Overview of methicillin resistant *Staphylococcus aureus* mediated bone and joint infections in India

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Abstract

Staphylococcus aureus is the most common pathogen causing bone and joint infections (BJI). In India, prevalence of Methicillin resistant *Staphylococcus aureus* (MRSA) is increasing at an alarming rate and emerged as an important contributor towards the difficult to treat BJI. Currently available anti-MRSA agents have their own limitations with regards to reduced susceptibility as well as safety and tolerability. Furthermore, biofilms over the prosthesis with invariably multi-drug resistant strains leads to complex treatment processes. This necessitates the need to develop and screen new antibiotics against MRSA that can easily penetrate the deep pockets of infection and take care of the challenges discussed. This review aims to discuss on MRSA infection in bone and joint infection, current antibiotic regimen, its associated limitations, and finally, the need to develop new antibiotic therapy for effective management of patients with BJI.

Introduction

Staphylococcus aureus (*S. aureus*) is an aerobic Gram-positive commensal known to colonize in human skin and anterior nares of about 20-25% of healthy humans and have been a cause of invasive infections where there is a breach in the immune system.[1-4] When exposed to an immunosuppressive environment, they make best use of the system to reach the nook and corner of human body like bones and joints via blood stream. An alternate point of entry has been through open fracture sites or to make situations more complex, they reside on the prosthesis forming a biofilm. The only cure to bacterial infections has been antibiotics, the wonder drug discovery of the 19th century. But, along with discovery arose an equally major concern of random use and misuse of antibiotics that promoted the bacteria to develop resistance. This increases the number of visits to hospitals, frequent change in antibiotics, and under worst conditions, lead to hospitalization. All these increase the financial burden upon patients and in a country like India, patients suffer immense loss in health due to the inability to afford for medical treatment and thus become the reservoirs of resistant bacteria. The need of the hour is continuous development of mutation-specific antibiotics that again is time consuming and expensive. But the relatively lower cost of antibiotics contrasts the manufacturing costs thus pushing big pharmaceutical companies to back out of antibiotic drug discovery research. But, despite the challenges, just like every cloud has a silver lining, 2014 witnessed approvals of four antibiotics.

Ever since the discovery of Penicillin and its derivative Methicillin, development of Methicillin resistant Staphylococcus *aureus* also called MRSA has become endemic in India.[5,6] Bacteria develop such resistance by either enzymatic inactivation of drug or increased drug efflux, preventing drug to bind by altering the binding site or horizontal transfer of genes from a resistant bacterium of any species.

Presently, in India, MRSA is no more restricted to hospitals (commonly known as hospital acquired MRSA, HA-MRSA), but they have spread widely across the community and known to be community-acquired MRSA (CA-MRSA).[7-9] Brief differentiation between HA-MRSA and CA-MRSA is given in Table 1.[10] Virulent strains reach the blood stream and find their entry into different organs via open sores and in course of time, invade bones (osteomyelitis), joints (septic arthritis), or form a biofilm over the prosthesis.[11-14] Children, elderly and patients with chronic illness, as well as patients on treatment with immunosuppressants or on cancer chemotherapy are more prone to MRSA infection. The surface of artificial implants for hips, knees, ankle, shoulder or elbows acts as a reservoir site for *S. aureus* where they are known to form a biofilm and colonize to promote the growth of highly resistant strains that are extremely difficult to eradicate by conventional antibiotics. These often lead to failure of surgery, more than one surgery in patients, and under extreme conditions patients undergo amputation of the infected organ and even cause mortality.[15]

MRSA has been a world-wide concern and recent trend of good practices in hospital has brought down the percentage of population suffering from HA-MRSA consistently. However, the CA-MRSA is on the rise and has become a big challenge in places where more people are in close contact. In either case, the cost of treatment, long term hospitalization and the psychological stress affects the health care systems and economy of every country where the prevalence of MRSA is high.

Antibiotic resistance in India

Among the Asian countries, India tops antibiotic consumption followed by China

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and Pakistan. A recent health economic research based on pharmaceutical sales data shows that between 2000 and 2015, an increasing trend by 103% in antibiotic consumption in India, more specifically towards last resort drugs were seen.[16] This leaves us with greatest issue of the 21st century, 'development of multi drug resistance (MDR)' as well as MRSA.

