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Research Article

Recent Update on MRSA

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Abstract

Methicillin-resistant *S. aureus* (MRSA) isolates are resistant to all available penicillins and other ß-lactam antimicrobial drugs. *Staphylococcus aureus* is the most commonly isolated human bacterial pathogen and is an important cause of skin and soft tissue infections (SSTIs), endovascular infections, pneumonia, septic arthritis, endocarditis, osteomyelitis, foreign-body infections, and sepsis. They were once confined largely to hospitals and health care environments, and patients frequenting these facilities, however, there has been an explosion in the number of MRSA infections in the community that have been responsible for a large proportion of the increased disease burden in the last decade. This review deals with the pathogenesis, virulence mechanisms, risk factors, and clinical presentation, diagnosis and treatment strategies for MRSA.

Keywords: Methicillin-resistant *S. aureus;* CA-MRSA; HA-MRSA

Introduction

Staphylococcus aureus is the most commonly isolated human bacterial pathogen and is an important cause of skin and soft tissue infections (SSTIs), endovascular infections, pneumonia, septic arthritis, endocarditis, osteomyelitis, foreign-body infections, and sepsis especially in immunocompromised and ill patients [1]. Methicillin-resistant *S. aureus* (MRSA) isolates are resistant to all available penicillins and other ß-lactam antimicrobial drugs. These isolates are also referred to as 'Oxacillin resistant *S.aureus*' as oxacillin is used as an alternative to methicillin in susceptibility tests. When methicillin and other antibiotics do not kill the bacteria causing an infection, it becomes harder to get rid of the infection. MRSA bacteria are more likely to develop when antibiotics are used too often or are not used correctly. MRSA and other antibiotic-resistant bacteria are also referred to as "super bugs." They were once confined largely to hospitals, other health care environments, and patients frequenting these facilities. Since the mid-1990s, however, there has been an explosion in the number of MRSA infections in population lacking risk factors in the community and is described as community- associated MRSA (CA-MRSA) strains that have been responsible for a large proportion of the increased disease burden in the last decade [2]. In *S. aureus* isolates, there can be alterations to existing PBPs leading to low level resistance to methicillin and termed as 'moderately resistant *S. aureus*' (MODSA). Under certain conditions, low-level resistance may also be seen in isolates producing excessive amount of penicillinase (penicillinase hyper-producers) and these isolates are referred as 'borderline oxacillin resistant *S. aureus*' (BORSAs) [3,4].This review deals with the pathogenesis, virulence mechanisms, risk factors, clinical presentation, diagnosis and treatment strategies for MRSA.

History of MRSA Strains

In 1940, Penicillin was used as an antibiotic agent for the treatment of *Staphylococcus aureus* and certain strains of *S. aureus* became resistant to penicillin by producing penicillinase in 1950s. In 1950s, Methicillin was introduced and used as a drug of choice to treat penicillin-resistant staphylococcal infections and first MRSA strain was reported in 1961 which was resistant to all β-lactams antibiotics including penicillin, methicillin, and cephalosporins due to altered penicillin-binding protein, PBP, which is encoded by *mec* genes [5]. Incidence of MRSA was increased in USA in 1970s and by 1990s MRSA was a worldwide problem [6]. In 1997, first case of Vancomycin Intermediate Staph. aureus was reported from Japan. By 2000s, MRSA strains became common in community-acquired cases. Many MRSA resistant phenotypes with multi resistance characteristic were reported worldwide e.g. MRSA-MLSB phenotypes i.e. MRSA were found resistant to other antibiotic classes i.e. macrolides, lincosamides, and streptogramines B [7]. Likewise, inducible clindamycin resistance was also reported in MRSA- MLSBi strains and vancomycin resistant *S.aureus* (VRSA) has been also described in 2002 which is the drug of choice to treat MRSA infections [8,9].

Pathogenesis and Virulence Mechanisms in MRSA

Nearly all MRSA strains contain the SCC*mec* (Staphylococcal chromosomal cassette) element, which is uniformly integrated into a specific *S. aureus* chromosomal site known as *orfx*. SCC*mec* carries the *mecA* gene which confers methicillin resistance,

