INTRODUCTION

Among Staphylococcal species, *Staphylococcus aureus* is one of the most important pathogen worldwide. It is found in 20%-30% of the population as part of normal flora, most often in the area of the nasal vestibulum, but it may be found in the mucous membrane of the throat, intestines, on the skin and in the groins.\cite{1, 2}

*Staphylococcus aureus* can colonize the host without causing any signs or symptoms of infection; can become pathogenic when it gains access through cuts, abrasions or other openings.\cite{3}

*Staphylococcus aureus* is a pathogen of major concern because of its ability to cause a diverse array of diseases ranging from minor infections of skin and soft-tissue to life threatening conditions like bacteremia, toxic shock syndrome etc and due to its ability to adopt to adverse environmental conditions. *S. aureus* has many virulence factors, which are also responsible for the occurrence of various clinical syndromes. So far more than 30 different virulence factors have been described, which lead to the occurrence of the disease in particular situations.\cite{4}

Despite changes in the medical field towards the reduction of infections, the rate of antibiotic resistant infections is on the rise. Majority of *S. aureus* infections were treatable with common antibiotics but emergence of drug resistance has become a major concern. *S. aureus* was originally sensitive to most of the commonly used antibiotics but Methicillin resistant *S. aureus* (MRSA) has a tendency to accumulate additional unrelated resistance determinants in its genome and hence it has become resistant to multiple antibiotics.

Resistance to penicillin first appeared in 1942. MRSA was first reported in 1961, became endemic in hospitals and has disseminated rapidly and unexpectedly in communities in the 1990s the world over.\cite{5} MRSA is not more virulent than other staphylococci species. However, they are much more dangerous because they do not respond to the mainstream of antibiotics, which are considered as safer and more effective than the hard line treatment offered to MRSA patients. *S. aureus* has ability to continue modifying itself, especially in hospital environment, a situation that may turn the still remaining effective antibiotics obsolete.
The prolonged hospital stay, lack of awareness, indiscriminate use of antibiotics, receipt of antibiotics before coming to the hospital etc. are the possible predisposing factors for MRSA emergence. [6]

Because of its resistance to multiple antibiotics, MRSA is difficult to eradicate and has become epidemiological risk in both developing and developed countries. The MRSA strain has been progressively causing increased mortality and morbidity in high risk areas like intensive care units with increase in health care costs also. [7] The action of antibiotics and resistance to these drugs are linked like light and shadow—one does not survive without the other.

Methicillin resistant *S. aureus* is difficult and expensive to treat, therefore early screening is necessary. The severity of infections by MRSA increases the economic burden of the patient due to longer hospital stay and prolonged antibiotic administration.

In India the prevalence and spread of MRSA has been recognized late, which led to its emergence as a real threat to community and hospital settings. The growing problem in the Indian scenario is that MRSA prevalence has increased enormously to the extent that some studies show prevalence up to 80.83% [8] with treatment options limited to few drugs. Moreover, since antibiotics of last resort such as Vancomycin or Linezoid are very expensive, infections caused by MRSA pose therapeutic challenge. [9, 10] However, epidemiological studies in such resource poor settings are largely lacking and there have been no documented attempts to quantify the nosocomial transmission of MRSA in India. [11]

Understanding the prevalence, antibiotic sensitive as well as resistance patterns of MRSA strain is necessary for appropriate antibiotic treatment and effective control measure because there are fewer options available for the treatment of MRSA infections.

Surveillance of MRSA locally, nationally and globally is also dependent on accurate laboratory reporting. Errors in determining methicillin resistance may have serious adverse clinical consequences. False negative susceptibility results may lead to treatment failure and the spread of MRSA, especially if appropriate infection control measures are not applied. On the other hand improper detection of resistance may increase health care cost following unnecessary isolation precautions for patients
and may lead to overuse of glycopeptides such as Vancomycin. Therefore rapid and accurate identification of MRSA is required for therapeutic and epidemiological reasons to start the appropriate antimicrobial therapy as well as to prevent its spread. [12]

The mechanism for MRSA is production of an auxiliary penicillin binding protein, PBP2a or the recently discovered PBP2c, which render the isolate resistant to all β-lactams except for the novel class of cephalosporins, which have sufficiently high affinity to PBP2a and probably also PBP2c to be active against MRSA. [13] The PBPs are encoded by the meca gene or the recently described mecC (formerly known as mecA LGA251) respectively. [4] meca is an additional gene found in MRSA and with no allelic equivalent in methicillin susceptible staphylococcus aureus (MSSA).

All bacterial cells in culture may carry the genetic information for resistance but a small number can express this kind of resistance in routine susceptibility testing performed in the laboratory. This phenomenon is termed as heterogeneous resistance and occurs in Staphylococci resistant to penicillinase stable penicillin such as oxacillin. [14] Strains which shows heterogeneous expression of the meca gene has low MICs to oxacillin; hamper the accuracy of susceptibility testing. However, some strains express low-level resistance but are meca negative and do not produce alternative PBPs. These strains are called as borderline oxacillin resistant S. aureus (BORSA). The mechanism of resistance in these isolates is often poorly characterized but may include hyperproduction of β-lactamases or alteration of the pre-existing PBPs. [15]

Detection of meca gene by the polymerase chain reaction (PCR) is considered as the “gold standard” for the detection of MRSA. [16, 17] But PCR for meca gene detection, still remains expensive method and is not yet available in most of the routine microbiology laboratories. Therefore phenotypic method still remains a method of choice for detection of MRSA in most of the laboratory setting in developing countries. [18]

Actually several phenotypic methods have been proposed to detect heterogeneous, low level resistance and borderline resistance. Previous Clinical
Laboratory Standards Institute (CLSI) recommendation for detecting MRSA included oxacillin agar dilution, oxacillin broth microdilution, oxacillin disc diffusion, oxacillin screen agar test, cefoxitin disc diffusion and detection of meca or its product-PBP2a by PCR and latex agglutination respectively. Some phenotypic methods used for detection of MRSA are prone for errors. Conventional phenotypic methods to detect methicillin resistance have many discrepancies because expression of resistance may be subject to environmental and conditional variations. However, there is no optimal phenotypic method for detection of methicillin resistance in S. aureus because some conventional methods require special conditions e.g. 2% - 4% NaCl enriched media, incubation time up to 48 hours, incubation temperature of ≤ 35°C etc. [19]

The laboratories that cannot afford to perform the PBP2a- latex agglutination test or do not have access to PCR, need alternative methods for detecting meca mediated resistance. Hence this study was planned to evaluate the efficacy of different conventional phenotypic methods by comparing with genotypic method (PCR for meca gene) which is considered as the gold standard for detection of MRSA.