In India, the earliest dominant strains of MRSA were Brazilian and Hungarian epidemic clone (ST239-MRSA-III). HA and CA infections in Mumbai showed 25% *SCCmec*III, 34% *SCCmec*IV and 41% *SCCmec*V. Shambat et al have reported two new MRSA clones, ST1208 (CC8) and ST672 from Bengaluru.[17] The *SCCmec* typing of Staphylococcus strain comes from the Staphylococcal cassette chromosome *mec*. This is the mobile genetic element that encodes *mecA* gene coding for altered penicillin-binding protein called penicillin-binding protein-2a (PBP2a). Staphylococcus is known to transfer this mutant gene into another susceptible strain via horizontal transfer. PBP2a shows reduced affinity towards penicillin, cephalosporin and carbapenems, except for ceftaroline and ceftobiprole. Another homolog, *MecC* that shares 69% homology with *MecA* fails to be easily detected for diagnostic purpose due to the lack in sensitivity testing using disk diffusion method. Most of the *mecC* MRSA strains express resistance to cefoxitin but not oxacillin.[18] Thus, MRSA clones are named by combining their *SCCmec* type and the chromosomal background (I to VI) based on the recommendations of International Working Group on the Classification of Staphylococcal Cassette Chromosome Elements (IWG-SCC).

Infection of bones and joints by *S. aureus*

S. aureus is the most common bacteria in bones and joint infections. It is equally prevalent among children and adults with weak immune system. In children the bacteria predominantly reside in the hip joints whereas in adults, they are found in both the knee and hip joints. The treatments at times are complex and depend on the sensitivity profile of the *S. aureus* to antibiotics, degree of infection and extent of bone penetration. The wide spectrum of bone and joint pathologies include infection of prostheses, osteomyelitis, and septic arthritis.[19]

Osteomyelitis

Osteomyelitis is infection of the bone. The skin forms a first line of defense for interior of the body and is the home of microflora of which 20-30% includes *S. aureus*. Any kind of breach in the epithelial barrier due to deep wounds, fractures, surgeries helps the bacteria to enter the body. The other common route of entry is through blood stream where *S. aureus* must fight against the host immune system and enter the circulation giving rise to bacteremia. In course of infection, if left untreated, minor breaches in the periosteum allows the bacteria from the blood stream to reach the metaphysis in the bone of children or vertebral discs of adults. Prolonged discitis leads to infection in the vertebral bodies above and below the vertebrae and in the long bones thereby causing osteomyelitis.[20,21] Therefore, bacteremia in course of time paves the way to bone and joint infections. Another cause of osteomyelitis is diabetic foot infection that usually occurs in neuropathic patients having skin ulcers in the foot. *S. aureus* is the known cause of 80-90% of osteomyelitis and the basic treatment includes long term antibiotic treatment and in many cases debridement of necrotic bones and soft tissues. For correct treatment, an essential factor involves understanding the disease stage. Osteomyelitis being a very complex infection is classified based on the criteria established by Waldvogel and Cierny-Mader way back in 1970 and 1984, respectively.[22,23]

Septic arthritis

In contrast to osteomyelitis that involves infection of bones, target site for septic arthritis are the joints. Diagnosis of septic arthritis calls for an emergency treatment that involves surgery in majority of instances followed by 6 weeks of *intravenous* antibiotic treatment. Normal synovial joints have significant number of phagocytic cells to take care of bacterial infections; however, the ones of arthritic patients have reduced chemotactic and phagocytic activity. On the contrary, their synovial membranes possess increased adhesion molecules and *S. aureus* readily binds to fibronectin, collagen, elastin and hyaluronic acid via the adhesive factors. The bound *S. aureus* then secretes chondrocyte protease thus damaging the articular cartilage.[24] A study among children with an average age of 7.5 years from a tertiary care unit in India found that 62% cases of septic arthritis was due to *S. aureus*, the other strains included Group B *Streptococcus* and *S pneumoniae*.[25] In the US, approximately 20,000 cases of septic arthritis occur each year (7.8 cases per 100,000 person/year), with a similar incidence occurring in Europe.

Prosthetic joint infection

Bone and joint infections are also known to occur after joint replacement surgeries. The infection may appear within three to twelve months or later, post-implantation. This includes both knee and hip replacement where treatment includes prolonged hospitalization, antibiotic therapy, prosthesis removal, replacement, and space debridement.[21] While the earlier type of infections described were due to floating bacteria, the prosthetic infection post-surgeries and implants results due to growth of planktonic bacteria that form a biofilm and prevent the permeability of antibiotics like penicillin and gentamycin. These bacteria are characterized by their ability to form a layer over the bones and artificial implants and are highly resistant to the patient's immune system. The scientific community has therefore classified the planktonic bacteria in a different class because of their ability to grow very slowly in colonies and increased tendency to develop resistance against antibiotics leading to chronic infection. The requirement of antibiotics to eradicate bacteria from a biofilm is known to be 100-1000 times higher in concentration as compared with floating and acute bacterial infections. It is estimated that in the developed world, 65% of orthopedic patients are

Table 1. Comparison between HA-MRSA and CA-MRSA.

