Integrative Approaches to Combat Methicillin-Resistant *Staphylococcus aureus* (*MRSA*): Novel Diagnostics, Therapeutic and Genomic Insights

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Abstract

Methicillin Resistant Staphylococcus aureus (*MRSA*) is a well-known and potent pathogen that can be found in both medical and community settings. To effectively battle *MRSA*, management, treatment, and preventative strategies are crucial. Prevention includes maintaining hygiene, taking care of wounds, avoiding sharing personal items, and adopting healthy habits. *MRSA* diagnosis and treatment require medical guidance, prompt antibiotic sensitivity testing, appropriate antibiotic use, and thorough clinical care. In healthcare settings, frequent screening, infection control practices, and antibiotic stewardship are essential. Long-term management includes education, awareness, and follow-up care. Molecular-based tests have improved *MRSA* screening accuracy and turnaround time, enabling early isolation and treatment. Whole-genome sequencing and genomic approaches enhance the management of resistant infections in hospital settings by predicting antibiotic resistance and finding resistance markers in clinical bacterial isolates. Additionally, the combination of transcriptome and proteome data has revealed immunologic patterns associated with persistent *MRSA* bacteremia outcomes, offering insights into potential therapeutic targets. By leveraging advancements in genetics, medicines, and diagnostics, a more comprehensive approach to the issues posed by *MRSA* infections can be developed.

Background on *MRSA* and its impact on public health

Staphylococcus aureus (S. aureus) continues to be the most common life-threatening pathogen causing both community acquired (CA) and Hospital Acquired Infections (HAI) worldwide[1]. The Centres for Disease Control and Prevention has classified methicillin-resistant *S aureus* (*MRSA*) as a serious public health threat that causes significant morbidity and mortality and increases healthcare expenditures. In India, gram-positive infections, particularly *MRSA* prevalence among invasive *S aureus* infections, have been reported to increase exponentially from 29 % in 2009 to 69 % in 2020 because the importance of *MRSA* as a problem has been recognized relatively late[2]. *S. aureus* is known to cause skin and soft tissue infections in the community settings and more of invasive infections in the healthcare settings. Among invasive infections such as bacteraemia, mortality rates range between 15% and 30%, particularly in hospitalized and immunocompromised patients[3]. Antibiotic resistance in Gram positive cocci has emerged as a major threat to human health. After introduction of penicillin in the infectious disease therapy in early 1940s, resistance to β- lactams started to develop among *S. aureus*. The first *Methicillin Resistant Staphylococcal aureus* (*MRSA*) was identified in 1960, harbouring the *mecA* gene encoding a modified penicillin binding protein (PBP)[4]. This gene is located on a mobile genetic element known as the staphylococcal cassette chromosome *mec* (SCC*mec*), enabling horizontal gene transfer and clonal dissemination[5]. *MRSA* is one of the well-known endemic pathogens in India. The first case of nosocomial *MRSA* infection was reported in 1988[6].

The incidence of *MRSA* varies with the wide range of 33% in western part when compared to 34 % in southern part of India[7]. Indian Network for Surveillance of Antimicrobial Resistance (INSAR) has taken various initiatives to network the prevalence and antimicrobial resistance data of *S. aureus* in the national level. The overall proportion of *MRSA* in India was 41.4%. *MRSA* colonization of the nose and other body sites of patients, nurses and other healthcare personnel were found to be the possible reservoirs of *MRSA*[8]. Colonization plays a crucial role in induction and spread of staphylococcal infections[9]. Emerged *MRSA* led to the clinical demand of effective novel therapeutic agents like vancomycin, teicoplanin, linezolid and mupirocin. The 2016 Indian infectious disease treatment guidelines recommend vancomycin and teicoplanin as first-line choice for *MRSA*, linezolid for *MRSA* induced skin and skin structure infections (SSSIs), and daptomycin for complicated SSSIs and bacteraemia due to *MRSA*[2]. Vancomycin is used in the treatment of

serious Gram-positive bacterial infections, including *MRSA*. It is in the cell wall synthesis inhibitor class of antimicrobial medications. Vancomycin is a tricyclic glycopeptide antibiotic that exerts the bactericidal effect by inhibiting polymerization of peptidoglycans in the bacterial cell wall[10]. Vancomycin is derived from the organism Streptococcus orientialis. Vancomycin is used to treat and prevent various bacterial infections caused by gram-positive bacteria. Vancomycin has been available for more than 70 years[11]. For the past 60 years, vancomycin has remained the first choice for *MRSA* infections in the majority of countries around the world[9]. However, with the extensive use of this drug, reports on the emergence of *MRSA* strains with reduced susceptibility to vancomycin have also increased[2].

