REVIEW OF LITERATURE -

Historical perspective

*Staphylococci* were first observed in human pyogenic lesions by von Recklinghausen in 1871. Pasteur (1880) obtained liquid cultures of the cocci from pus and produced abscesses by inoculating them into rabbits. Rosenbach provided the first formal description of the genus *Staphylococcus* and divided the genus into two species, *Staphylococcus aureus* and *Staphylococcus albus*. Zopf placed the staphylococci and group of saprophytic, tetrad-forming micrococci back into the genus *Micrococcus* and Flugge rearranged the cocci and separated the genus *Staphylococcus* from the genus of *Micrococcus*. Evans et al. (1955) proposed separating staphylococci from micrococci on the basis of their relation to oxygen using a standard oxidation-fermentation test for glucose fermentation. The facultative anaerobic cocci were placed in the genus *Staphylococcus* and obligate aerobes were placed in the genus *Micrococcus*. Early medical microbiologist placed major emphasis on distinguishing the pathogenic species *S. aureus* from presumed commensal staphylococci, referred to as *S. albus*, *S. epidermidis*. *S. aureus* is major cause of morbidity and mortality, however clinical specimen often found to carry both types of organisms. Meyer (1967) and Hajek (1971) divided the species *S. aureus* into several biotypes on the basis of physiological, biochemical and phage typing properties and host preferences.

Morphology and cultural characteristic

*S. aureus* are spherical cocci, approximately 1µm in diameter arranged characteristically in grapes like clusters (FIGURE: 1). Cluster formation is due to cell division occurring in three
planes, with daughter cells tending to remain in close proximity. They may also be found singly, in pairs and in short chains of three or four cells, especially when examined from liquid culture.

A.  

B.  

FIGURE: 1. (A). Gram positive cocci arranged in cluster  

(B). Electronmicrograph of *Staphylococcus aureus*.

They are nonsporing, nonmotile and usually non capsulate with the exception of rare strains. *S. aureus* are aerobes and facultative anaerobes, optimum temperature for growth is 37°C (range 12°C - 44°C), pH 7.5, can grow on ordinary medium. On nutrient agar colonies are 1-3 mm in diameter, have smooth, glistening surface, entire edge with soft butyrous consistency and show pigmented appearance. Most strain shows golden–yellow pigment, though some strains may form white nonpigmented colonies. They show zone of β-hemolysis on blood agar (if sheep, human or rabbit blood is used instead of horse blood). Colonies are smaller and pink in colour due to lactose fermentation in MacConkey agar.  

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Biochemical characters

*S. aureus* ferments a range of sugars, including manitol. On manitol salt agar it forms 1mm diameter yellow colonies surrounded by yellow medium due to acid formation and are tolerant to sodium chloride concentrations (7-10%) that inhibit most other bacteria. The tube coagulase and clumping factor (bound coagulase) test, catalase, acetoin production (Voges-Proskauer), and gelatinase are all typically positive. Most strains of *S. aureus* have bound coagulase or ‘clumping factor’ react directly with fibrinogen in plasma, causing rapid cell agglutination. This test can be performed from blood agar or any non selective medium. Coagulase test is the standard criterion for the identification of *S. aureus* isolates. The tube coagulase test detects free coagulase which is secreted extracellularly and reacts with a substance in plasma called coagulase reacting factor (CRF) to form a complex which in turn, reacts with fibrinogen to form fibrin (clot formation). All stains of *S. aureus* produce phosphatase which liberates phenolphthalein from sodium phenolphthalein diphosphate which can be detected in an indicator medium Phenolphthalein phosphate agar, where colonies will turn pink. *Staphylococcus* shows resistant to bacitracin (0.04-U) and susceptible to furazolidone (100μg) impregnated disk placed on the surface of a 5% sheep blood agar plate that has been previously streaked in three directions with a cotton-tipped swab that has been dipped in a bacterial suspension prepared to match the turbidity of the 0.5 McFarland standard.

Habitat

*Staphylococci* are one of the major groups of bacteria inhibiting the skin and mucous membrane. The common resident staphylococcal species on the human skin in terms of population size is *S. epidermidis*. The nose provides a major habitat for *S. epidermidis* and is
the dominant ecological niche for \textit{S. aureus}. The host may also modulate carriage through immune regulation. Unlike other bacterial species that colonize nasopharyngeal tissue, \textit{S. aureus} is predominantly localized to the anterior nares. The epithelium in this region is stratified, keratinized, nonciliated epithelium, contrasting with rest of the nasal cavity and respiratory tract which is lined with ciliated columnar epithelium. Three pattern of carriage can be distinguished: (1) persistent carriers, (2) intermittent carriers, and (3) noncarriers. Approximately 20% of healthy people are persistent carriers, 60% are intermittent carriers and 20% are noncarriers. Most infants become colonized shortly after birth, but carriage decreases with age.

