at the prevention and eradication of infections. Therefore, it should aim to minimize repeated and extended hospital admissions by optimal use of home intravenous antibiotic management, invasive treatment and broad spectrum antibiotic treatment. MRSA spread often through person-to-person via hand contact. Patients with MRSA infection should be separated from others, taking care to minimize stigma and sense of resolution. The importance of hand washing and of observance to basic hygiene must be regularly and frequently emphasized. The policy of handling and cleaning of equipments should be agreed with the hospital infection control team.[33]

In the early 1962, the resistance of bacteria to the penicillins emphasized the difficulties of obtaining an estimate of the incidence of methicillin-resistance in *Staphylococcus aureus*. There is little evidence that the use of methicillin has so far played much part in the spread of resistant strains. The hospitals in which resistant strains were widespread were not ones in which methicillin has been used excessively for therapeutic or prophylactic purpose. The antibiotic appears to have been used with resistant in most hospitals. The proportions of methicillin received patients were probably higher than in certain special units. But the methicillin appears seldom to have used as freely as other antibiotics to which *Staphylococcus aureus* has frequently been found to be resistant.[34]

Vancomycin and other glycopeptides antibiotics are recent mainstay of treatment for infections caused by methicillin-resistant *Staphylococcus aureus* (MRSA). The high occurrence of MRSA has lead to increase use of vancomycin in the chronic and seriously ill patients and has resulted in the emergence of MRSA with reduced vulnerability to glycopeptides. The detection of reduced vulnerability to vancomycin by routine susceptibility testing is unreliable and vancomycin non-susceptible is most probably being underreported. All MRSA strains recovered from patients whose infections do not respond to vancomycin treatment should be tested accurately for vancomycin susceptibility. The treatment option of infection due to MRSA with reduced susceptibility to vancomycin is limited.[35]

In vitro and in vivo experiment reported in 1992 demonstrated that vancomycin resistance genes from *Enterococcus faecalis* could be transferred by horizontal gene transfer to *S. aureus*, conferring high-level vancomycin resistance to *S. aureus*.[36] Vancomycin usually reserved for the treatment of serious infections including those caused by multidrug-resistant *Staphylococcus aureus* which is a clinical isolates of *S. aureus* with high-level resistance to

vancomycin. The genetic analyses suggest that the long-anticipated transfer of vancomycin resistance to a methicillin-resistant *S. aureus* occur in vivo by interspecies transfer of Tn1546 from a co-isolate of *Enterococcus faecalis*.[37]

During January 1993 to November 1994 were tested for antimicrobial susceptibility using Kirby-Bauer disc diffusion technique. Among 1382 isolate of *Staph. aureus*, 332 (24%) were MRSA. All tested strains were susceptible to vancomycin. Therefore, when new antimicrobials other than vancomycin are considered for therapy, their choice requires the results of in vitro susceptibility testing of every isolate of MRSA.[38]

Over the past two decades, in 1999 vancomycin has been considered the antibiotic of preference for methicillin-resistant *Staphylococcus aureus* (MRSA) infections. Indeed, multidrug-resistant clones of MRSA for which the only available effective antibacterial agent in vancomycin have been identified. Some reports described the therapeutic failure of vancomycin for MRSA infections that have aroused considerable concern regarding the emergence of MRSA strains for which there will be no effective therapy.[39-41] The method of reduced susceptibility in the staphylococcal strains has not been identified, although the data indicate that it is not the same as vancomycin-resistance mechanisms in enterococcal strains..[42]

In India, Methicillin-resistant *Staphylococcus aureus* (MRSA) has proved to be the most nosocomial pathogen of the late 20th century.[43] The hospital-based studies described the incidence causing MRSA infections.[44-47] It has only few years where nosocomailly acquired isolates were found to methicillin-resistance, but now even community acquired strains have shown methicillin-resistnce.[48]