Linezolid (LZD) the first member of oxazolidinone class of antibiotics that has been approved for clinical use in 2000. It has broad spectrum of activity against variety of Gram-positive pathogens[2]. It acts by inhibiting protein synthesis via binding to the peptidyl transferase centre of the 50S ribosomal subunit and preventing formation of the fMet-tRNA-30S ribosome-mRNA initiation complex. Nowadays Linezolid resistance (LR) was also reported, the first case of Linezolid-resistant staphylococcus was reported in peritonitis patients undergoing oral linezolid treatment during peritoneal dialysis.
Mupirocin (pseudomonic acid A) is approved antibiotic for decolonization of *MRSA* and methicillin susceptible *S. aureus* (*MSSA)* both in patients and healthcare personnel. This acts by binding to the enzyme leucine-specific tRNA aminoacyl synthetase, inhibiting protein synthesis. After widespread use of mupirocin for prolonged periods, especially for decolonization of healthcare personnel and for bedsores and other skin lesions, resistance emerged. High level mupirocin resistance is associated with plasmid mediated *mupA* gene. Topical use of mupirocin does not eliminate the *MRSA* strains with high-level mupirocin resistance[12].

The Daptomycin (2003), Retapamulin (2007), and Fidaxomicin (2011) were developed resistantce in the same year of their discovery[13].

In addition to antimicrobial resistance, virulence factors like hemolysin, lipase, protease, gelatinase and lecithinase also adds more challenges in managing patients with *MRSA* infections[14].

Diagnostic Methods:

Traditional/Conventional methods

Traditional diagnostic techniques for *Methicillin-resistant Staphylococcus aureus* (*MRSA*) typically involve culture-based methods (Swab samples were cultured in Mannitol Salt Agar (HiMedia; SPH118) plates (MSA plates) for selective isolation of *Staphylococcus aureus*[15]. Isolated *Staphylococcus aureus* colonies can also be cultured in HiCrome-Rapid *MRSA* Agar plates (HiMedia; M1974) to isolate methicillin-resistant *Staphylococcus aureus* (*MRSA*). The Minimum inhibitory concentration (MIC) of cefoxitin was also checked for the *S. aureus* to detect methicillin resistance or susceptibility of all *S. aureus* culture isolates[1]. The yellow-coloured colonies on mannitol salt agar and cream to golden yellow colonies with *β* or weak haemolysis on blood agar, biochemical (the isolated colonies from NA plates were used for biochemical tests including catalase, coagulase (slide and tube coagulase test), and DNase tests for the identification of *S. aureus* isolates[4]. Morphological identification (Gram staining, Blood agar plates were incubated for 24 and 48 hours at 35 °C with 5% CO2. The plates were visullay examined. If needed, a magnifying glass was used to assist in visualizing the colonies. The colony shapes were classified as target-shaped (T1) or dome-shaped (T2). T1 is an elevated colony center encircled by a pale zone, which is surrounded by a single ring of peripheral enhancement giving a 'target' appearance[6], and genetic testing targeting *MRSA*-specific genes such as *mecA* and SCC*mec*. The *mecA* gene remains the predominant determinant of methicillin resistance in *MRSA*; however, *mecC*-containing *MRSA* strains have been increasingly. Studies have shown prevalences of approximately 31.9% for *mecA* alone, 7.9% for *mecC* alone, and 57.1% for combined *mecA/mecC* positivity in *MRSA* isolates. *mecC*-mediated *MRSA* was initially reported mainly in livestock-associated infections in Spain but has now also emerged in several Asian countries[7]. Methicillin resistance in *Staphylococcus aureus* is primarily mediated by the *mecA*-encoded PBP2a protein, an altered penicillin-binding protein with low affinity for β-lactam antibiotics. The *mec* genes are carried on SCC*mec* chromosomal elements and can spread through horizontal gene transfer. With the exception of more recent drugs like ceftaroline and ceftobiprole, *MRSA* strains are resistant to the majority of β-lactam antibiotics. This resistance mechanism is intimately associated with modified PBP-mediated peptidoglycan production[10].

For identification, these techniques rely on traits such coagulase tests, Gram staining, colony morphology, and antibiotic susceptibility testing. Chromogenic medium and modified conventional techniques have demonstrated improved *MRSA* detection sensitivity and specificity[7]. Clinical samples were cultivated using mannitol and blood agar. Staphylococcus species were isolated by incubating salt agar at 37 °C for 24 hours. Gram staining and biochemical assays, such as the catalase and coagulase tests, were used to identify suspected Staphylococcus aureus colonies with golden-yellow colouring. Both tests were positive for S. aureus. However, these conventional techniques can be laborious, requiring several days to yield findings, which could postpone the start of treatment[16]. Promising developments in *MRSA* detection and management are being made possible by ongoing attempts to enhance traditional diagnostics using cutting-edge techniques like MALDI-TOF MS for accurate species identification and protein expression analysis.[17].