	HA-MRSA	CA-MRSA
Anti-microbial resistance	Multi drug	β-Lactam resistance
Staphylococcus Cassette chromosomes mec.	Type I, II, III	IV and V
PVL gene	Weakly expressed	Highly expressed (100%)
Population at risk	Patients in long term care facilities, with diabetes, undergoing regular dialysis, exposed ulcers, eczema and erosions	Health care personal, relatives of infected patients, those with open wounds

treated for biofilms.[26] Thus, the eradication of biofilm requires a multi-step process starting with the aim to convert planktonic bacteria to floating species. This requires disruption in their property to adhere, disturb their quorum sensing and finally develop biofilm resistant surfaces.[27]

Epidemiology of MRSA affecting the bone and joint infection in India

India is a diverse country with respect to lifestyle, weather conditions that control diseases, bacterial strains and the healthcare model. Therefore, epidemiological studies carried out at different isolated hospitals at different corners of the country have shown variation in incidences of MRSA from 12-80%.[27] A unique study carried by INSAR consortium formed by a group of microbiology laboratories from premiere medical colleges in India with the support of WHO presented 25% and 50% MRSA positive population in Western and Southern India, respectively.[28] Furthermore, with an increase in elderly population, increased number of hospitalizations, longer duration of stays in hospital and ICU, application of catheters, ventilators along with indiscriminate use of antibiotics by patients of all ages has resulted in MRSA endemic in India. Survey on the patients tested for MRSA prior to admission in hospitals showed that *S. aureus* infection stands second to *E. coli*, with a robust 74% of the cases were community acquired of which 54% were resistant to Methicillin.[29] A portion of these patients had visited hospitals earlier, undergone knee replacement or underwent MRI interventions for spine, rendering them to be carriers. A statistical analysis on samples collected from different units in hospitals showed Methicillin resistant strains higher in orthopedic units as compared with other specialties.[30] Although lack of proper documentation and variability among studies conducted at different parts of the country creates a challenge in concluding the actual burden of methicillin resistance in India, however it rings the alarm on increase in CA-MRSA.

Antibiotic resistance in bone and joint infections: an Indian perspective

Infection in bone and joints may arise due to deep external injury, stitch abscess, fractures or formation of biofilms over prosthetics and/or exposed bones. All of these require long hospitalization and treatment. Screening of such patients for antibiotic resistance at the time of admission can help in better understanding the treatment paradigm and isolating the resistant types. While the prior process is cumbersome, it gives a better picture of the proportion of CA-MRSA over HA-MRSA. A study by Patted et al. from an orthopedic ward showed 67% infected at the time of admission of which 10 out of 16 suffered from osteomyelitis. Also, 6/8 patients with open fracture and 2/8 with closed fracture that were negative for MRSA turned positive during the course of stay in hospital.[30] It is known that *S. aureus* reside in the external nares and such subjects are known to be carriers. In an interesting study to understand whether the patients treated in the orthopedic units for osteoarticular infections after invasive or surgical procedure were carriers of resistant strain, nasal swabs test was carried out to evaluate MRSA nasal colonization. This population included the elderly and 0.45% was found to be MRSA positive.[31] Different opinion exists among the medical fraternity regarding the role of nasal carriers in promoting surgical site infection. One school of thought believes that treatment with mupirocin prior to surgery reduces surgical site infection. On the contrary, some studies report no difference while another group emphasizes that re-colonization happens post-3 months of surgery. Swabs collected from the site of surgery post-procedure showed 57% positive for MRSA that was close to reports of 58% by Thool V.U. in 2012.[32] On the contrary, a far lower number of 24% was observed by Mallick and Basak (2010) from a study from Central part of India.[33] This heterogeneity in study reports evidently suggests a plausible reason to be difference in site of sample collection, type of sample, as well as type of test done to evaluate the genotype. The age group of patient population makes a great difference in inference as elderly and children are affected more by HA-MRSA while CA-MRSA is equally spread across the population. Furthermore, samples collected from restricted hospitals also bring in the regional variance but very clearly indicates the increase in CA-MRSA over HA-MRSA in India.[34] Furthermore, because of biofilm formation, it is trickier to eradicate MRSA in BJI. Lack of understanding of biofilm cultures makes it complex to evaluate its epidemiology.