A study was carried out to find the presence of VISA and VRSA in the northern part of India. Glycopeptides such as vancomycin are the frequent antibiotics of choice for the treatment of infections caused by methicillin resistant Staphylococcus aureus (MRSA). The study revals for the first time about the emergence of VISA/VRSA from this and indicates the magnitude of antibiotic resistance in and around the study area. The major cause of infection may be unawareness and indiscriminate use of broad-spectrum antibiotics. Strains of Vancomycin Intermediate *S. aureus* (VISA) with vancomycin MIC of 8 µg/ml have been reported from different countries. This is the first report of VRSA emergence from tertiary care hospital from this part of world.[49]

Hososaka Y et. Al, in 2007 described in their paper, that oxacillin susceptible MRSAs (OS-MRSAs) were least resistant to oxacillin among the MRSAs tested and they were within the susceptible range to seven other beta-lactam antibiotics tested. Thus, OS-MRSA may become a high-resistant MRSA upon the treatment of patients with beta-lactam antibiotics. To distinguish wheather these OS-MRSAs were hospital-acquired or community-acquired MRSAs, they tested for the OS-MRSAs were hospital-acquired or community-acquired MRSAs, they tested for the presence of the genes encoding toxins. These results discovered that OS-MRSAs could be classified as a new type of MRSA that exhibits properties distinguishable from either hospital-or community-acquired MRSA.[50]

Vancomycin indeed is considered to be the antibiotic of choice for MRSA. Multidrug-resistance clones of MRSA for which the only available effective antibacterial agent is vancomycin. The therapeutic failure of vancomycin for MRSA infections have aroused considerable concern regarding the emergence of MRSA strains for which there will be no effective therapy. The incidence of MRSA varies from 25 per cent in western part of India[51] to 50 per cent in South India.[52] *S. aureus* infection caused by antibiotic-resistant strains often occurs in the epidemic waves initiated by a few successful clones. MRSA is emerged widespread to cause community infection. So community or community-associated MRSA spreads rapidly among healthy individuals. Community MRSA infections have been reported worldwide and community MRSA strains are epidemic even in the United States. Therefore, it is a region of concern because MRSA often can readily become resistant to multiple antibiotics, thus limiting treatment options.[53]

MRSA may be associated with stigmatization and social isolation with resulting adverse psychological effects.[54] MRSA infection is a relative contraindication to lung transplantation. There is still no consensus on the best method of treatment and on whether eradication is effective.[55] Methicillin resistant *Staphylococcus aureus* (MRSA) the nosocomial pathogen cause significant mortality and morbidity. Within a year of the use of methicillin, methicillin resistant *Staphylococcus aureus*(MRSA) strains were reported worldwide and over the next few decades, MRSA has reached epidemic proportions.[56-57] MRSA is a resistant variant of *Staphylococcus aureus* which has evolved an ability to survive treatment with beta lactam antibiotics which includes penicillin, methicilllin and cephalosporins and to various other groups of antimicrobial agents. They are often referred to as super bugs. Methicillin resistance in *S. aureus* is conferred by the gaining of one of several

staphylococcal cassette chromosome *mec (SSCmec)* elements, which carry the *mecA* gene encoding a penicillin-binding protein homologue (PBP2a) with reduced affinity for β-lactam antibiotics.[58]

Penicillin-binding proteins (PBPs) are set of membrane-associated proteins that catalyze the final step of murein biosynthesis. The protein function either as transpeptidases or carboxypeptidases or in few cases as demonstrates transglycosylase activity. The transpeptidase and carboxypeptidases activities of PBPs occur at the D-Ala-D-Ala terminus of murein precursor containing a disaccharide pentapeptide comprising N-acetylglucoseamine and N-acetyl-muramic acid L-Ala-D-Glu-L-Lys-D-Ala-D-Ala. β-lactam antibiotics inhibit these enzymes by competing with the pentapeptide precursor for binding the active site of the enzyme. It describes the crystal structure, biochemical characteristics, and expression profile of PBP4, which is a low molecular mass PBP from Staphylococcus aureus strain COL.[59]