New diagnostic methods and technologies for rapid detection of *MRSA*.

Next Generation Sequencing (NGS) is an effective tool for diagnosing and understanding *MRSA*, with advantages such as detecting *MRSA* strains using whole genome sequencing and targeted sequencing, profiling antibiotic resistance, tracking epidemiology, understanding virulence factors, elucidating resistance mechanisms, enabling rapid diagnosis, and facilitating customised treatment strategies. Compared to conventional diagnostic techniques, NGS offers comprehensive genomic data that can enhance *MRSA* infection management and control[18].

Antibiotic-based therapy for Methicillin-Resistant *Staphylococcus aureus* (*MRSA*)

Treatment options include vancomycin, daptomycin, linezolid, tigecycline, clindamycin, trimethoprim-sulfamethoxazole (TMP-SMX), doxycycline, minocycline, and ceftaroline[19]. The choice of antibiotic depends on the severity and location of the infection. Susceptibility testing is crucial for determining the most effective treatment and preventing further resistance development. *MRSA* identification and antimicrobial susceptibility testing were performed according to the 2020 Clinical and Laboratory Standards Institute guidelines using the Kirby–Bauer disk diffusion method. Cefoxitin resistance was used for phenotypic detection of *MRSA*, With isolates showing a zone of inhibition ≤19 mm classified as *MRSA*. *MRSA* isolates demonstrated high resistance to β-lactam antibiotics and several other agents including fluoroquinolones and macrolides, while vancomycin, teicoplanin, linezolid, clindamycin,

chloramphenicol, fusidic acid, gentamicin, and tetracycline retained relatively good activity and remaied useful treatment options in clinical settings[7].

Present status of *MRSA* resistance *Staphylococcus aureus*

Methicillin-resistant Staphylococcus aureus (*MRSA*) is resistant to a variety of antibiotics due to the *mecA* gene, which provides resistance to methicillin and other beta-lactam antibiotics[19]. The expression of *mecA* is regulated by the *mecI* and *mecR1* regulatory genes, which *control* transcription in response to beta lactam exposure[20]. *MRSA* is typically resistant to antibiotics such as methicillin, oxacillin, penicillin, ampicillin, amoxicillin, cloxacillin, and dicloxacillin. Additionally, it may exhibit resistance to monobactams, carbapenems, and cephalosporins, however strain and location-specific resistance patterns may differ[21].

Vancomycin, daptomycin, linezolid, tigecycline, and quinupristin-dalfopristin are effective antibiotics for treating *MRSA* infections. Due to possible differences in antibiotic susceptibility and changing resistance patterns, susceptibility testing is essential for choosing the right course of treatment[21].

Novel Antibiotics

New *MRSA* antibiotics, such as oxazolidinones, lipopeptides, dalbavancin, oritavancin, ceftaroline, and inhibitors that target resistance pathways, are being researched. While the creation of new anti-*MRSA* medications is still a long-term objective, combination therapy—which combines current antibiotics with other synergistic substances—has shown promise as a quicker and more effective way to treat drug-resistant infections. Antimicrobial resistance may be mitigated by restoring the efficacy of current medications through synergistic interactions[11]. The World Health Organization has highlighted how urgently new antimicrobials are needed to combat serious diseases like *MRSA*. Because they target the ribosome to prevent bacterial protein synthesis and exhibit little cross-resistance with other antibiotics, oxazolidinones are promising agents. The therapeutic efficacy of oxazolidinones against *MRSA* infections may be increased by creating novel compounds with improved anti-biofilm action[8]. A more recent oxazolidinone that has been licensed to treat acute bacterial skin and soft tissue infections brought on by susceptible bacteria is called tedizolid. In order to stop bacterial protein synthesis, oxazolidinones attach to the 50S ribosomal subunit and hinder the development of the 70S initiation complex. Daptomycin, a cyclic

lipopeptide used for complicated skin infections, bacteremia, and right-sided infective endocarditis caused by *S. aureus*, acts by inserting itself into the bacterial cell membrane, causing membrane depolarization, potassium leakage, and rapid cell death. Although daptomycin effectively penetrates most tissues, it is inactivated by pulmonary surfactant and is therefore unsuitable for primary lung infections[9]. Combining antibiotics, alternative therapies like bacteriophage therapy and host-directed therapies and exploring natural products are also promising approaches. Plants are important sources of bioactive compounds with significant anti-*MRSA* activities. Secondary metabolites, such as alkaloids, flavonoids, phenolics, and terpenoids have a variety of antibacterial characteristics and may improve the efficacy of conventional antibiotics through synergistic interactions. Combination therapy using natural products and antibiotics has shown potential in re-sensitizing resistant *MRSA* strains and may contribute to the development of novel therapeutic strategies against antimicrobial resistance[11].