Drug management

The first line of treatment includes the β-Lactams, Clindamycin and quinolones. β-Lactams were the earliest group of antibiotics that possessed slow acting property when present in the body at a concentration above the minimum inhibitory concentration (MIC). They acted by inhibiting bacterial cell wall synthesis. Penicillin with a β-lactam ring was the first-generation antibiotic that faced antimicrobial resistance and was soon replaced by Methicillin and other isoxazoyl penicillins like oxacillin and flucloxacillin. *S. aureus* that is sensitive to any of the Methicillin derivative are known as Methicillin Sensitive *S. aureus*.[35] But most *S. aureus* infections have been registered to develop resistance to Methicillin or its derivatives by low binding affinity of Penicillin Binding Protein 2a (PBP2a) encoded by *MecA* and *MecC* genes.

The quinolones are another class of drugs that target DNA Gyrase and Topoisomerase IV and inhibit DNA replication. These include Nalidixic acid (1st generation), Ciprofloxacin (2nd generation), Levofloxacin (3rd generation) and Trovafloxacin (4th generation). While Nalidixic acid targeted gyrase to inhibit DNA replication, Norfloxacin blocked DNA replication by acting upon Topoisomerase IV. Ciprofloxacin targets both the enzymes to inhibit bacterial DNA replication. But Gram-positive bacteria including *S. aureus* developed mutations to gyrase and topoisomerase IV thus weakening the quinolone-enzyme interaction.[36]

Besides Quinolones, the third type of antibiotic that acts by inhibiting bacterial protein synthesis is Clindamycin, a synthetic analog of Lincomycin that shows a MIC of 1.5 mg/mL against almost all *S. aureus*.[37] The rate of resistance against Clindamycin varies between different countries. In India, a study from 2010 reported 9% cases of constitutive resistance and 10% inducible resistance among Methicillin-sensitive *S. aureus* isolates.[38]

Present treatment approaches for MRSA in bone and joint infections

The glyco-peptide, Vancomycin is the next line of treatment in cases of MRSA that acts by inhibiting the bacterial cell wall synthesis. The MIC of Vancomycin was initially proposed to be ranging between 0.5-2 mg/L but slowly in course of time the MIC for Vancomycin on *S. aureus* have been raised to 8-16 mg/L and classified as Vancomycin-intermediate *S. aureus* (VISA) Recent study has shown, vancomycin MIC to be >32 mg/l and ths strain is thus referred as Vancomycin resistant *S. aureus* (VRSA).[39] Besides Vancomycin, Daptomycin (a lipopeptide), and linezolid (an oxazolidinone), have been approved for the treatment of serious infections caused by MRSA but resistance and other limita-

tions has been reported against them too, thus creating an uncertain future for antibiotics (Table 2).[40] Among the tetracyclines, a new glycylcycline, named Tigecycline is administered for MRSA related to abdominal infection, but is not used to treat bone and joint infections and also known to be highly protein bound, thus, reducing serum levels.[41]

MRSA and economic burden

Infection of bone and joints by *S. aureus*, including MRSA has created considerable burden on health care system and on the Indian economy. Late and incomplete diagnosis of MRSA leads to prolonged hospital stay for infected patients, thus increasing hospital cost along with increased risk of mortality. The situation becomes more complex with the knowledge of HA-MRSA and CA-MRSA. While the latter is not a multi-drug resistant strain, it has become more virulent and the infection is no more restricted to hospital patients but both health care providers and household contacts are equally at risk.

Challenges in treatment for MRSA and way forward

S. aureus has been the most common cause of bone and joint infection all over the world and an endemic in India. Besides India, the other Southeast Asian countries with higher cases of MRSA are Sri Lanka, Taiwan, Philippines, Vietnam and Korea.[21] With an easy availability of over-the-counter (OTC) antibiotics, and irrational prescription of antibiotics for upper respiratory tract infections and diarrhea in India, has led to the spread of resistance followed by China. Therefore, the first challenge is to reduce the rate of inappropriate antibiotic usage and completion of antibiotic dosing with frequent screening to test for complete eradication of infection from the body. This otherwise when left incomplete has been the prominent reason behind development of resistance. Therefore, the strict implementation of schedule of Drugs & Cosmetics Act is worth appreciable.