Penicillin binding protein (PBPs) are the vital components of the cell wall synthesis of machinery in bacteria. These membrane associated protein are largely classified as low-molecular-mass (LMM) PBPs that are monofunctional D, D-carboxypeptidase enzymes or multilocular high-molecular-mass (HMM) PBPs with multiple functional roles. PBPs, are anchored to the cytoplasmic membrane by a noncleavable pseudo signal peptide. In case of the HMM PBPs, the cytoplasmic C-terminal domain binds penicillin and catalyzes peptidoglycan cross-linking, whereas the juxtamembrane N-terminal domain participates in transglycosylation.[60] The catalytic penicillin-binding (PB) module also occur as part of penicillin sensor transducers, such as *Staphylococcus aureus*.[61] The carboxypeptidase domain of PBPs is the target for β-lactam antibiotics in susceptible staphylococci.

The β-lactam resistance of MRSA which is caused by the making of a novel penicillin-binding protein (PBP) selected by PBP 2' or PBP 2a and which is different to the inherent set of PBPs (PBP 1 to 4) of *S. aureus*, has extremely reduced binding affinities to β-lactam antibiotics.[61-63] PBP 2' is determined by a *mecA* gene positioned on the chromosome of MRSA. The *mecA* gene is extensively distributed among *S. aureus* and coagulase-negative staphylococci.[64 - 65]

Methicillin resistance in staphylococci is determined by mec, composed of DNA found only in methicillin-resistant strains. mec contains mecA, the gene for penicillin-binding protein 2a

(PBP2a). A low level type of resistance to methicillin exhibited by strains lacking mecA, is related with the modifications in native PBPs, a beta-lactamase hyperproduction, or possibly a methicillinase. Vancomycin is the drug used for the treatment of infection caused by methicillin-resistant strains. PBP2a confers a cross-resistance to most beta-lactam antibiotics. Alternative to vancomycin are little because of the numerous drug resistances usual of methicillin-resistance staphylococci.[66]

Methicillin-resistant strains were identified immediately after introduction of drugs. The methicillin-resistant strains have unique properties. The conditions related with the heterogeneous terms of resistance were described where the methicillin-resistant is related with production of unique penicillin-binding protein PBP2a which are bounded and inactivated at high concentration of beta-lactam antibiotics. PBP2a emerge to be encoded by *mec* determinant, which are unique to the methicillin-resistant strains. The relationships among PBP2a emerge and the expression of resistance and inference for the mechanism of resistance were discussed where heterogeneous appearance of methicillin-resistance by staphylococci pose problem in the recognition of resistant strains. The practice with several susceptibility test method is reviewed. The alternatives are few because methicillin-resistant strains frequently are resistant to multiple antibiotics in addition to beta-lactam antibiotics.[67]

The target alteration underlies resistance to beta-lactam antibiotics in Staphylococcus species. The methicillin resistance in staphylococci is due to expression of PBP 2a, which is a novel, low affinity PBP for which there is no homologue in methicillin-susceptible strains. PBP 2a is encoded by mecA. It is a highly conserved gene most likely acquired by a rare transposition from Staphylococcus. expressions can be highly variable, but this seems not to be determined by PBP modifications. Several more non-PBP factors are required for high-level resistance.[68]

A low-affinity penicillin-binding proteins (PBPs), participate in the beta-lactam resistance of several pathogenic bacteria that have different origins. The natural transformation and recombination events with DNA acquired from neighbouring intrinsically resistant organisms are responsiblefor the appearance of mosaic genes encoding two or three low-affinity PBPs in highly resistant strainsof transformable microorganisms. Methicillin-resistant Staphylococcus aureus and coagulase-nagative staphylococcal strains posses the mecA determinant gene, which probably evolved within the Staphylococcus genes from closely related and physiologically functional gene. Enterococci have a natural low susceptibility to beta-lactams

related to the presence of an intrinsic low-affinity PBP. The highly resistant enterococcal strains overexpressses the PBP and reduce its affinity.[69]

