INTRODUCTION - *Staphylococcus aureus* is one of the most frequent bacterial pathogens in humans.¹ *Staphylococcus aureus* is a major cause of hospital- and community-acquired infections worldwide.¹ It has an intrinsic arsenal of virulence determinants that contribute to disease, including a suite of toxins that facilitate tissue destruction and a number of adhesins and surface proteins that mediate tissue adherence and colonization.² It causes skin infections, osteoarthritis and respiratory tract infections in the community, and moreover, postoperative and catheter-related infections in hospitals.¹ Methicillin was introduced in 1959 to treat infections caused by penicillin-resistant *Staphylococcus aureus*. There were reports from the United Kingdom of *S. aureus* isolates that had acquired resistance to methicillin (methicillin-resistant *S. aureus*),³ and MRSA isolates were soon recovered from other European countries, and later from Japan, Australia, and the United States.² MRSA is now a problem in hospitals worldwide and is increasingly recovered from nursing homes and the community.⁴,⁵ Methicillin resistant *Staphylococcus aureus* (MRSA) are often resistant to multiple classes of antibiotics. In hospitals, transmission occurs from a colonized or an infected individual to others, mainly via the hands of transiently-colonized healthcare workers.⁶ MRSA has been associated with many infection sites including bones and joints, lungs, and the urinary tract.⁷ Bacteremia is common, possibly leading to endocarditis and osteomyelitis.⁷ Hospital-acquired MRSA is typically resistant to classes of antimicrobials other than β-lactams.⁸ Methicillin resistant *S. aureus* (MRSA) has become a major public health problem worldwide.⁹ The rising colonization rates lead to the increasing of infection rates in the community and in hospitals.⁹ The consequence to the health care system is longer hospital stays and greater costs, which approximately double the expenditure per patient.¹⁰ The patient risks include significantly higher mortality and morbidity
rates with invasive MRSA infection.\textsuperscript{11,12} Within U.S. hospitals, nearly 60\% of nosocomial \textit{S. aureus} infections acquired in intensive care units are methicillin resistant.\textsuperscript{13} Health care workers may carry MRSA on their hands or clothes following their contact either with to asymptomatic carriers or patients who have clinical infection.\textsuperscript{13} Health workers may then, unknowingly transmit the organism to other patients. The contaminated environmental surfaces also contribute to the MRSA transmission.\textsuperscript{13} Thus; symptomatic patients constitute a small portion of the actual reservoir of MRSA within hospitals resulting in an iceberg phenomenon.\textsuperscript{14} The world wide emergence of community acquired methicillin resistant \textit{S. aureus} (CA-MRSA) can have severe public health implications.\textsuperscript{15} The differentiation between community-acquired MRSA and hospital acquired MRSA (HA-MRSA) is becoming difficult to understand, since CA-MRSA could spread into hospitals.\textsuperscript{16} The risk of the acquiring MRSA in the hospitals increased by severity of illness,\textsuperscript{17} length of stay,\textsuperscript{18} use of intravascular devices\textsuperscript{19} and the intensity of exposure to infected patients.\textsuperscript{20} Infection control measures include screening,\textsuperscript{21,22} and segregation of positive patients,\textsuperscript{23} eradication of carriage\textsuperscript{24} and good standards of general hygiene.\textsuperscript{25} During the last 45 years, various hospital-associated methicillin resistant \textit{S. aureus} (HA-MRSA) clones disseminated worldwide.\textsuperscript{24} In addition, since the 1990s, virulent community-associated MRSA (CA-MRSA) clones, characterized by the presence of the toxin Panton-Valentine leukocidin (PVL), spread worldwide, first in the community, but later on also in healthcare facilities.\textsuperscript{26} In general CA-MRSA is more virulent compared to HA-MRSA due to presence of various virulence factors.\textsuperscript{26} The first report of CA-MRSA came in 1993 from Western Australia, and described the observation of CA-MRSA in Aboriginal patients in remote communities.\textsuperscript{26} Many MRSA isolates are multiply resistant and are susceptible only to glycopeptide antibiotics such as vancomycin and investigational drugs.\textsuperscript{26} MRSA isolates that have decreased susceptibility to
glycopeptides (glycopeptide intermediately susceptible *S. aureus*, GISA), reported in recent years, are a cause of great public health concern. Many studies have characterized MRSA isolates from individual hospitals or countries and have identified strains that appear to be well adapted to the hospital environment, are established in several hospitals within a country, or have spread internationally (epidemic MRSA, EMRSA). ^28_