Alternative Therapies

Bacteriophage therapy for *MRSA*

One promising treatment strategy for *MRSA* and other antibiotic-resistant infections is bacteriophage therapy. It entails the use of viruses that selectively target and destroy bacteria, providing advantages such biofilm disruption, specificity, and the ability to overcome resistance[22]. Phage selection, immunological reaction, and regulatory concerns are obstacles. Promising findings from research on bacteriophage therapy for *MRSA* have been reported in controlled clinical studies and case reports. Because they are selective, bacteriophages only target specific strains of bacteria while preserving helpful microorganisms[12]. Phage therapy has shown successful in treating *MRSA* infections, including those involving biofilms and persistent wounds, particularly when customised phage mixtures are used. Biofilms are particularly problematic, particularly in long-term infections[12]. Bacteria that are frequently resistant to traditional treatments can be specifically killed thanks to phage's ability to pierce and break bacterial biofilms. The process of identifying and isolating particular bacteriophages from natural sources, including soil, sewage, and the human microbiome, is known as phage treatment[23]. Following administration, bacteriophages use the host's mechanism to replicate by attaching to vulnerable bacteria and injecting their genetic material. This leads in bacterial cell lysis and release of additional phages, which subsequently infect neighbouring bacterial cells and intensify the therapeutic impact at the

infection site[14]. But issues like phage isolation, resistance, and regulatory obstacles still exist. In order to better understand the safety and ideal application of phage therapy for *MRSA*, recent advancements concentrate on increasing clinical studies and improving phage efficacy through engineering. Even though the evidence is mounting, more study is required to resolve issues and improve treatment plans in this developing sector[14].

Antimicrobial peptides-based Therapy for *MRSA*

Antimicrobial peptides (AMPs) exhibit potential as a treatment for methicillin-resistant Staphylococcus aureus (*MRSA*) because of their distinct modes of action, which include immunological regulation and membrane disruption. naturally occurring peptides that have the ability to circumvent conventional resistance mechanisms and show broad-spectrum effectiveness against bacteria, including *MRSA*. Global focus is required to address the problem of antibiotic-resistant bacteria and the concerning increase in infectious illnesses brought on by AMR bacteria[24]. According to recent studies, some AMPs are more effective against *MRSA*, they can be used in combination treatments with conventional antibiotics, and they have a lesser chance of developing resistance than antibiotics. AMPs are amphipathic, highly conserved peptides with strong antibacterial properties. They create ionic pores or temporary gaps that significantly change the permeability of bacterial membranes through electrostatic interactions with internal bacterial components and cell membranes[25]. Ongoing research is concentrated on creating new peptides and derivatives for enhanced activity, and safety assessments show no harm to human cells. Recent studies have shown that two antimicrobial peptides (AMPs) from a rumen microbiome metagenomic dataset, HG2 and HG4, are active against multidrug-resistant (MDR) bacteria, particularly *MRSA* strains[26]. These lead AMPs are fast-acting and have demonstrated anti-biofilm and anti-inflammatory properties in vitro. Moreover, they demonstrated low toxicity to human primary cell lines. Animal models and early clinical trials support the effectiveness of AMPs in vivo, while research into formulation and delivery methods is ongoing. The peptides have also been found to be effective *in vivo* in a Galleria mellonella model of *MRSA* infection. The AMPs, HG2 and HG4, appeared to interact with the cytoplasmic membrane of target cells and may inhibit other cellular processes. They also appear to bind preferentially to bacterial lipids over human cell lipids[27,28]. Challenges include cost-effective production and the need for continuous monitoring of resistance mechanisms in these bacteria. It should be noted that there are challenges in developing

and selling AMP resistance molecules, whether for topical or systemic treatment. Polyphor conducted phase 3 clinical trials to assess the safety and effectiveness of intravenous murepavadin. However, the trials had to be stopped prematurely due to elevated serum creatinine levels in patients treated with AMP, which is an indication of renal failure[27,28]. AMP-based therapies offer a promising approach to combat *MRSA*, with ongoing research aimed at optimizing their efficacy, safety, and application[29].