In a study report the design, synthesis, along with the structure-activity relations of a sequence of 5-amido-1-(2, 4-dinitrophenyl)-1H-4-pyrazolecarbonitriles as DD-carboxypeptidase/penicillin-binding protein (PBP) inhibitors with Gram-positive antimicrobial activity. The result have shown that the compounds with superior, extra polarizable, and electron-rich substituted benzamide moieties as *para*-dimethyaminobenzamide (3j) and para-methoxybenzamide (3i) will exhibit better antibacterial activity against methicillin-susceptible *Staphylococcus aureus* and methicillin-resistant *Staphylococcus aureus* with minimum inhibition concentration (MIC) values. These outcome are in accordance with predictable inhibition constants (K_i) that have obtained from docking with PBP2 and PBP4 of *Staphylococcus aureus.*[70]

Despite the modern antibiotics, infectious disease are still one of the most urgent health-problems and are responsible for nearly one third of human deaths worldwide. The rapid emergence of bacterial resistance such as penicillin producing isolates of *Staphylococcus aureus* were found in the very same year as penecillins were introduced in the market. These enzymes are capable of hydrolyzing the β-lactam moiety of penecillins, leading to their inactivation.[71]

The goal of this project was to built a model of the protein-DNA complex which reconciles existing biochemical knowledge such as resistance mutations and partial structures. This model shows the predictive power for previously unknown residues involved in the reaction mechanism, quinolone binding, and the development of resistance.[72]

Staphylococcus aureus is a familiar human pathogen responsible for a wide array of diseases from superficial skin infections to life-threatening pneumonia, septicemia, and endocarditis.[73-74] This bacteria is also one of the most common ophthalmic pathogens recovered from conjunctivitis and other more serious ocular infections.[75] The eye is a unique organ that is almost impermeable to all external agents. Methicillin-resistant *S. aureus* (MRSA) have been reported through the media as to blame for more deaths than acquired immune insufficiency syndrome in the USA, insufficient information is known about the occurrence and epidemiology of eye infections because of the methicillin-sensitive *S. aureus* (MSSA) or MRSA. Methicillin resistance is conferred on the organism by the presence of a

unique mobile genetic element called the staphylococcal cassette chromosome carrying the *mecA* gene (SCC*mec*).

After a literature review of the laboratory diagnosis and susceptibility testing of methicillin-resistant *Staphylococcus aureus* (MRSA) it have considered the detection of reduced susceptibility to glycoproteins in *S. aureus*. It have considered the detection of MRSA in the screening samples and the detection of reduced susceptibility to glycopeptides in *S. aureus*. The recommendations are given for the identification of *S. aureus* for suitable method of susceptibility testing and screening for MRSA and for *S. aureus* with reduced susceptibility to glycopeptides. The guidelines specify what tests should be used and not when the test are applicable, as aspects of this deal with in guidelines on control of MRSA. So there are at present several developments in the screening media and molecular methods. It is possible that some of our recommendation will need modification as the new methods become available.[76]

A work carried out on resistance to beta-lactam class of antibiotics in methicillin-resistance *Staphylococcus aureus* (MRSA). It is mediated by PBP2a, a synthetic bacteria cell wall penicillin-binding protein with low affinity of binding to beta-lactams which is encoded by mecA. Beta-lactams that bind to PBP 2a with a high affinity and that are highly active against MRSA are under growth. The emergence of resistance to such compounds was investigated by homogenous MRSA strain COL in L-695,256. A high resistant mutant, COL52, expressed PBP 2a in which a two-amino-acid deletion mutation and three single-amino-acid substitution mutations are present. The highest level o resistance to beta-lactams that bind to PBP 2a with high affinity is likely to require multiple mutations in mecA, chromosomal mutations appear to have a minor role.[77]