Methicillin resistance first appeared among nosocomial isolates of *S. aureus* in 1961. ^29_ The incidence of methicillin resistance of *S. aureus* (MRSA) in India ranges from 30-70%. ^30_ MRSA are implicated in serious infections and nosocomial outbreak. These strains shows resistance to wide range of antibiotics, thus limiting the treatment options to very few agents such as vencomycin and teicoplanin. ^30_ The mechanism of methicillin resistance in heterogeneous and homogeneous population of MRSA is diverse. ^30_ Primarily these include the production of a low affinity penicillin binding protein (PBP) which is an altered form of penicillin Binding Protein required for bacterial cell wall synthesis termed PBP2a in addition to usual PBPs and the production of β-Lactamase. ^30_ The gene encoding the altered PBP is mecA. ^30_ It is clinically crucial to determine rapidly whether clinical isolates are methicillin resistant of not, as this is a paramount importance for both treatment and control. ^30_ Diagnostic methods used to detect MRSA in clinical samples should be highly sensitive and specific and most important the result should be available within a short time. Accurate detection of methicillin resistance can be difficult due to presence of two subpopulations, one susceptible and the other resistant that may coexist within the culture of staphylococci. ^30_ All cells in culture may carry the genetic information for resistance but only a small number may express resistance in vitro. ^31_ This phenomenon is termed heteroresistance for distinction with homogenous resistance which is easily detectable, variations of growth condition influences PBP2a production. ^31_ Classic disk diffusion methods with oxacillin disk or automated system allow only detection of high level PBP2a producing strains. ^31_ Some strains show a heterogeneous resistance and more often strains producing low level of PBP2a are reported, these classes of strains escape classic detection and
are misidentified as methicillin sensitive.\textsuperscript{31} The clinical consequences may be fatal.\textsuperscript{29} Actually several phenotypic methods have been proposed to detect heterogeneous and low level resistance and to discriminate borderline resistance. Borderline resistance to oxacillin is attributed to the hyper production of normal staphylococcal β-lactamase but borderline strains remain sensitive to oxacillin.\textsuperscript{31} Japanese studies reported that cefoxitin induced greater production of PBP2a that oxacillin. MRSA contain one resistance island called SCC\textit{mec}, where SCC stands for staphylococcal cassette chromosome and mec for genetic element conferring resistance to methicillin. SCC\textit{mec}, is an exogenous piece of DNA that may vary between 15-60 kb and is absent from methicillin susceptible staphylococci.\textsuperscript{31} Its boundaries are demarcated by direct and inverted repeats, which allow integration at a homologous site into the site into the chromosome. The SCC\textit{mec} critical genes are the recombinases \textit{ccrA} and \textit{ccrB}, which can mediate mobilization of the whole elements and the \textit{mecA} gene which mediates β- lactam resistance.\textsuperscript{31} The rest of SCC\textit{mec} contains various additional determinants and each referred to as “J” for junkyard.\textsuperscript{32} \textit{MecA} encodes a particular penicillin-binding protein called PBP2A, which has a very low affinity of methicillin and most other β-lactam drugs.\textsuperscript{32} Hence, PBP2A is responsible for the intrinsic resistance of MRSA to almost all β-lactams.\textsuperscript{32}

The \textit{mecA} gene is regulated by the repressor \textit{MecI} and the transmembrane β-lactam sensing signal transducer \textit{MecR1}, which are both divergently transcribed.\textsuperscript{32} \textit{MecI} repressed both the transcription of \textit{mecA} and \textit{mecR1-mecI} in the absence of β-lactam antibiotic.\textsuperscript{32} However, in the presence of a β-lactam antibiotic, \textit{MecR1} is autocatalytically cleaved and the metalloprotease domain, which is located in the cytoplasmic part of \textit{MecR1}, becomes active.\textsuperscript{26} This metalloprotease cleaves \textit{MecI}, which, in turn, is bound to the \textit{mecA} operator region, allowing the transcription of \textit{mecA} and the subsequent production of PBP2a to occur.\textsuperscript{26}
Methicillin resistance among nosocomial isolates of S. aureus in India ranges from 30-70%.