Immunotherapy for *MRSA*

Recent research on immunotherapy for *MRSA* has shown progress in vaccine development, monoclonal antibodies, immune modulation, and combination therapies. Monoclonal antibodies that neutralise toxins or mark bacteria for destruction are vaccines that target particular *MRSA* antigens. The high specificity of mAbs is a major benefit that enables targeted treatments that reduce off-target effects frequently seen with broad-spectrum antibiotics, particularly in cases involving antibiotic-resistant strains like *MRSA*[30] and immuno-modulating substances to improve the immune response are being investigated. Additionally being researched is the combination of immunotherapy and antibiotics. Additionally, combining mAbs with conventional antibiotics may improve therapeutic effectiveness and reduce required dosages, which would lessen the likelihood of resistance[31]. There is hope for more potent treatments against *MRSA* as clinical trials are being conducted to evaluate the safety and effectiveness of these novel medications in humans. Although preclinical research is promising, thorough clinical trials are necessary to verify the efficacy, safety, and dosage of mAb treatments for *S. aureus* infections. The best usage of these treatments will depend on a number of variables, such as patient demographics and the severity of the infection[31.] Researchers are constantly finding new virulence factors to target with monoclonal antibodies (mAbs), which could lead to more therapy options for infections caused by *S. aureus* [32].

Innovations in drug delivery systems (Nanotechnology)

To overcome the drawbacks of conventional antibiotic therapies for *MRSA*, novel drug delivery methods are essential. They enhance patient outcomes and assist in addressing the particular difficulties presented by this resistant infection. To enhance treatment results, researchers are investigating novel medication delivery methods. Notable developments include hydrogel systems

with smart and injectable options, microneedle arrays for targeted delivery, antibody-conjugated carriers for specific *MRSA* targeting, nanomaterial-enhanced antibiotics using silver nanoparticles and graphene oxide, and nanoparticle-based delivery systems like liposomes and polymeric nanoparticles[33.] Nanoparticles (NPs) are showing promise as a means of combating antibiotic resistance and *MRSA*. Numerous NPs have shown strong antibacterial and anti-biofilm activity against *MRSA* both in vitro and in vivo, including phospholipid, chitosan, silver, alginate, copper-ferrite, hyaluronic acid, and mesoporous silica-based nanoparticles. These techniques improve the efficacy of treatment for resistant *MRSA* infections by improving drug administration, intracellular drug uptake, wound healing, biofilm destruction, reactive oxygen species production, and bacterial cell membrane damage. Additionally, research is being conducted on bacteriophage therapy and responsive drug delivery systems. Moreover, biofilm-targeting techniques, gene editing, and RNA-based therapies show promise in the battle against *MRSA* resistance. These advancements aim to enhance treatment efficacy, fight antibiotic resistance, and improve patient outcomes in *MRSA* infections[34].

Genomic Insights

Advances in genomic studies revealing resistance mechanisms.

The biology of *MRSA* is better understood because to advances in genetic research, which is crucial for controlling outbreaks, developing potent medications, and enhancing public health responses to this challenging virus.. *MRSA* genetics, resistance mechanisms, and epidemiology have all been better understood thanks to recent developments in genomic technologies. These developments have made it possible to track genetic changes between strains, identify resistance genes and genetic variations, do thorough whole-genome sequencing, and comprehend virulence aspects[19]. Additionally, strain typing, epidemic investigations, and the creation of novel treatment and diagnostic tools have all benefited from genomic research[19]. The integration of genomic data into clinical practice holds promise for personalized medicine approaches in treating *MRSA* infections, however it is unlikely that WGS will be able to replace phenotypic methods entirely, and some form of phenotypic surveillance will need to be maintained, for example based on clusters of treatment failures. Ongoing genomic surveillance is crucial for monitoring the emergence of new *MRSA* strains and resistance patterns[47].

Recent findings on the genetic basis of *MRSA* pathogenesis.

The genetic basis of Methicillin-resistant *Staphylococcus aureus* (*MRSA*) pathogenesis has identified key factors contributing to its antibiotic resistance and virulence[34]. *MRSA*'s resistance to methicillin is primarily due to the *mecA* gene, which encodes a modified penicillin-binding protein. Macrolide resistance in *MRSA* is mainly mediated by *erm* genes, which encode ribosomal methylases causing resistance to macrolides, lincosamides, and streptogramin B antibiotics, including inducible clindamycin resistance detectable by the D-test. Fluoroquinolone resistance occurs through mutations in *gyrA* and *grlA* genes, while aminoglycoside resistance is mediated by aminoglycoside-modifying enzymes. Trimethoprim-sulfamethoxazole resistance is caused by modified dihydrofolate reductase enzymes, whereas tetracycline resistance includes *tetK*-mediated efflux pumps or *tetM*-encoded ribosomal protection proteins. *MRSA* isolates have significant rates of resistance, especially to erythromycin, ciprofloxacin, and clindamycin, according to surveillance studies[21].

