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Molecular Epidemiology of Methicillin Resistant *Staphylococcus aureus* (MRSA) in India.

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ABSTRACT

Staphylococus aureus is a common microflora present principally on skin and nasal tissues of the human body. It is an opportunistic pathogen capable of causing diseases varying from minor to major clinical implications. The emergence of antibiotic drug resistance in this organism in the form of Methicillin Resistant *Staphylococcus aureus* (MRSA) strains and its spread has been documented on a global scale in both diseased individuals in health care centers and healthy individuals in the community. Recent findings of Vancomycin Resistant *Staphylococcus aureus* (VRSA) strains has led to the necessity for regular screening of environment and individuals, particularly in health care settings as well as pharmaco-vigilance in administration of drugs for treatment of individuals infected with these strains. In addition to phenotypic methods of resistance screening, genotypic methods are widely followed in order to establish the relationship between strains found in different settings as well as determining the spread pattern of the strains across geographical locations. The availability of phenotypic and genotypic data for the strains allows for focused medical planning and action to prevent the spread. This review assesses the survey data for MRSA in India in various settings and the molecular epidemiology established by these studies.

Keywords: *Staphylococcus aureus*, MRSA, Molecular Epidemiology, India.

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INTRODUCTION

Staphylococcus aureus is a nosocomial pathogen present primarily in the anterior nares of the nasal passage and also on skin of humans. It is an opportunistic pathogen which has lately become of importance in the medical scenario due to its increasing antibiotic resistance which leads to difficulty in treatment of infections which may range from mild to fatal outcomes.

Presence of chromosomal and plasmid genes coding for penicillinases which were effective in breaking down early generation beta-lactams was found in *S.aureus* rendering penicillins ineffective for treatment [1]. Discovery of methicillin in 1960 which was able to withstand penicillinase activity was a landmark in treatment of antibiotic resistant *S.aureus*. By 1961, however, the first case of methcillin resistant *S.aureus* was reported in the UK [2]. Additional antibiotic resistance was found due to production of low affinity pencillin binding proteins such as PBP2a encoded by the mecA gene found on the novel Staphylococal Cassette Chromosome mec (SCCmec) which is found in an unique location on the *S.aureus* chromosome [3,4]. SCCmec contains regulatory genes and recombinase genes which allow expression and site specific integration and excision of SCCmec. It also contains several open reading frames and pseudogenes of unknown benefit or function. Additionally, SCCmec also possesses genes coding for resistance to other non beta lactam antibiotics which contributes to the multi drug resistance of MRSA [5,6]. Horizontal transfer of SCCmec has been documented [7] but is restricted due to host specificity and stability [8] which results in limited number of closely related MRSA clones worldwide [9]. Several genes involved in either regulatory or other roles (direct or indirect) of peptidoglycan synthesis whose loss negatively impacts resistance such as fem/fmt genes [4], pbpB [10], murE [11] and murF [12] are also responsible for antibiotic resistance. Methicillin resistance independent of mecA has also been observed [13] and is attributed to alterations in or overproduction of other PBPs [14].

Two types of MRSA are currently present – Community Acquired MRSA (CA-MRSA) and Healthcare Acquired MRSA (HA-MRSA) [15]. HA-MRSA was found in 1961, shortly after the introduction of methicillin and CA-MRSA was identified in the United States in the 1990s [16].

A variety of phenotypic and genotypic typing methods are available to identify and type the strains. A good typing method must be simple to perform, inexpensive, reproducible with sufficient discriminatory power and must be widely available [17]. In order to differentiate between the types, genotypic or molecular methods are used as they have have good reproducibility and high discriminative power and are thus used for epidemiological studies [18].

The most commonly used phenotypic method is antibiogram typing which involves comparison of susceptibility of strains to various antibiotics. Strains differing in susceptibility to antibiotics are considered to be different. The technique is established, easy to perform, inexpensive, rapid and is readily available in routine microbiology laboratories [19]. However, its result cannot be solely used for interpretation due to low discriminatory power as antibiotic resistance patterns vary due to environmental factors, selective antibiotic pressure, acquisition and loss of plasmids that carry resistance genes and other genetic mechanisms. This method is successfully used to screen epidemic strains [20]. Other phenotypic typing mechanisms include Phage typing [21], Serotyping [22], Biotyping [23] and Zymotyping [24]. Most of these methods are labour intensive and are expensive and are limited in their use due to poor discrimating power.

Several genotyping methods are used for typing MRSA strains which are better than phenotyping methods primarily due to their high discriminatory power and rapidity. The most commonly used methods are Multi Locus Sequence Typing [25], SCCmec typing [26], Toxin Gene typing for genes like Panton Valentine Leucocidin (PVL) [27], *spa* gene typing [28] and Pulsed Field Gel Electrophoresis [29], all of which are widely followed for molecular discrimination and epidemiology studies of MRSA strains on broad scale.