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encodes for altered penicillin binding protein 2a (PBP2a), a cell wall transpeptidase, with decreased affinity for β-lactam antibiotics [10]. SCC*mec* elements also carry the *mecR1,* which is the regulatory element controlling mecA transcription and *mecI*, which is mecA repressor gene and they are collectively called as *mec* complex [11]. There are four complex types: A, B, C and D. Class B *mec* complex express PBP2a constitutively while class A *mec* complex express PBP2a only when induced by β-lactam antibiotics, and class D complex has been found only in coagulase-negative *Staphylococcus* species. SCC*mec* is flanked by cassette chromosome recombinase genes (*ccrA*/*ccrB*/*ccrC*) that permit mobilization of SCC and responsible for intra- and interspecies horizontal transmission of SCC*mec*. Also there is presence of *fem* gene in SCCmec complex which is the factor essential for expression of methicillin resistance. Till date, nine types of SCC*mec* (types I to VIII and VT) have been defined, which can be distinguished by the type of *ccr* gene complex and the class of *mec* complex that they bear [10,12]. The large SCC*mec* types I to III are present in HA-MRSA strains and these include sites for insertion of genes conferring non-β-lactam resistance phenotypes to *S. aureus* strains. The smaller SCC*mec* types IV and V are present in CA-MRSA which lack genes conferring non β-lactam resistance and hence these strains are less commonly MDR.

Other Virulence Determinants in MRSA

PVL (Panton-Valentine Leukocidin)

PVL has a strong epidemiological association with CA-MRSA but not with HA-MRSA [13]. The outbreaks of skin and soft-tissue infections and necrotizing pneumonia were caused by PVL-positive strains. PVL was significantly associated with community-acquired pneumonia (85% of strains) and invasive skin infections such as furunculosis (93%) and cutaneous abscess (50%). PVL is secreted as bicomponent toxins consisting of S and F proteins [14]. Depending on the combination of particular S and F proteins, a toxin is formed with varying leukocytolytic, erythrocytolytic, and dermonecrotic properties [15]. PVL forms pores in the membranes of leukocytes, causing their lysis. It causes neutrophils to release inflammatory enzymes and cytokines causing dermonecrosis. Production of PVL is increased in vitro by β-lactam antibiotics while antibiotics that inhibit protein synthesis, like clindamycin and linezolid, decrease the production of PVL, suggesting role of these antibiotic agents in the early therapy of severe CA-MRSA infections [16].

α-Toxin

It is a pore-forming leukocyte toxin that lyses macrophages and lymphocytes and alters platelet morphology thereby contributing to increased thrombotic events in *S.aureus* sepsis [17]. Antibodies produced against α-toxin were found protective against skin and soft tissue infections in CA-MRSA in animal model and may lead to new treatment options for human skin infections from MRSA [18].

PSMs (Phenol-Soluble Modulins)

These are secreted *S. aureus* peptides, which are produced in high concentration in CA-MRSA strains compared to HA-MRSA [19]. PSMs recruit, activate and lyse human neutrophils. The human formyl peptide receptor 2 (FPR2/ALX) senses PSMs at nanomolar concentrations and initiates proinflammatory neutrophil responses to CA-MRSA [20].

MRSA Superantigens

Many species of *S. aureus* are capable of producing super antigens that initiate a cytokine storm, cause serious toxinoses, including toxic shock syndrome and necrotizing pneumonia. The superantigen genes se and tst-1 were linked to SCCmec type I and type II, which may contribute to the biological fitness of MRSA [21].

Biofilms

The ability of MRSA to form biofilms is an important virulence mechanism that complicates infections involving foreign materials like catheters and prosthetic joints. Biofilms are surface-attached communities of cells encased in an extracellular polymeric matrix that are more resistant to antibiotics and also protected against the host's immune response [22]. Once biofilm forms, the easiest way to treat the infection is to remove the infected device. Biofilm formation starts with the adherence of the bacteria either directly to artificial surfaces or through host factors such as fibrinogen or fibronectin [23]. Bacteria can adhere to components of the extracellular matrix of host tissues via microbial surface components recognizing adhesive matrix molecules (MSCRAMM) family, leading to colonization. Colonization is followed by proliferation of bacteria and accumulation into a biofilm requiring intercellular adhesion which is promoted by polysaccharide intercellular adhesin (PIA). MRSA transitions between planktonic and biofilm stages occur through quorum sensing (QS), defined as a multicellular response to coordinate expression of genes required for biofilm in a population density dependent manner [24].