**Staphylococcal diseases**

Staphylococcal infections are among the most common of bacterial infections and range from the trivial to the fatal. Staphylococcal infections are characteristically localized pyogenic lesions, in contrast to spreading nature of streptococcal infections. \textit{S. aureus} causes disease through the direct invasion and destruction of tissue or through production of toxin. The common pyogenic staphylococcal infections are skin and soft tissue, musculoskeletal, respiratory, central nervous system endovascular, urinary tract infections. The common toxin-mediated staphylococcal diseases are food poisoning, toxic shock syndrome and scalded skin syndrome. Staphylococci have become one of the most common causes of nosocomial infections. Multidrug-resistant staphylococci pose a growing problem for human health. The rise of drug-resistant virulent strains of \textit{Staphylococcus aureus}, particularly methicillin-resistant \textit{S. aureus} (MRSA) is a serious problem in the treatment and control of staphylococcal infections. Methicillin-resistant staphylococci (MRSA) cause hard-to-treat infections because these are resistant to most of the antibiotics such as \(\beta\)-lactams, aminoglycosides, and macrolides. The most important mechanism of resistance to penicillin is production of \(\beta\)-lactamase which inactivates penicillin by
hydrolysis of its β-lactam ring. Another mechanism is associated with penicillin-binding protein 2a (PBP2a), encoded by mecA. Another gene involved in penicillin resistance in staphylococci is blaZ which encodes β-lactamase. β-lactam antibiotics inhibit bacteria by binding covalently to PBPs in cytoplasmic membrane. These target proteins catalyze the synthesis of the peptidoglycan that forms cell wall of bacteria. Alteration of PBPs can lead to β-lactam antibiotic resistance. The resistance to β-lactam antibiotics may be associated either with a decrease in the affinity of the PBP for an antibiotic or with a change in the amount of PBP produced by the bacterium. Multiple mechanisms seem to be present in some of the clinical isolates. The induction of low-affinity PBP in methicillin-resistant S. aureus occurs to a larger extent when the microorganisms are grown at 32°C rather than 37°C, conditions known to favor the expression of methicillin resistance. The structural gene (meca) that determines the low affinity PBP of methicillin-resistant S. aureus shares extensive sequence homology with a PBP of E. coli, and the genes that regulate the production of low-affinity PBP have considerable sequence homology with the genes that regulate the production of staphylococcal penicillinase. The production of this low affinity PBP in methicillin-resistant S. aureus may be mediated by a fusion of gene segments from E.coli and S. aureus. Methicillin-resistant isolates with alterations to existing PBPs have been described. These isolates have been termed ‘moderately resistant S. aureus’ (MODSA). They are not frequently reported, the resistance is low-level and their clinical significance is unclear. Under some test conditions, low-level resistance may also be seen in isolates which produce large amounts of penicillinase (penicillinase hyper-producers). These isolates have been referred to as ‘borderline oxacillin resistant S. aureus’ (BORSAs). Erythromycin (a macrolide, ERY) and clindamycin (a lincosamide, CLI) represent two
distinct classes of antimicrobial agents that act by binding to the 50s ribosomal subunit of bacteria to inhibit its protein synthesis.\textsuperscript{59} Macrolide resistance in Staphylococcus aureus is by diverse mechanisms.\textsuperscript{59} The resistance to macrolide can arise by efflux mechanism, classically mediated by msr A gene.\textsuperscript{59} Another mechanism is via erm gene, which encodes enzymes that confer inducible or constitutive resistance to macrolide, lincosamide and Type B streptogramin (MLS\textsubscript{B} resistance).\textsuperscript{60} This resistance mechanism can be constitutive, where rRNA methylase is always produced (cMLS\textsubscript{B} ) or can be inducible where methylase is produced only in the presence of an inducing agent (iMLS\textsubscript{B} ).\textsuperscript{60} ERY is an effective inducer whereas CLI is a weak inducer.\textsuperscript{60} In vitro Staphylococcus aureus isolates with constitutive resistance are resistant to both ERY and CLI whereas those with inducible resistance are resistant to ERY and appear sensitive to CLI (iMLS\textsubscript{B} ).\textsuperscript{61} If clindamycin is used for treatment of such an isolate (iMLS\textsubscript{B} ), selection for constitutive erm mutants occurs which may lead to clinical failure.\textsuperscript{61} This inducible MLS\textsubscript{B} resistance can be detected by a simple disc approximation test, commonly referred to as D-test. For this test, an ERY (15\textmu g) disc is placed 15-26 mm (edge to edge) from a CLI (2 \textmu g) disc in a standard disc diffusion test.\textsuperscript{61} Following incubation, a flattening of the zone in the area between the discs where both drug have diffused indicates that the organism has inducible clindamycin resistance.\textsuperscript{61} Increasing prevalence of Methicillin–resistant Staphylococcus aureus (MRSA) worldwide is a growing public health concern.\textsuperscript{62} MRSA typing is an essential component of an effective
surveillance system to describe epidemiological trends and infection control strategies.\textsuperscript{62}

Current challenges for MRSA typing are focused on selecting the most appropriate technique in terms of efficiency, reliability, ease of performance and cost involved.\textsuperscript{62} The phenotypic methods in general are easier to perform, easier to interpret, cost effective and are widely available, however less discriminatory.\textsuperscript{62} The genotyping methods are expensive and technically demanding, however less discriminatory.\textsuperscript{63} The infections caused by MRSA are serious and are difficult to treat.\textsuperscript{63} Only a few antimicrobial agents are available for treatment of such infections and none of these possesses ideal characteristics.\textsuperscript{63} The reports from India suggest increasing incidence of MRSA.\textsuperscript{64,65} Hence accurate and rapid identification of MRSA in a clinical specimen is essential for timely decisions on isolation procedures and effective antimicrobial therapy.\textsuperscript{65}