The virulence and antibiotic resistance profiles of *MRSA* are influenced by genetic heterogeneity, particularly variations in mobile genetic elements (MGE). Bacterial evolution and horizontal gene transfer are significantly influenced by mobile genetic elements (MGEs), including insertion sequences, transposons, integrons, plasmids, integrative conjugative elements, and bacteriophages. They can either irreversibly or reversibly integrate into the bacterial chromosome and aid in the dissemination of antibiotic resistance genes across bacteria. Integration by integrase enzymes that are either encoded by the MGEs or acquired from the host cell. Reversible integration allows for excision and further horizontal transmission, which promotes the dissemination of resistance determinants[35]. Examples of virulence factors that increase *MRSA*'s pathogenicity include toxins and surface proteins. Clonal complexes, which categorise *MRSA* strains based on genetic fingerprints, have an impact on clinical results. According to studies, the CC22-*MRSA* lineage is made up of different genotypes that carry different antibiotic resistance determinants, SCCmec elements, and toxins such toxic shock syndrome toxin-1 (TSST-1) and Panton-Valentine leukocidin *(pvl)*[5]. Genetic regulation mechanisms control the generation of virulence components, and *MRSA* adaptability allows it to rapidly adapt to selective pressures. The primary regulator of *Staphylococcus aureus* virulence is the accessory gene regulator (Agr) quorum-sensing system, which uses autoinducing peptides (AIPs) to sense bacterial population density and control the

transition from colonisation to invasive toxin-producing phases. Toxin-neutralizing monoclonal antibodies (mAbs), which target released toxins and leukocidins, are an essential anti-virulence strategy that prevents host cell damage without eradicating the bacteria or promoting antibiotic resistance[36].

Identification of new therapeutic targets based on genomic data

Multiomics research offers a thorough understanding of *MRSA* aetiology and resistance mechanisms by combining proteomics, metabolomics, transcriptomics, and genomes, potentially leading to the discovery of new treatment targets. Genomic investigations identify strain diversity and resistance genes, whereas transcriptomics shows alterations in gene expression in response to antibiotics37. Proteomics studies find overexpressed proteins linked to resistance, while metabolomics explains metabolic alterations[38]. However, because of their complexity, heterogeneity, and high dimensionality, omics data require advanced computational methods and tools for analysis and interpretation. Integrated insights reveal new pharmacological targets, combination treatments, and tailored medical approaches. Future directions include target validation, technological advancements, and clinical trials to translate multiomics findings into effective *MRSA* treatments[38].

Conclusion and Future Directions

Future developments in the treatment of methicillin-resistant *Staphylococcus aureus* (*MRSA*) will concentrate on improving genetic knowledge, treatments, and diagnostics through a variety of creative strategies. Next-generation sequencing and CRISPR-based diagnostics are two fast and accurate detection techniques being developed. New antibiotics, bacteriophage therapy, immunotherapies, and combination treatments are examples of emerging therapeutics that attempt to address resistance. In order to enable more individualised and successful treatments, genomic research aims to identify the genetic pathways underlying *MRSA*'s resistance and adaptability. Systems biology and AI-driven big data analysis are examples of integrative approaches that have the potential to advance our knowledge of and ability to treat *MRSA* infections, improving patient outcomes. By facilitating early detection through predictive

analytics, optimising antibiotic stewardship, improving infection control with surveillance systems, customising treatment plans based on genetic information, supporting clinical decision-making, expediting the development of new therapies, and engaging patients with educational tools, artificial intelligence (AI) and machine learning (ML) are revolutionising *MRSA* management. However, for these technologies to realise their full potential, issues including bias, data privacy, and integration into clinical practice must be resolved. Healthcare professionals and technologists must work together for implementation to be successful.

Table 1: List of conventional and recent diagnostic methods in MRSA.