The multidrug resistant bacteria such as methicillin-resistant *Staphylococcus aureus* (MRSA), are endemic in healthcare settings in the United States and many countries all over the world. The nosocomial transmission of MRSA serves as a source of hospital outbreaks, and the recent report of vancomycin-seristant *Staphylococcus aureus* strains in the United States emphasize the need for better control of MRSA and the other resistant bacteria of healthcare settings. The colonization of *S. aureus* or MRSA is relatively common in both healthy and hospitalized individuals. Colonization increases the risk of infection. MRSA get transmitted through patient-to-patient within the healthcare settings. It primarily occurs via carriage on the hands of healthcare workers. To prevent the transmission of MRSA and vancomycin-

resistant enterococci within healthcare settings have developed the guidelines and chief among the recommendations is an emphasis on adherence to hand hygine guidelines. Some other measures which can prevent the nosocomial transmission of MRSA include improved antibiotic stewardship, staff cohorting, maintenance of appropriate staffing ratios, reductions in length of hospital stays, contact isolation, active microbiologic surveillance, and better staff education.[78]

The colonization of MRSA was detected using nasal samples in all the patients plus wound samples in surgical patients. It is checked within 48 hours of admission or within the first 48 hours of ICU stay and weekly thereafter. MRSA infections was defined using Centers for Disease Control and Prevention standard definitions. The MRSA colonization greatly improved the risk of S. aureus infection and of glycopeptide use in colonized and non-colonized patients, without influencing ICU mortality even when an MRSA infection was not demonstrated. Thus the MRSA control program is warrented to decease the use of vancomycin and to limit glycopeptides resistance in gram-positive cocci.[79]

In microbiology laboratory it is of great importance for quality control, teaching and research and freezing is a very common method of preservation and storage of microorganisms. The new diagnostic evaluation *in vitro* antimicrobial susceptibility tests for methicillin-resistant *Staphylococcus aureus* (MRSA) requires well defined strain collections. The study aimed at determining whether the loss of *mecA* gene in MRSA is related to the storage method. A total of 1692 non-duplicate *S. aureus* isolates were collected from different human clinical specimens at 8 diufferent health institutions in Northwestern Nigeria from February, 2008 to April, 2010. The isolates were screened for methicillin resistance using disc diffusion method (DDM), screen agar method (SAM) and latex agglutination techniques (PBP). Therefore, the isolates were stored in 16% v/v glycerol broth at -80°C. In December, 2011, the isolates were retested by polymerase chain reaction (PCR) which was used to amplify both the *S. aureus* specific sequence gene and *mecA* gene of 100 isolates, with the amplicon size of 107 and 532 bp. The prevalence rate of MRSA on DDM, SAM, and PBP were 26.3, 24.2 and 25.0%, respectively. The mecA gene was lost in 95.0% of 100 MRSA isolates after 2 years of storage at -80°C with the Minko bank system. This study demonstrates that *mecA* can be lost from MRSA strains stored at -80°C with the Micro bank system. The finding has important implications for the management of strains collections and is of use for future biobanking projects.[80]

A review carried out on Nasal carriage of methicillin-resistant *Staphylococcus aureus*. Methicillin-resistant *Staphylococcus aureus* (MRSA) nasal carriage is a recognized risk factor for successive endogenous infections. The association between MRSA carriage and patient survival in hemodialysis patients has not been established. The major limitations found are relatively small size of MRSA carriers. There may also be an outcome of association between MRSA nasal carriage and should alert the physicians of a group at high risk of morbidity and mortality.[81]