Expression of methicillin resistance in the clinical laboratory setting is depends on following factors:

- Environmental conditions i.e., temperature, pH, incubation time, and salt concentration in the medium. Conditional expression of PBP2a antigen may cause ambiguity in susceptibility tests.\textsuperscript{65}

- Methicillin resistance is often expressed heterogeneously, in that only 1 in $10^4$ to $10^7$ cells of the population is phenotypically resistant.\textsuperscript{65}
These factors emphasize the need to develop a rapid, standardized, accurate and sensitive method for detection of methicillin resistance in staphylococci which is not dependent on growth conditions. The conventional methods to detect MRSA in the laboratory include oxacillin agar screen, disk diffusion using one microgram oxacillin disk as well as oxacillin MIC by agar or broth dilution methods. Numerous approaches that improve the turnaround time for the detection of MRSA have been described. Polymerase chain reaction (PCR) is considered the gold standard. It is rapid with a high degree of sensitivity and specificity, but is expensive. A combination of primers for genes responsible for coagulase activity (femB) and methicillin resistance (mecA) would identify all the isolates correctly and can be used to perform a single step multiplex PCR. MRSA strains produce toxins including toxic shock syndrome toxin (TSST), enterotoxins and exfoliative toxins. Gene encoding for enterotoxins are carried on Staphylococcal pathogenicity islands. Other toxin genes like gene for Panton Valentine Leucocidin (PVL) are carried on bacteriophages and are easily transferred between lineages. Thus toxin gene profile of the strains can be used as an important epidemiological marker for typing of MRSA strains. Studies have been shown that MRSA strains possess more toxin genes as compared to MSSA strains. Some studies have shown that certain clinical MRSA strains may produce more enterotoxin A and B as well as coagulase, compared with MSSA but these findings is not consistant. Mixed result have also been observed for binding to extracellular matrix proteins and similar adhesion properties to human epithelial cells have been observed. Of the various detection methods available, multiplex PCR technique is recommended for detection of toxins in MRSA. It is rapid, reproducible relatively inexpensive, easier to interpret and provides a high degree of discrimination. The technique is useful for studying the chromosomal diversity and evolutionary history of MRSA strains. Since the emergence of
MRSA, a large number of observational studies support the idea that patients infected with MRSA have a higher mortality than do those infected with MSSA.\textsuperscript{70} There are many potential factors in the pathway between methicillin resistance and mortality.\textsuperscript{70}

(FIGURE: 2).

\textbf{FIGURE: 2. Factors that may contribute to the relationship between infection with MRSA and increased mortality}\textsuperscript{70}

The resistance of \textit{S. aureus} to methicillin is caused by the presence of the \textit{mecA} gene, which encodes the 78-kDa penicillin-binding protein (PBP) 2a (or PBP2\textsuperscript{*}).\textsuperscript{70} \textit{β}-Lactam antibiotics normally bind to PBPs in the cell wall, resulting in the disruption of synthesis of the peptidoglycan layer and death of the bacterium. Since \textit{β}-lactam antibiotics cannot bind to PBP2a, synthesis of the Peptidoglycan layer and cell wall synthesis are able to continue.\textsuperscript{70} PBP2a is
responsible for the intrinsic resistance of MRSA to all β-lactams. The mecA gene is preceded or not by the mecR1 and mecI regulatory determinants, which are homologues of the blaR1 and blaI regulators of penicillinase (bla) genes. mecR1 (and blaR1) encodes a membrane receptor, and mecI (blaI) encodes a gene repressor.\textsuperscript{32} In presence of penicillin, the extracellular portion of membrane mecR1 (blaR1) receptor triggers an autolytic cleavage of its intracytoplasmic portion.\textsuperscript{32} The mecA gene is bracketed by one or two copies of IS431, which are believed to serve as a gene collector and may promote the local insertion of additional determinants, such as antibiotic resistance gene.\textsuperscript{32} The mecA gene is regulated by the repressor MecI and the transmembrane β-lactam-sensing signal transducer MecR1, both of which are transcribed divergently.\textsuperscript{32} However, in the absence of a β-lactam antibiotic, MecI represses the transcription of both mecA and mecR1–mecI. In the presence of a β-lactam antibiotic, MecR1 is cleaved autocatalytically, and a metallo-protease domain, which is located in the cytoplasmic part of MecRI, becomes active.\textsuperscript{32} The metallo-protease cleaves MecI bound to the operator region of mecA, which allows transcription of mecA and subsequent production of PBP2a.\textsuperscript{71} Both mecI and mecR1 can be truncated by insertion sequences IS431 or IS1272, and this result in derepression of the mecA gene.\textsuperscript{72} Transcription of mecA, the gene mediating oxacillin resistance in staphylococci, is regulated by a repressor, mecI that is divergently transcribed from mecA as the second gene in a two-gene operon that also includes mecR1.\textsuperscript{71} More than 90% of staphylococcal isolates also produce β-lactamase, the product of blaZ, and contain blaZ regulatory sequences (blaI and blaR1) that are similar in sequence and function to mecA regulators.\textsuperscript{72} In addition to regulating blaZ transcription, BlaI also binds to mecA-mecR1 promoter-operator (P-O) sequences and regulates their transcription.\textsuperscript{72} Coregulation of mecA by both MecI and BlaI has been
demonstrated in defined laboratory strains, but neither the presence nor the nucleotide sequences of the two coregulators have been investigated in clinical isolates.\textsuperscript{73}