Diagnostic method	Advantage	Limitations	References
CONVENTIONAL METHODS			
Culture on Selective Media	Low cost, widely available, allows antimicrobial susceptibility testing (AST)	Slow (24–72 h), labor-intensive, may miss low bacterial loads	Brown et al.,[15] [44]
Gram Staining	Rapid, inexpensive, simple	Cannot distinguish *MRSA* from *MSSA;* low specificity	David & Daum, 2010[39] [45]
Biochemical Tests (Coagulase, Catalase)	Inexpensive, easy to perform	Cannot confirm methicillin resistance	Forbes et al., 2017[40] [46]
Disk Diffusion (Cefoxitin/Oxacillin Test)	Standardized, economical, reliable	Requires culture isolation; results take 18–24 h	CLSI standard M100; 2025[41]. [47]
Broth Microdilution/MIC Testing	Quantitative resistance data, gold standard for susceptibility testing	Time-consuming, requires technical expertise	CLSI standard M07. Wayne, PA: CLSI; 2024[42] [48]
Latex Agglutination for PBP2a	Faster than molecular methods, good specificity	Costlier than routine culture; may miss rare variants	Lee AS et al.,[3] [22]
RECENT ADVANCES			
PCR for *mecA/mecC* Genes	High sensitivity and specificity, rapid (2–6 h)	Requires specialized equipment, expensive	Paterson GK et al.,[43] [49]
Real-Time PCR (qPCR)	Very rapid, highly sensitive, reduced contamination risk	High cost, skilled personnel required	Lee AS et al.,[3] [22]
Multiplex PCR	Detects several targets in one assay, saves time	Complex assay design, expensive reagents	Lee AS et al.,[3] [22]
Loop-Mediated Isothermal Amplification (LAMP)	Rapid (<1 h), no thermal cycler needed, suitable for point-of-care testing	Risk of contamination, limited multiplexing capability	Misawa et al.,[44] [50]
DNA Microarray	Detects resistance, virulence, and strain types simultaneously	Very expensive, requires advanced infrastructure	Cuny C et al.,[45] [51]

MALDI-TOF Mass Spectrometry	Rapid species identification, high throughput	Generally cannot directly determine methicillin resistance without additional testing	Patel et al.,[46] [52]
Whole-Genome Sequencing (WGS)	Highest resolution, detects resistance and epidemiological markers	Expensive, complex data analysis, longer turnaround time	Köser et al.,[47] [53]
Next-Generation Sequencing (NGS)	Comprehensive resistance and outbreak investigation	Requires bioinformatics expertise and significant resources	Maljkovic et al.,[48] [54]
CRISPR-Based Diagnostics (e.g., Cas12/Cas13 systems)	Ultra-sensitive, rapid, potential point-of-care use	Limited clinical validation, not widely available	Chen JS et al.,[49] [55]
Biosensor-Based Detection	Fast, portable, minimal sample preparation	Still under development; standardization challenges	Pérez-Rodríguez et al.,[50] [56]
Microfluidic Lab-on-a-Chip Systems	Rapid, low sample volume, potential bedside testing	High development cost; limited commercial availability	Wang X et al.,[51] [57]

Table 2: Novel Antibiotics for *Methicillin-Resistant Staphylococcus aureus (MRSA)*

Antibiotic	Drug Class	Mechanism of Action	Advantages	Limitations	Reference
Linezolid Oral, IV	Oxazolidinone	Inhibits protein synthesis by binding to the 50S ribosomal subunit	Excellent oral bioavailability, effective for skin and lung infections	Myelosuppression, peripheral neuropathy with prolonged use	Greenfield et al.,[52]
Tedizolid Oral, IV	Oxazolidinone	Inhibits protein synthesis at the 50S ribosome	Once-daily dosing, lower hematologic toxicity than linezolid	Limited long-term clinical experience	Greenfield et al.,[52]
Daptomycin IV	Cyclic Lipopeptide	Disrupts bacterial cell membrane causing depolarization	Rapid bactericidal activity, effective for bacteremia and endocarditis	Not suitable for pneumonia due to lung surfactant inactivation	Terver et al.,[53]
Ceftaroline fosamil IV	Advanced Cephalosporin	Binds PBP2a and inhibits cell wall synthesis	Only β-lactam with reliable *MRSA* activity	Requires parenteral administration	Shirley et al.,[54]
Telavancin IV	Lipoglycopeptide	Inhibits cell wall synthesis and disrupts membrane integrity	Dual mechanism of action	Nephrotoxicity risk, pregnancy precautions	Yan et al.,[55]
Dalbavancin IV	Lipoglycopeptide	Inhibits cell wall synthesis	Very long half-life, once-weekly dosing	High acquisition cost	Yan et al.,[55]
Oritavancin IV	Lipoglycopeptide	Inhibits transglycosylation, transpeptidation, and membrane function	Single-dose therapy possible	Expensive, limited indications	Yan et al.,[55]
Delafloxacin Oral, IV	Fluoroquinolone	Inhibits DNA gyrase and topoisomerase IV	Active against some *MRSA* strains, oral option available	Potential fluoroquinolone-associated adverse effects	Turban et al., [56]
Omadacycline Oral, IV	Tetracycline Derivative	Inhibits protein synthesis by	Active against multidrug-	Gastrointestinal adverse effects	Zhanel et al.,[57]