Risk Factors for the Acquisition of MRSA

Various risk factors contributing to increase in incidence of MRSA include:

- Frequent antimicrobial therapy or long term antibiotic use
- Direct contact with an infected or colonized individual
- Previous hospitalization and prolonged hospital stay or frequent outpatient visits
- Underlying chronic illnesses like autoimmune diseases, HIV/AIDS and cancer patients
- enteral feeding, mechanical ventilation, implantation of prosthetic devices and
- nasal carriage of MRSA
- Crowded and unhygienic living conditions such as prisons, dormitories, army barracks and child care settings.
- Injection or intravenous drug use and homosexuals [25,26,27]

Table 1: Difference between CA-MRSA and HA-MRSA [28,29,30]:

Characteristic	CA-MRSA	HA-MRSA
Clinical infections	Skin and soft tissue infections. Also cause severe infections like necrotizing pneumonia and severe sepsis	Pneumonia, urinary, bloodstream, surgical site infections
At-risk populations	Clusters and outbreaks in closed populations (prisoners, athletes, military, selected ethnic populations, intravenous drug users, men who have sex with men, soldiers)	Outbreaks health care-associated
Underlying conditions	None	Health care-associated risk factors (Diabetes, dialysis pts, prolonged hospitalization, ICU pts)
Age-group	Younger (previously healthy persons)	Older persons with other co-morbid conditions
Antimicrobial resistance	Resistant to other beta-lactam antibiotics but sensitive to non β- lactam groups	Resistant to many classes of non β- lactam antimicrobials like fluoroquinolones, aminoglycosides, macrolides, lincosamides, streptogramin etc.
Genotype resistance	SCCmec type IV, V (Staphylococcal chromosomal cassette)	SCCmec type I, II, III (smaller and more mobile)
PVL Toxin (Panton-Valentine leukocidin)	Present	Absent
Phenol soluble modulins (PSMs)	Less expression	Increased expression responsible for more severe disease

Laboratory Diagnosis of MRSA

Diagnostic microbiology laboratories and reference laboratories are key for identifying outbreaks of MRSA. New rapid techniques for the identification and characterization of MRSA have been developed however, culture takes time. Therefore, initial treatment is often based upon 'strong suspicion' by the treating physician, since any delay in treating this type of infection can have fatal consequences.

Identification of *S. Aureus*

Speciation of isolates is essential to distinguish *S. aureus* from coagulase-negative staphylococci (CoNS) on the basis of production of protein A, cell-bound clumping factor, extracellular coagulase, heat-stable nuclease and molecular methods. Tube coagulase test for the detection of extracellular coagulase is the standard test for routine identification of *S. aureus*. However, rare strains of *S. aureus* are negative in coagulase tests and some other species like S. schleiferi and S. intermedius, may also give positive results but are not common isolates from human infections. The slide agglutination test (slide coagulase test) for detection of clumping factor is very rapid but up to 15% of *S. aureus* strains are negative and these need to be confirmed with tube coagulase test. Commercial latex agglutination tests for *S. aureus* detect protein A and/or clumping factor. However, some MRSA strains produce little or no clumping factor and protein a giving false negative results. Heat-stable nuclease tests can also be used to identify *S. aureus*, although some rare coagulase-negative species can be positive. Occasional isolates of *S. aureus* give equivocal results in coagulase or other biochemical tests, and there is a need for confirmation of such isolates by molecular methods like PCR. Primers designed to amplify species-specific targets include the nuclease (nuc), coagulase (coa), protein A (spa), femA and femB, Sa442, 16S rRNA and surface-associated fibrinogen-binding protein genes [31].

Methicillin (Oxacillin) Susceptibility Testing

Disc diffusion method, broth dilution and agar dilution methods are commonly used methods for diagnosis of MRSA. Use of both resistant and susceptible control strains is required to ensure that the method is performing correctly, and also participation in an external quality assessment scheme will provide an independent assessment of performance.

i. Disc diffusion method: Cefoxitin disc is used as surrogate for detection of oxacillin resistance. 30µg cefoxitin disc is applied over lawn culture of 0.5 McFarlard inoculum on MHA plate incubated at 33- 35°C for 16-18 hrs. Zone diameter of ≤ 21 mm is considered as oxacillin resistant while ≥ 22mm as oxacillin sensitive [32].

ii. Agar screening method: Mueller Hinton agar (MHA) with 2% NaCl and 6µg/ml oxacillin concentration is inoculated with 1µl of 0.5 McFarlard inoculum incubated at 33- 35°C for 24 h is examined for growth. >1 colony or light film of growth seen under transmitted light is considered as oxacillin resistant as per CLSI 2014 [32].

iii. Broth microdilution method: Cation adjusted Mueller Hinton broth (CAMHB) with 2% NaCl for oxacillin and CAMHB for cefoxitin is inoculated with 0.5 McFarlard inoculum incubated at 33- 35°C for 24 h. MIC (Minimum Inhibitory Concentration) for oxacillin is ≤ 2µg/ml is considered sensitive and MIC≥ 4 µg/ml is considered resistant, while for cefoxitin, MIC ≤ 4µg/ml is considered sensitive and MIC≥ 8 µg/ml is considered resistant [32].