**FIGURE: 3.** (a) *Induction of staphylococcal β-lactamase synthesis in the presence of the β-lactam antibiotic penicillin.* I. The DNA-binding protein BlaI binds to the operator region, thus repressing RNA transcription from both blaZ and blaR1- blaI. In the absence of penicillin, β-lactamase is expressed at low levels. II. Binding of penicillin to the transmembrane sensor-transducer BlaR1 stimulates BlaR1 autocatalytic activation. III–IV. Active BlaR1 either directly or indirectly (via a second protein, BlaR2) cleaves BlaI into inactive fragments, allowing transcription of both blaZ and blaR1-blaI to commence. V–VII. β-Lactamase, the extracellular enzyme encoded by blaZ (V), hydrolyzes the β-lactam ring of penicillin (VI), thereby rendering it inactive (VII). (b) *Mechanism of S. aureus resistance to methicillin.* Synthesis of PBP2a proceeds in a fashion similar to that described for β-lactamase. Exposure of MecR1 to a β-lactam antibiotic induces MecR1 synthesis. MecR1 inactivates MecI, allowing synthesis of PBP2a. MecI and BlaI have coregulatory effects on the expression of PBP2a and β-lactamase.\textsuperscript{74}

The 21-60kb mecA gene is located on a mobile genetic element, designated the Staphylococcal Cassette Chromosome mec (SCCmec).\textsuperscript{74} Although the origin of SCCmec remains unknown, the cassette could originate from staphylococci other than S. aureus.\textsuperscript{74} It is believed that Staphylococcus sciuri harboured the ancestor of PBP2a, since a PBP was found in S. sciuri that showed 87.8% amino-acid sequence identity with PBP2a.\textsuperscript{74} These strains were all susceptible to methicillin, but became resistant to methicillin following growth of the strains in the presence of methicillin because of an increase in the transcription rate of the mecA homologue, subsequent to a point mutation in the promoter.\textsuperscript{74} Furthermore, a strain of methicillin-sensitive S. aureus (MSSA) became resistant to methicillin following introduction of this mecA homologue, and could thus be classified as MRSA. MRSA strain had emerged in vivo following horizontal
transfer of mecA between the two staphylococcal species.\textsuperscript{75} Currently, five main types of SCCmec (type’s I–V) have been distinguished, ranging in size from 20.9 to 66.9 kb. SCCmec types I (34.3 kb), IV (20.9–24.3 kb) and V (28 kb) encode exclusively for resistance to β-lactam antibiotics.\textsuperscript{75} In contrast, SCCmec types II (53.0 kb) and III (66.9 kb) determine multiresistance, as these cassettes contain additional drug resistance genes on integrated plasmids and a transposon.\textsuperscript{75} Besides the resistance genes on SCCmec, \textit{S. aureus} can carry resistance genes inserted at other sites of the chromosome and on plasmids.\textsuperscript{76} SCCmec also carries insertion sequences, e.g., IS431, as well as genes responsible for the regulation of mecA transcription, i.e., DmecRI (on SCCmec types I, IV and V) or mecRI and mecI (on SCCmec types II and III).\textsuperscript{76,77} These genes are situated in mec complexes. Besides MRSA, methicillin-resistant coagulase-negative staphylococci can harbour SCCmec.\textsuperscript{76} It has been shown that methicillin-resistant \textit{Staphylococcus epidermidis} isolates from the 1970s harboured SCCmec type’sI–IV.\textsuperscript{77} MecA gene is widely disseminated in \textit{Staphylococcus aureus} population.\textsuperscript{77} The mecA gene is part of a 21- to 60-kb staphylococcal chromosome cassette mec (SCCmec), a mobile genetic element that may also contain genetic structures such as Tn554, pUB110, and pT181 which encode resistance to non-β-lactam antibiotics.\textsuperscript{75} Two hypotheses have been raised to explain the evolutionary origin of Methicillin-resistant \textit{S. aureus} (MRSA) strains.\textsuperscript{77}

- The single clone hypothesis, based on early analyses of the restriction fragment length polymorphisms obtained for MRSA isolates collected worldwide by using probes for mecA and Tn554, suggests that mecA entered the \textit{S. aureus} population on one occasion and resulted in the formation of a single MRSA clone that has since spread around the world.\textsuperscript{77}
The second hypothesis, based on the detection of meca in diverse S. aureus multilocus enzyme electrophoresis types, proposes that MRSA strains evolved a number of times by means of the horizontal transfer of meca into phylogenetically distinct methicillin-susceptible S. aureus (MSSA) precursor strains. 77