		binding to the 30S ribosome	resistant Gram-positive bacteria		
Lefamulin Oral, IV	Pleuromutilin	Inhibits protein synthesis via the 50S ribosomal subunit	Novel mechanism, active against resistant Gram-positive pathogens	Limited *MRSA*-specific indications	Paukner et al.,[58]
Contezolid Oral	Oxazolidinone	Inhibits bacterial protein synthesis	Reduced hematologic toxicity compared with linezolid	Availability varies by country	El-Kimary et al., [59]
Levonadifloxacin Oral, IV	Benzoquinolizine	Inhibits DNA gyrase and topoisomerase IV	Active against quinolone-resistant *MRSA* strains	Limited global approval and availability	Kumari et al.,[60]

Table 3: List of alternative therapies for *MRSA*

Alternative Therapy	Mechanism of Action	Advantages	Limitations	Current Status	Reference
Bacteriophage Therapy	Uses viruses (phages) that specifically infect and lyse *MRSA* cells	Highly specific, effective against multidrug-resistant strains, minimal effect on normal microbiota	Narrow host range, potential bacterial resistance, regulatory challenges	Clinical trials and compassionate-use cases	Agarwalla et al.,[61]
Phage-Derived Endolysins	Enzymes that degrade the bacterial cell wall	Rapid bactericidal activity, low resistance development	Stability and delivery challenges	Preclinical and early clinical studies	Taati Moghadam et al., [62]
Antimicrobial Peptides (AMPs)	Disrupt bacterial membranes and cellular functions	Broad-spectrum activity, low likelihood of resistance	Potential toxicity, high production cost	Experimental and clinical development	Zhang et al.,[63]
Nanoparticle-Based Therapy	Metal or polymer nanoparticles damage bacterial membranes and biofilms	Effective against biofilm-associated *MRSA*, targeted delivery possible	Potential cytotoxicity and environmental concerns	Research and preclinical stage	Mohanta et al.,[64]
Photodynamic Therapy (PDT)	Photosensitizers activated by light generate reactive oxygen species that kill bacteria	Effective against resistant strains and biofilms	Requires light exposure and specialized equipment	Investigational use	Tanu et al.,[65]
Photothermal Therapy (PTT)	Nanomaterials convert light into heat, destroying bacterial cells	Localized treatment with minimal systemic effects	Limited penetration depth and specialized equipment	Experimental	Mei et al.,[66]

Probiotic Therapy	Beneficial bacteria inhibit *MRSA* colonization through competitive exclusion and antimicrobial production	May reduce *MRSA* carriage, safe for many patients	Variable efficacy, limited clinical evidence	Investigational	Zhao et al.,[67]
Vaccines	Stimulate host immunity against *MRSA* antigens	Potential prevention of infection	No approved *MRSA* vaccine available yet	Clinical research ongoing	Scully et al.,[68]
Monoclonal Antibodies	Target *MRSA* toxins or virulence factors	Highly specific, can enhance immune clearance	Expensive, limited spectrum	Clinical and preclinical studies	Piscaglia et al.,[69]
CRISPR-Cas Antimicrobials	Gene-editing systems target and destroy resistance genes or bacterial DNA	Highly specific, can eliminate resistance determinants	Delivery challenges and regulatory hurdles	Experimental	Moitra et al., [70]
Plant-Derived Phytochemicals	Bioactive compounds inhibit growth, virulence, or biofilm formation	Natural source, multiple mechanisms	Variable potency, lack of standardization	Preclinical research	Rafeeq et al.,[71]
Essential Oils	Contain antimicrobial compounds that disrupt bacterial membranes	Natural and readily available	Variable composition, potential irritation/toxicity	Complementary research	Lopes et al.,[72]
Quorum Sensing Inhibitors	Block bacterial communication and virulence regulation	Reduce biofilm formation and pathogenicity	Often not directly bactericidal	Experimental	Singh et al.,[73]
Anti-Biofilm Agents	Disrupt biofilm matrix or prevent biofilm formation	Enhance effectiveness of antibiotics	Limited clinical data	Investigational	Abd El-Hamid et al.,[74]
Fecal Microbiota	Restores healthy microbiota to	May reduce resistant	Limited evidence specifically for *MRSA*	Experimental	Woodworth et al.,[75]

Transplantation (FMT)	reduce pathogen colonization	organism colonization			
Combination Therapy (Antibiotics + Adjuncts)	Combines conventional antibiotics with alternative agents such as phages, nanoparticles, or AMPs	May improve efficacy and reduce resistance development	Complex treatment protocols	Active area of research	Kumar et al.,[12]

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