Methicillin-resistant *Staphylococcus aureus* (MRSA) study was undertaken to genotype clinical MRSA isolates collected from hospitals in different parts of India. One hundred and eighty-six isolates were collected and characterized by phenotypic and genotypic methods using published protocols. Majority of isolates were positive for *mecA* gene. In Indian hospitals, MRSA is one of the frequent causes of hospital-acquired infections and different hospitals have reported anywhere from 30 to 80% methicillin resistance based on antibiotic sensitivity tests.[82] It leaves only vancomycin as the drug of preference in India and vancomycin-resistant MRSA has already been reported in Japan, the United States and several other countries. Nosocomial MRSA isolates are mostly multidrug resistant. Methicillin resistance is due to the presence of *mecA* gene coding for penicillin-binding protein (PBP2A) with a low affinity for *β*-lactam antibiotics.[83]

Frequency of methicillin resistant *Staphylococcus aureus* from a referral hospital in Assam was studied. The methicillin resistance among the *Staphylococcus aureus* isolates was 52.9% and 15% between the coagulase negative staphylococci. Resistance to all antibiotics tested with the methicillin resistant and methicillin sensitive staphylococci was found to be 23.2% and 6.6% respectively. Higher resistance to multiple antibiotics in methicillin resistant strains as compared to methicillin sensitive strains was found to be statistically important. Ciprofloxacin resistance among the strains was still lower in comparison to the findings from other parts of the country.[84]

The multidrug-resistant *Staphylococcus aureus* (*S. aureus*) makes the treatment of infectious disease in hospitals more difficult and increases the mortality of the patients. This attempt to identify novel potent antibiotic candidate compounds against *S. aureus* dihydrofolate reductase (saDHFR). It performed in three-step in silico structure-based drug screening (SBDS) based on the crystal structure of saDHFR using a chemical compound library. It subsequently evaluated whether candidate chemical compounds exhibited inhibitory effects

on the growth of the model bacterium. It performed structure-activity relationship (SAR) analysis of active chemical compounds and observed a correlative relationship among the IC50 values, interaction residues and structure scaffolds. The structural and experimental information regarding these novel chemical compounds will likely to contribute to the development of new antibiotics for both wild-type and drug resistant *S. aureus*.[85]

MRSA is a most important pathogen and are reported from hospitals in various parts of India.[86] MRSA is an major pathogen causing pyogenic, disseminated, and toxin-mediated infections.[87-89] MRSA bacteremia is related with considerably higher mortality than is known for methicillin-susceptible *S. aureus* bacteremia. MRSA isolates has reached extraordinary proportions in Indian hospitals, with some cities reporting that upto 70% of the strains are resistant to methicillin.[90] The development of drug resistant strains of *Staphylococcus aureus* has initiated the need for search of new drug targets.[91] Infectious diseases are one of the leading cause of death all over the world. So, the population of the world is likely to get infected by bacterial infections. Unfortunately, the emergence of antibiotic resistance due to Gram-positive bacterial pathogens such as Methicillin-resistance *Staphylococcus aureus* (MRSA) has put a great pressure on public health and there is an urgent need to develop new chemical entities for the eradications of infectious diseases.[92]

S. aureus strains are resistant to penicillin, and many are resistant to methicillin-related drugs (MRSA strains). *S. aureus* are part of our normal flora, but cause fatal disease as a result of the expression of multiple virulence factors. A work is done on 2′, 5-di-*O*-galloyl-d-hamamelose (hamamelitannin) as a nonpeptide analog of RIP by virtual screening of a RIP-based pharmacophore. Staphylococci are also a common cause of infections related to bacterial biofilm formation on implanted devices. Infections may result in longer hospitalization time, or need for surgery, and they can even cause death. The spread of drug-resistant strains of staphylococci and the ineffectiveness of treatments in cases of biofilm-related infections underscore the necessity to find new modes of prevention and effective alternatives to antibiotic treatment. A novel way would be to interfere with bacterial cell-to-cell communication that leads to virulence.[93-95]