The second challenge in the treatment is time taken to categorize patients based on their type of infection. This requires a culture of sample for 48-72 h and the chances of positive tests being 50%. This is because compared to CA-MRSA, HA-MRSA has a very slow duplication time and to make matters more complex, culture of bacteria from biofilms is far more complex. It is therefore recommended to evaluate MRSA based on serum parameters for inflammation markers. This can be valid for older children and adults, but in children <1 years of age with immature immune system and lack of good serum profile for biomarkers are highly susceptible to MRSA. Studies have been reported using 4 to 7 parameter criteria for patients to diagnose for primary treatment and therefore, observed 94% positive prediction with 7 conditions - hemoglobin <9.5 gm/dL, PCV <34, CRP >32, ANC >65, ESR>35, WBC >14000/mm^3 and temperature >100.4°F.[42] CRP levels have been good predictors in segregating MRSA from MSSA. As rightly pointed by Rahul et al. in 2015, need of the hour is to develop a database of the predictive markers thereby generating a predictive algorithm for acute MRSA by categorizing the different age groups. This would form the basis of diagnosis for an efficacious treatment strategy.[43]

The third challenge is the restricted treatment options during life-threatening situations. Under such conditions, Vancomycin is the first line of therapy and pharmacodynamic studies indicate the clinically effective AUC/MIC ratio to be 400 that requires dosing of Vancomycin at 3-4 gm per day, thus raising concern for nephrotoxicity. Therefore, Vancomycin cannot be the ideal mode of treatment in bone and joint infections. But, Daptomycin that comes up as the next alternative has its own disadvantages because of the development of cross resistance between daptomycin and vancomycin.[44]

The only other option, therefore, is to increase the pace of new drug discovery research with the aim to synthesize efficacious new molecules against novel targets. Despite low returns, big pharmaceuticals should religiously concentrate on antibiotic drugs.

Treatment of the future

From 1968 to 2003, Linezolid (2000) and Daptomycin (2003) were the only two new class of systemic anti-MRSA agents developed. This is because of pharmaceutical companies staying away from the antibiotic research due to low profit motivations and difficulty in conducting clinical trials against drug resistant strains. Later, due to the regulatory agencies providing incentives for the development of antibacterial agents, research initiatives re-emerged. One important breakthrough and the first one from India has been a novel anti-infective, which is the arginine salt of levonadifloxacin, and its pro-drug developed by Wockhardt Limited (Mumbai, India) that received Qualified Infectious Disease Product (QIDP) status from USFDA for both the chemical entities.[45] These new anti-infective agents are new benzoquinolone subclass of quinolones that have a broader anti-microbial spectrum with improved pharmacokinetic properties. WCK 771 (IV) and WCK 2349 (Oral) inhibits NorA efflux pumps in *S. aureus* and are able to overcome mutations in both DNA gyrase and Topoisomerase IV. They are highly effective against MRSA, CA-MRSA, VISA and VRSA.[46,47] WCK 771 when dosed at 800 mg intravenously and its pro-drug (WCK 2349) dosed at 1000mg orally in clinical trials attained a half-life of 6 h, a concentration that when dosed BID systemically has been effective to treat MRSA infections.[48,49]

Table 2. Limitations of commonly used anti Methicillin resistant *Staphylococcus aureus* (MRSA) agents.

Anti MRSA agents	Type	Limitation
Vancomycin	Glycopeptide	A progressive increase in minimum inhibitory concentration (MIC) Variability in tissue penetration due to its strong protein binding property In order to achieve clinical effectiveness, (AUC/MIC ratio of 400), maintaining high trough levels is nephrotoxic
Daptomycin	Lipopeptide	Ineffective in patients suffering from pneumonia because of being rendered inactive when coming in contact with pulmonary surfactant Prone to decrease in susceptibility with increase in MIC of Vancomycin
Tigecycline	Glycylcycline	Increased risk of mortality Ineffective in HA-MRSA or patients with pneumonia Low serum levels due to high protein binding
Linezolid	Oxazolidinone	Causes bone marrow suppression, lactic acidosis and peripheral and optic neuropathy

Conclusions

MRSA is a clinically important pathogen, playing a major role in bone and joint infections. The rising prevalence of MRSA is directly associated with the increasing morbidity and mortality rates in India. This can be partly attributed to the increasing resistance of MRSA to multiple antibiotics and formation of biofilms which is one of its virulence factors. Furthermore, BJI due to MRSA requires antibiotics for longer duration which cannot be instituted safely with the current anti-MRSA agents due to the adverse events pertaining to safety and tolerability of these agents. The lack of novel antibiotic therapies in the past decade has increased the need for newer antibiotic regimens in order to treat drug resistant infections in BJI patients. Therefore, novel drug therapies like WCK771/2349 that can escape the existing resistant mechanisms like Nor A efflux pump, DNA Gyrase / Topo IV mutations and biofilm production can help the physicians to overcome the challenges of treating BJI.

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