By using DNA microarray technology, meca has been detected in at least five divergent lineages, implying that horizontal meca transfer has played a fundamental role in the evolution of MRSA. 76 The transfer of meca from S. epidermidis to S. aureus was recently witnessed in vivo, suggesting that meca may transfer more frequently to MSSA. It has been proposed that CoNS serve as donors for the transfer of the meca gene to S. aureus. 76 In this context, it is noteworthy that 70 to 75% of all CoNS worldwide are now resistant to methicillin, thus representing a huge potential reservoir of resistance. 77 The mechanism of transfer, however, remains unclear. There is evidence that meca resides within a mobile genetic element, SSCmec, that encodes recombinases for its excision from and integration into the staphylococcal chromosome. 77 This element may also contain other genetic elements, like Tn554, pUB110, and pT181, which encode resistance to non-β-lactam antibiotics, causing multiresistance. 77 Thus, while new MRSA strains will continue to emerge by the horizontal transfer of the meca gene, those strains disseminated widely possess additional resistance traits and are favored most by antibiotic selection pressure. 78 Cefoxitin, a cephamycin, is a more potent inducer of the meca regulatory system and an accurate surrogate marker for the detection of MRSA in the routine susceptibility testing. 78 It has been suggested that no special medium or incubation temperature is required for cefoxitin as is required for oxacillin. 78 Recent studies have indicated that disc diffusion testing by using the cefoxitin disc is far superior to most of the currently recommended phenotypic methods like oxacillin disc diffusion and oxacillin screen agar testing and that it is
now an accepted method for the detection of MRSA by many reference groups including CLSI.\textsuperscript{79}

Clindamycin belongs to the macrolide, lincosamide and streptogramin B (MLS\textsubscript{B}) family, act through inhibition of protein synthesis, resistance may be expressed through ribosomal target site modification (macrolide-lincosamide-streptogramin B [MLS\textsubscript{B}] resistance; usually encoded by \textit{erm} A or \textit{erm} C) cause production of methylase enzymes (methylation of the 23S rRNA) reduces binding of the drug to the rRNA target, macrolide efflux pump (encoded by \textit{msr}A) and enzymatic antibiotic inactivation.\textsuperscript{80} Mechanism of ribosomal target modification causes resistance either constitutive or inducible, if the \textit{erm} genes are consistently expressed, isolates shows in vitro resistance to erythromycin (E), CD, and to other members of MLS\textsubscript{B}, known as constitutive resistance phenotype (cMLS\textsubscript{B}).\textsuperscript{80} In case of inducible resistance, the \textit{erm} genes require an inducing agent to express resistance to CD (iMLS\textsubscript{B}).\textsuperscript{79} Erythromycin acts as a strong inducer of methylase synthesis.\textsuperscript{80} These isolates known as inducible resistance phenotype (iMLS\textsubscript{B}) show in vitro resistance to Erythromycin and are susceptibility to Clindamycin.\textsuperscript{81} Clindamycin therapy in this phenotype can lead to clinical failure.\textsuperscript{81-84} \textit{S. aureus} also have isolated macrolide resistance because of the presence of an efflux pump, the MS phenotype (resistance to erythromycin, inducible resistance to streptogramins and susceptibility to clindamycin), encoded by the \textit{msr}A gene.\textsuperscript{85} Clindamycin therapies can be safely given in infections with this phenotype without the risk of clinical failure.\textsuperscript{84} Therefore, it is important to differentiate these two mechanisms of resistance.\textsuperscript{84} Phenotypic detection of inducible resistance can be made by double disk diffusion test (D-test), a distorted ‘D-Shaped’ zone of inhibition around clindamycin if an erythromycin disc is placed adjacent.\textsuperscript{86} D-test is a simple, reliable, inexpensive, sensitive, specific and easy to interpret test.\textsuperscript{86} Low levels of Erythromycin
resistance is the most effective inducers of iMLSB resistance. The molecular markers for the erm genes are presently available, but costly and inconvenient for daily use. The inducible clindamycin resistance in various clinical isolates of S. aureus by the disc diffusion induction test (modified D test) along with azithromycin. Development of drug resistance in S. aureus has led to the use of older antibiotics such as macrolide, lincosamide, and streptogramin B (MLS\textsubscript{B}) antibiotic. However, extensive use of these antibiotics in serious staphylococcal infections has caused the emergence of S. aureus resistant to MLS\textsubscript{B} antibiotics. There are three different mechanisms of resistance to MLS\textsubscript{B} 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 MLS\textsubscript{B} antibiotics in staphylococci, which can be constitutive or inducible. In vitro, S. aureus isolates with constitutive MLS\textsubscript{B} (cMLS\textsubscript{B}) resistance are resistant to erythromycin and clindamycin but isolates with inducible MLS\textsubscript{B} (iMLS\textsubscript{B}) resistance are resistant to erythromycin and susceptible to clindamycin. In this condition, treatment of patients with clindamycin can lead to the emergence of resistant mutants to cMLS\textsubscript{B} from iMLS\textsubscript{B}-resistant strains and treatment failure. On the other hand, assigning all erythromycin-resistant S. aureus as clindamycin resistant strains may cause to avoid the use of clindamycin in the treatment of S. aureus infections. For this reason, careful screening of iMLS\textsubscript{B}-resistant strains is very important. While constitutive resistance is detectable by routine antimicrobial susceptibility tests, inducible resistance to clindamycin is not detectable by standard methods. For detection of iMLS-resistant strains, Clinical and Laboratory Standards Institute (CLSI) developed a phenotypic method called the double disk diffusion test (D-test). The
aim of this study will be to determine the frequency of inducible resistance to clindamycin using D-test and polymerase chain reaction (PCR) with specific primers to confirm the presence of the *erm* genes in these isolates.⁹⁶