Recent Studies

Several computer-aided drug design approaches like molecular docking studies and QSAR studies can be used in drug discovery in several ways. The search for new compounds with a given biological activity requires huge effort in terms of manpower and cost. This effort

arises from the large number of compounds that need to be synthesized biologically evaluated. For this reason the pharmaceutical industry has shown great interest in theoretical methods that facilitate the rational design of pharmaceutical agents. In the last years bioinformatics has experienced a great evolution due to the development of specialized software and the increasing computer power. The codification of the structural information of molecules through molecular descriptors and the subsequent data analysis allow establishing QSAR models (Quantitative Structure-Activity Relationship) that can be applied to the design and the virtual screening of new drugs.[96-98]

Kahlon AK et al during 2010 studied about Molecular docking carried out on *Staphylococcus aureus* with Dehydrosqualene synthase inhibitors. It involved in the synthesis of golden carotenoid pigment staphyloxanthin. The pigment of *S. aureus* provides the antioxidant property to the bacterium to survive inside the host cell. Dehydrosqualene synthase have structural similarity with the human squalene synthaseenzyme which is involved in the cholesterol synthesis pathway in humans. Cholesterol lowering drugs were found to have inhibitory effect on dehydrosqualene synthase enzyme of *S. aureus*. This study attempts to focus on squalene synthease inhibitors, lapaquistat acetate and squalestatin analogs on dehydrosqualene synthase enzymes of *S. aureus*. Then mode of binding of lapaquistat actate and squalestatin analogs on dehydrosqualene synthase enzyme of *S. aureus* was performed by molecular docking analysis.[99]

B Hariprasath in 2011 studied about the molecular docking of plant derived compounds against two target proteins which are helpful for MRSA infection. Docking studies was done with the help of discovery studio to understand the applicability of the method to differentiate between the active and the inactive compounds. The docking studies of two structurally diverse inhibitors were carried out ad was observed through some false positive were also obtained. The result obtained were the high molecular weight compound with heterocyclic rings showed very low binding energy. The active constituents that were docked with the protein are Baicalein, Biochanin, Carnosol, Genistein, Orobol, Resveratrol, Rhein, Gallic acid, Pyrithione, Resveratrol and Linozolid . The compound Orobol was found to interact more towards the target protein like showing highest Dock score.[100]

Mehta et al. in 2012 woked on pharmacophore mapping and 3D-QSAR analysis of *S. aureus* as it causes variety of human infections, ranging from superficial abscesses to life threatening bacteremias. S. *aureus* Sortage A inhibitors are broadly used for the treatment of bacterial

infections.[100] The 3D-QSAR analysis has been applied to a structurally diverse set of 34 compounds as S. *aureus* Storage A inhibitors. This study has been focused on pharmacophore mapping study that can explore 3D features and configurations responsible for biological activity of structurally diverse compounds. A four point pharmacophore with one hydrogen bond acceptor (A), one hydrogen bond donor and two aromatic rings (R) as pharmacophore features was developed. The generated best pharmacophore hypothesis yielded a statistically significant 3D-QSAR model, with correlation coefficient of R2=0.929 for training set molecules. The model generated showed excellent prediction power, with Q2=0.887 for an external set of 10 test set molecules. The geometry and its features of pharmacophore are expected to be useful for design of selective *Staphylococcus aureus* Sortase A inhibitors.[101]

Scotti L. et all in 2014 studied about the docking and PLS studies on *Staphylococcus aureus*, as *Staphylococcus aureus* lives in commensalism with majority of population being recognized as an important pathogen in patients with chronic liver diseases and causes deadly infections. The use of antibiotics as rifampin for the chemotherapy of infections caused by *S. aureus* has resulted in the selection of mutants with resistance. After seeking further information on existing scientific literature, the compounds under study were applied the methodologies of PLS, docking and calculation.[102]

ACKNOWLEDGEMENT

Authors are thankful to the Director, Centre for Bioinformatics Studies, Dibrugarh University. Department of Pharmaceutical Sciences is gratefully acknowledged. The authors are also grateful for the immense help received from the Researchgate.

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