iv. Etest method: E test strips can also be used for determination of MIC levels and it is comparatively simple compared to broth microdilution method. However, E test strips are expensive [33].

v. Latex agglutination: A rapid slide latex agglutination test based on detection of PBP2a is commercially available. The method involves extraction of PBP2a from suspension of colonies and detection by agglutination with latex particles coated with monoclonal antibodies to PBP2a. The test is very sensitive and specific with *S. aureus*, however isolates producing small amounts of PBP2a may give weak agglutination reactions or agglutinate slowly [31,34].

vi. Automated methods: Vitek/Vitek2 (bioMe´rieux), Phoenix (Becton Dickinson) and Microscan (Siemens) include tests for methicillin/ oxacillin susceptibility and are generally reported to be reliable for S. aureus, although false resistance has also been reported [35].

vii. Quenching fluorescence method: In Quenching fluorescence method (Becton Dickinson), inhibition of growth of an isolate by oxacillin is indicated by the quenching of fluorescence of an oxygen-sensitive fluorescent indicator by oxygen remaining in the broth [36].

Molecular Methods

PCR-based methods for detection of mecA gene have been used routinely by reference laboratories as standard method. Presence of mecA is generally considered as a marker to identify MRSA [31]. Borderline resistance, which is not mediated by mecA, will not be detected by mecA gene. However, MRSA PCR assays are vulnerable to the presence of inhibitors, which will lead to a false-negative result, and the addition of a second set of primers like nuc, coa and gyrA genes to amplify a gene which is always present within staphylococci has been used as a control method. Real-time PCR and Quantitative PCR are increasingly being employed in clinical laboratories for the rapid detection and identification of MRSA strains. Multiplex PCR procedure targeting the femA and mecA genes has been used successfully to identify MRSA [37].

Treatment

CA-MRSA strains are susceptible to a wide variety of non–β-lactam antibiotics. Drugs like clindamycin, tetracyclines, and trimethoprim-sulfamethoxazole (TMP-SMX) have activity against CA-MRSA and can be used in treating CA-MRSA infections. In hospitalized patients with severe infection, vancomycin is the drug of choice; however isolates with intermediate susceptibility to vancomycin (VISA) and Vancomycin resistant (VRSA) strains are isolated these days. Newer agents, such as linezolid, teicoplanin, quinupristin-dalfopristin, daptomycin, tigecycline, otravancin and dalbavancin can be feasible options in VISA and VRSA cases [28,38].

Prevention

The spread of MRSA between patients can be minimised if several steps are undertaken:

- Hospital staff should wash their hands scrupulously before and after any contact with patients, using soap and water or alcohol based rubs.
- Patients colonised or infected with MRSA should be isolated from other patients and access to that room should be restricted.
- Hospital staff should wear personal protective equipment prior to having physical contact with MRSA patients. Before leaving the room, they should discard these safely, and wash their hands.
- Visitors should also wear disposable gloves and gowns when coming in contact with MRSA patients. They should wash their hands before leaving the room.
- Areas where MRSA patients are nursed should be thoroughly cleaned using disinfectants as it can survive on inanimate objects or surfaces such as linen, sinks, floors etc. for long time.
- Nasal carriers should be treated with topical mupirocin and chlorhexidine washes [39].

Vaccines

The challenge of developing an effective anti- S. aureus vaccine has been an elusive goal for researchers over many years. For CA-MRSA infections, one specific target is PVL toxin, however antibody levels against PVL in children with PVL-positive MRSA infections, was not protective against primary or recurrent CA-MRSA skin infection [40]. Peptidoglycan-based vaccine against S. aureus, A170PG, was shown to be protective in a mouse model against several strains of MRSA and the protection lasted for atleast 40 weeks. However, increase in overall mortality and multi-organ dysfunction in the vaccine recipients compared to those who received placebo was a major concern. Future vaccines targeting multiple antigens (e.g. surface proteins, toxoids and capsular polysaccharides) are under trials [29].

Conclusion

The increasing prevalence of MRSA infections in the hospitals and in the community has become a worldwide phenomenon. The wide spread dissemination of multiple - drug resistant strains complicates diagnosis and management of these patients. Increased virulence and increased risk of transmission in the hospital compounds morbidity. Appropriate antibiotic policy and strict implementation of infection control measures are essential to prevent the transmission of MRSA in hospitals. Spread of MRSA

in the community is worrisome due to the potentially large host population and it emphasises the need to control widespread or irrelevant use of antibiotics.

Conflict of Interest

There is no conflict of interest from other co-authors in the publication of this manuscript in this journal. All the co-authors have contributed in the preparation of the manuscript up to the submission stage.

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