**Prevalence of MRSA** - *Staphylococcus aureus* continues to be a dangerous pathogen for both community-acquired as well as hospital-associated infections. *S. aureus* resistant to methicillin was reported soon after its introduction in October 1960.¹⁰⁰ Methicillin resistant *S. aureus* (MRSA) is now endemic in India. The incidence of mrsa varies from 25 per cent in western part of India¹⁰¹ to 50 per cent in south India.¹⁰² Community acquired mrsa (CA- MRSA) has been increasingly reported from India.¹⁰³

A network of microbiology laboratories (Indian network for surveillance of antimicrobial resistance - insar) at premier medical colleges and hospitals in India was formed with support from the world health organization. The network aims to monitor antimicrobial resistance and to
review the magnitude of its problem in India.

Figure: 4. Places marked with Red Dot are INSAR members whose data included in the study. Places marked * are INSAR members whose data are not included in the study.

In India, Methicillin-resistant Staphylococcus aureus (MRSA) represents a challenge for all healthcare institutions. Previously it was limited to large institutions; now quite common in all
hospital settings. MRSA is now one of the commonest nosocomial pathogens, and S. Aureus asymptotically colonizing healthcare workers are the major sources of MRSA in the hospital environment. The prevalence of MRSA strains has increased worldwide. This increase has been associated with the reorganization of new community-associated MRSA (CA-MRSA) strains, which has increased the disease burden in general population with or without exposure to the health care environment. Beside this, prolonged hospitalization, use of invasive medical devices, healthcare workers, suppressed immune system, prolonged use of antimicrobials, living in crowded or unsanitary conditions are some risk factors for MRSA infection. According to European Antimicrobial Resistance Surveillance System report, there has been shown significant increases in methicillin resistance Staphylococcus aureus between 1999 and 2002 with the highest incidence of 44% in Greece and lowest of 0.5% in Iceland. Whereas in the similar study by Brog M et al. in 2006 in Jordan, 65% MRSA prevalence was reported. According to National Nosocomial Infection Surveillance System (NNIS) report, 50% of hospital acquired infections in ICUs in the USA are due to MRSA. The growing problem in the Indian scenario is that MRSA prevalence is rapidly increasing with the time. In a study conducted in Indore the MRSA has been increased to 80.83% in 1999 which was 12% in 1992. The same was also observed in Uttar Pradesh where the incidence of MRSA was as low as 6.9% in 1988 which raised to 32.8% in 1994 and 59.3% in 2008. In addition to the terrible situation, MRSA strains are important for their resistance to many other commonly used antibiotics and the emergence of resistance to Vancomycin, the drug which is considered as last resort for these strains. It is essential to control the emerging problem of Vancomycin resistance and multidrug resistance in MRSA strains. Reports of emergence of Vancomycin resistance in S. Aureus from India further justify this necessity.
### Prevalence Rate of MRSA in India

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<th>YEAR</th>
<th>INCIDENCE RATE OF MRSA %</th>
<th>STATE</th>
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<tbody>
<tr>
<td>1988</td>
<td>6.9%</td>
<td>UP</td>
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<tr>
<td>1994</td>
<td>12%</td>
<td>Indore</td>
</tr>
<tr>
<td>1994</td>
<td>24%</td>
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<td>32.8%</td>
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<td>1996</td>
<td>87%</td>
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<td>23.2%</td>
<td>Assam</td>
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<tr>
<td>2012</td>
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<td></td>
<td>Western States&lt;sup&gt;109&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>25%</td>
<td>Southern State&lt;sup&gt;109&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>50%</td>
<td>Akola&lt;sup&gt;100&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>67.85%</td>
<td>Puducherry&lt;sup&gt;99&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>33.3%</td>
<td>Karnataka&lt;sup&gt;99&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>47%</td>
<td></td>
</tr>
</tbody>
</table>

**Prevalence Rate of MRSA IN Worldwide**

<table>
<thead>
<tr>
<th>YEAR/ORGANIZATION</th>
<th>INCIDENCE RATE OF MRSA %</th>
<th>COUNTRY</th>
</tr>
</thead>
<tbody>
<tr>
<td>1999 TO 2000, European Antimicrobial Resistance Surveillance System Report</td>
<td>44%</td>
<td>Greece&lt;sup&gt;108&lt;/sup&gt;</td>
</tr>
<tr>
<td>1999 TO 2000, European Antimicrobial Resistance Surveillance System Report</td>
<td>0.5%</td>
<td>Iceland&lt;sup&gt;108&lt;/sup&gt;</td>
</tr>
<tr>
<td>2006, Antibiotic Resistance &amp; Control In The Mediterranean Region (ARMed)Project</td>
<td>65%</td>
<td>Jorden&lt;sup&gt;109&lt;/sup&gt;</td>
</tr>
<tr>
<td>National Nosocomial Infection Surveillance System (NNIS)</td>
<td>50%</td>
<td>USA¹¹⁰</td>
</tr>
<tr>
<td>--------------------------------------------------------</td>
<td>-----</td>
<td>--------</td>
</tr>
</tbody>
</table>