It is clinically crucial to determine rapidly whether clinical isolates are methicillin resistant or not, as this is of paramount importance for both treatment and control. A few options are available for the treatment of methicillin resistant (MRSA) staphylococcal infections, such as macrolides, lincosamides and streptogramin B (MLSB) with clindamycin being one of the good alternatives, particularly for skin and soft tissue infections and work as an alternative in penicillin allergic patients. There are two primary mechanisms provides resistance to macrolide antibiotics. Among S. aureus the gene msr A encodes efflux pump which is a primary mechanism of defense and quite common in some geographical areas. The second mechanism includes modification of drug binding sites on the ribosomes that also enhances resistance to macrolides. These two mechanisms promote resistance to macrolides, lincosamides and streptogramins B group of antibiotics and termed as MLSB resistance. An erm gene usually erm A or erm C encodes methylation of 23S rRNA- binding which is shared commonly by these three drug classes. Development of drug resistance in S. aureus has led to the use of older antibiotics such as macrolide, lincosamide, and streptogramin B (MLSB) antibiotic. However, extensive use of these antibiotics in serious staphylococcal infections has caused the emergence of S. aureus resistant to MLSB antibiotics. There are three different mechanisms of resistance to MLSB antibiotics including: (1) Active efflux mechanism encoded by msr gene, (2) drug inactivation encoded by lun gene and (3) ribosomal binding site modification (by methylation or mutation in the 23s rRNA gene) encoded by erm genes (ermA, ermB, ermC, and ermF) among which, ermA and ermC are predominant genes responsible for resistance to MLSB antibiotics in staphylococci, which can be constitutive or inducible.

Prevalence rate of Methicillin resistant S. aureus (MRSA) has dramatically increased in recent years as it varies with geographical location and bacterial species. For MRSA infection, vancomycin considered
as drug of choice, however vancomycin usage is associated with considerable side effects and cost as well as overuse of vancomycin has led to the emergence of resistant strains with reduced susceptibility. In the past decades MRSA, emerged as prevalent pathogen for community acquired infections (CA-MRSA), unlike hospital acquired MRSA, the CA-MRSA are sensitive to drugs other than vancomycin, such as, ciprofloxacin, trimethoprim sulphamethoxazole and clindamycin (CD). The increasing frequency of MRSA infections and rapidly changing patterns in antimicrobial resistance, led to renewed interest in the use of macrolide lincosamide – streptogramin B (MLSB) antibiotics to treat such infections.

### Justification of discrepancies

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<tr>
<th>Phenotypically MRSA but mecA(^-)</th>
<th>Phenotypically MSSA mecA(^+)</th>
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<tr>
<td>Hyperproduction of (\beta)-lactamase, production of normal PBP with altered binding capacity, and /or other as yet unidentified factors(^144)</td>
<td>Over-expression of (mecR1) and (mecl) genes which are co-repressors of mecA genes(^144)</td>
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The regular surveillance of hospital associated infections including monitoring antibiotic sensitivity pattern of MRSA and formulation of definite antibiotic policy may be helpful for reducing the incidence of MRSA infection.
Prevention & control measures include:

Eradication of MRSA carriage (decolonization) by the -:

- Use of topical nasal ointment (Topical nasal mupirocin; 2% in paraffin base) and body wash/shampoo, (Topical 4% chlorhexidine bodywash/ shampoo or 7.5% povidone iodine is equally efficacious for decolonization of non-nasal sites.)
- Use of systemic antibiotics to clear persistent carriage, for example persistent throat carriage.
- Proper hand hygiene is the single most important, simplest, and least expensive means of reducing the prevalence of health care associated infection and the spread of antimicrobial resistance.

Significance of Study - Increasing prevalence of Methicillin-resistant Staphylococcus aureus (MRSA) worldwide is a growing public health concern.

- MRSA typing is an essential component of an effective surveillance system, infection control and treatment strategies.
- The phenotypic methods in general are easier to perform, easier to interpret, cost effective and are widely available, however less discriminatory.
- The genotyping methods are expensive and technically demanding, however less discriminatory.