India faces unique challenges in tackling AMR due to its geography and vast population, low healthcare spending and inappropriate/overuse of antimicrobials. AMR surveillance constitutes the backbone for programs aimed at tackling AMR by providing *in vitro* evidence and trend of resistance for further endeavours.¹¹⁴ The ICMR-AMRSN is a uniform system of AMR surveillance with a network of quality assured laboratories (the nodal and regional centres), which not only give reliable, rapid AMR data but also monitor resistance pattern over a longer period of time. An Europe-wide survey found the most common infections associated with *S. aureus* to be SSTIs (71% cases), 22.5% of them being MRSA.¹¹⁶ The observed resistance was lower than what was reported in an earlier study from several centres across India, where rates in 2008 and 2009 were 42% and 40%, respectively.¹¹⁵ The proportion of MRSA varies among countries ranging from 0.4% in Sweden to 48.4% in Belgium.¹¹⁶ The differences in the prevalence of resistance phenotypes to almost all the antimicrobials tested between different centres of the surveillance network during the study period, notably, MRSA prevalence which ranged from as low as 21% to as high as 45% between the centres, may be indicative of localised differences in the antibiotic prescription practices and the infection control measures employed.¹¹⁶ The high and increasing level of resistance to ciprofloxacin, a routine antimicrobial, in all the centres was perhaps expected because of its pervasive use. It is heartening to note that resistance to MUP was very low despite this drug being extensively used both for decolonisation as well as a topical antibiotic.¹¹⁶
**Drug resistance**

The introduction of penicillin in the early 1940s dramatically improved the prognosis of patients with staphylococcal infection. However, as early as 1942, penicillin-resistant staphylococci were recognized, first in hospitals and subsequently in the community. By the late 1960s, more than 80% of both community and hospital-acquired staphylococcal isolates were resistant to penicillin. Methicillin, introduced in 1961, was the first of the semisynthetic penicillinase resistant penicillins. Its introduction was rapidly followed by reports of methicillin-resistant isolates. The therapeutic outcome of infections that result from methicillin-resistant S. aureus (MRSA) is worse than the outcome of those that result from methicillin-sensitive strains. As noted earlier, the mecA gene is invariably part of a larger unique, mobile genetic element, SCCmec. These islands may also contain additional genes for antimicrobial resistance and insertion sequences, as well as genes of uncertain function.

**Quinolone resistance**

Fluroquinolines initially a potentially important therapeutic alternative for methicillin-resistant staphylococci have become increasingly compromised by burgeoning resistance. Fluroquinolones resistance is more common in strains that are resistant to methicillin than the susceptible strains. Quinolone resistance among S. aureus emerged quickly, more prominently among the methicillin-resistant strains. The quinolones act on DNA gyrase, which relieves DNA supercoiling, and topoisomerase IV, which separates concatenated DNA strands.
Mechanisms of resistance

- One contributing factor is likely antibiotic selective pressure, especially in the hospital setting, resulting in the selection and spread of the more antibiotic-resistant MRSA strains.

- Resistance to quinolones results from the stepwise acquisition of chromosomal mutations. Fluoroquinolone resistance develops as a result of spontaneous chromosomal mutations in the target of the antibiotic, topoisomerase IV or DNA gyrase.

- An additional mechanism of resistance in S. aureus is induction of the NorA multidrug resistance efflux pump. Increased expression of this pump in S. aureus can result in low-level quinolone resistance.74

Vancomycin resistance

The VRSA strains acquire resistance by conjugal transfer of the vanA operon from an Enterococcus faecalis, raising the specter of a far more efficient means for dissemination of the resistance gene among strains of staphylococci.74 S. aureus with intermediate resistance to vancomycin has been given the mnemonic “GISA” for gycopeptide intermediate S. aureus as these strains have diminished susceptibility to both vancomycin and teicoplanin glycopeptides antibiotics. There is evidence that MRSAs and VISA strains may respond synergistically to combination of vancomycin and other β-lactam agents.74

Mechanisms of resistance

- VRSA strains are resistant to vancomycin because of the acquisition of the vanA operon from an enterococcus that allows synthesis of a cell wall precursor that ends in D-Ala-D-Lac dipeptide rather than D-Ala-D-Ala.76 The new dipeptide has dramatically reduced affinity for vancomycin. In the presence of vancomycin, the novel cell wall precursor is synthesized, allowing continued peptidoglycan assembly.74
In 1997, vancomycin intermediate-resistant was first reported. A pre-VISA stage of resistance, heterogeneously resistant, has also been identified. The heteroresistant strains remain susceptible to vancomycin but contain resistant subpopulations. It is hypothesized that, on exposure to vancomycin, the VISA isolates are selected from the vancomycin-resistant subpopulations. The reduced susceptibility to vancomycin appears to result from changes in peptidoglycan biosynthesis. The VISA strains are notable for the additional quantities of synthesized peptidoglycan that result in irregularly shaped, thickened cell walls. There is also decreased cross-linking of peptidoglycan strands, which leads to the exposure of more D-Ala-D-Ala residues. The altered cross-linking results from reduced amounts of L-glutamine that is available for amidation of D-glutamate in the pentapeptide bridge. As a result there are more D-Ala-D-Ala residues available to bind and trap vancomycin. The bound vancomycin then acts as a further impediment to drug molecules reaching their target on the cytoplasmic membrane. HPLC provided further proof of this novel resistance mechanism by showing that large quantities of vancomycin become trapped in the abnormal peptidoglycan.

Clindamycin (Cd), a lincosamide was widely used to treat Staphylococcus aureus in case of intolerance to penicillin or resistance to methicillin. However, recent reports indicate that failure may occur in the case of inducible Cd resistance inspite of invitro susceptibility to clindamycin. Cd inhibits the production of toxins and virulence factors in Gram positive organism through inhibition of protein synthesis. Resistance mechanism to Cd in Staphylococcus is mediated by a methylase encoded by erythromycin resistant methylase (erm) gene and macrolides streptogramins resistance (msrA) genes. Bacteria resist macrolide and lincosamide antibiotics in 3 ways: (1) through target-site modification by methylation or mutation that prevents the binding of the antibiotic to its ribosomal target, (2) through efflux of the antibiotic, and (3) by drug inactivation. In pathogenic microorganisms, the impact of the 3 mechanisms is unequal in terms of incidence and of clinical implications. Modification of the ribosomal target confers broad-spectrum resistance to macrolides and lincosamides, whereas
efflux and inactivation affect only some of these molecules.\textsuperscript{118} Macrolides, lincosamides and group B streptogramins (MLSB), have similar inhibitory effects on bacterial protein synthesis, but widely used in the treatment of Gram positive infections.\textsuperscript{119} The resistance mechanism is methylation of the 23s binding site. If this occurs then the bacteria are resistant to both the macrolides and the lincosamides.\textsuperscript{119} As a consequence of methylation, binding of erythromycin to its target is impaired. Expression of MLSB resistance can be constitutive or inducible.\textsuperscript{119} Prevalence of induced clindamycin resistance in \textit{S. aureus} was reported from many countries.\textsuperscript{120-123} Many reports from India also recorded the emergence of induced clindamycin resistance in \textit{S. aureus}.\textsuperscript{124-128} The D-test identifies inducible resistance that might presage mutational clindamycin constitutive resistance.\textsuperscript{128} The D-test is performed by placing clindamycin and erythromycin disks at an edge-to-edge distance of 15 to 20 mm and looking for flattening of the clindamycin zone nearest the erythromycin disk.\textsuperscript{129} A positive D-test suggests the presence of an \textit{erm} gene that could result in inducible clindamycin resistance and clinical failure. There are few published clinical failures of clindamycin with emergence of resistance.\textsuperscript{1130-134} Erythromycin (Ery) induces the production of this methylase, which is why these strains are Ery-resistant, but mutations in the promoter region of \textit{erm} allow production of methylase without an inducer.\textsuperscript{118} Methylation results in impaired binding of clindamycin that share this residue as a common binding site. MRSA are increasingly being reported as multidrug resistant with high resistance to macrolides and lincosamides leaving very few therapeutic options.\textsuperscript{135} Low levels of erythromycin are the most effective inducer of inducible macrolide, lincosamide and streptograminB (MLS\textsubscript{Bi}) resistance. To detect MLS\textsubscript{Bi} strains, there are special disk approximation tests that incorporate Ery induction of Cd resistance.\textsuperscript{135} These strains involve the placement of an erythromycin disk in close proximity to a disk containing Cd or lincomycin.\textsuperscript{135} As the Ery
diffuses through the agar, resistance to the lincosamides is induced, resulting in a flattening or blunting of the lincosamide zone of inhibition adjacent to the Ery disk, giving a D shape to the zone (D zone effect). In January 2004, NCCLS published a procedure for Cd induction testing in which Cd disks are placed 15 to 26 mm from an Ery disk either as part of a standard disk diffusion procedure or on an inoculum check agar plate.

**Prevention and control measures**

The prevention and control measures can improve patient care, minimize patient mortality and morbidity, and to help contain healthcare costs. In hospitals where MRSA is endemic, the objective is to minimize spread and in particular to avoid as far as possible the clinical impact of systemic or deep infection in high-risk patients such as those in the intensive care unit (ICU) or other key clinical areas. Under-dosing, multiple courses and excessive duration of antibiotic therapy and the over-use of broad-spectrum agents are major factors in the spread of antibiotic resistance in healthcare settings. Numerous antibiotic classes have been associated with MRSA colonization and infection in different studies. Exposure to broad-spectrum antibiotics, particularly third generation cephalosporins and fluoroquinolones, has been shown to be an independent risk factor for MRSA colonisation and infection in numerous studies. Prevention can be achieved by 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. More recently, the potential use of novel agents for this purpose, such as endopeptidase, lysostaphin, or phage lytic enzymes has also been considered.

One of the important recommendations for reducing health care associated infection is compliance with hand hygiene practices. The purpose of hand hygiene is to prevent
colonization and infection in the patient, colonization and infection in the health care worker and contamination of the environment.\textsuperscript{137} Therefore, an opportunity for hand hygiene arise every time that there is a possibility of microorganism transfer from one skin or inanimate surface to another surface.\textsuperscript{138}

Promoting the application of the "5 moments" at the point of care is one simple step that each health care worker and health-care facility can act.\textsuperscript{139}

(FIGURE: 5).

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{hand_hygiene.png}
\caption{five moments of hand hygiene\textsuperscript{139}}
\end{figure}