Numerous studies have been conducted around the world in order to determine the molecular epidemiology of both CA-MRSA and HA-MRSA strains by national surveys [30,31] which have been reviewed extensively for global level assessment [32,33]. To date, few studies have been done to assess the molecular epidemiology of MRSA in India.

CURRENT SCENARIO IN INDIA:

This review presents genotypic studies and recent phenotypic studies in chronological order From 2000-2003, a prevalence study was conducted in Aligarh Hospital to determine the molecular epidemiology of clinical and carrier S.aureus and to study the molecular mechanisms involved in transfer and dissemination of antibiotic resistance in strains. From a sample population of 750 (175 controls, 575 patients), 513 samples were confirmed to have presence of *S.aureus*. Of these, 180 samples were confirmed to be MRSA with highest resistances to pencillin, ampicillin and co-trimoxazole. Highest sensitivity was determined to be toward vancomycin. 61 samples subjected to PFGE revealed banding type A to be the highest and banding type I to be the lowest. Conjugation experiments revealed the transfer of antibiotic resistance genes from clinical MRSA to carrier *S.aureus* with two antibiotic resistances (ciprofloxacin and erythroycin) being chromosomally mediated. The major reservoir for MRSA was determined to be infected patients and colonized hospital workers. The carriage of MRSA was determined to be maximal via hands. The MRSA strains were determined to be HA-MRSA type due to low prevalence in adult outpatient population [34].

In 2004, a study conducted in two hospitals of Bangalore for molecular epidemiology of strains by genotyping of 82 MRSA single patient samples. All the isolates showed resistance to five antibiotics – Penicillin G, Methicillin, Erythromycin, Gentamicin and Tetracycline when tested by Kirby-Bauer method. All strains had type III or type IIIA SCCmec cassettes. Multi Locus Sequence Typing (MLST) and staphylococcal protein A (spA) typing of selected isolates showed same patterns for all strains. PFGE patterns for the same strains were diverse and the combined profile of MLST, PFGE and spA typing showed that the strains are related to Brazilian and Hungarian MRSA clones [35].

In 2005, a study was carried out in Chandigarh to assess the prevalence of MRSA in carriers among healthy children. Nasal swabs were obtained from 482 children. Overall prevalence of *S.aureus* was determined to be 52.3% (256/489) with 3.89% (19/489) confirmed to be MRSA by antibiotic screening methods. Maximum resistance was toward amoxicillin (20.9%) and minimum resistance was towards rifampicin (97.6%). An inhouse PCR screening method to determine MRSA by SCCmec gene typing revealed only 60% of phenotypically detected samples, which was reported to be poor and thus required improvement to increase the detection rate of MRSA. The study showed a carriage rate of 52.3% which is higher than earlier reports of 20-40% as well as higher MRSA prevalence which can cause spread to other niches like hospitals leading to outbreak [36].

In 2006, a study for nationwide prevalence of MRSA in hospitals was conducted. 186 samples were collected from eight major hospitals from various areas in India. All the strains were determined to be SCCmec type III or IIIA after SCCmec typing. All strains showed resistance to penicillin, methicillin, oxacillin and erythromycin and all were sensitive to vancomycin. MLST and spA typing patterns were same for most isolates. PFGE patterns were diverse for all isolates and indicated a variety of short term genetic changes. The study indicated that MRSA spread in hospitals is a serious healthcare problem and epidemiological studies at local and national levels are required to develop strategies for preventing spread [37].

From 2006-2007, a study was conducted in a tertiary hospital in Lucknow to assess the prevalence of CA-MRSA in the community. 200 health individuals with no history of recent hospitalization or antibiotic intake were randomly enrolled for the study; in addition, 100 inpatients were enrolled as controls to assess for HA-MRSA. Samples were taken from nose, throat and axilla (n=600). 204 *S.aureus* isolates were obtained from 116 of 200 individuals. Throat was the most common site (107/200) followed by anterior nares (71/200). 84 of 204 isolates were detected as MRSA by oxacillin MIC by agar dilution method. CA-MRSA was detected by PVL gene typing. 47 of 200 individuals had CA-MRSA in one or more sites. 28 of 100 hospitalized inpatients were detected with HA-MRSA. HA-MRSA had 100% resistance to ciprofloxacin and 86% resistance to clindamycin as compared to CA-MRSA which had 23% resistance to ciprofloxacin and 25% resistance to clindamycin respectively; this difference was statistically significant. The study concluded that significant high proportion of the study population of healthy individuals were carriers of CA-MRSA (23.5%) [38].

From 2008-2011, a study was conducted in a tertiary care center in Delhi to assess the prevalent organisms and their antibiotic resistances in intensive care unit patients. Out of 22491 blood cultures, 2846 samples were positive for cultures and 3771 microorganisms were isolated. 764 of these were Coagulase Negative *S.aureus*. 107 of these were Coagulase Positive *S.aureus*. Both had nearly complete resistance to

pencillin whereas the former had more resistance to clindamycin, gentamicin and oxacillin as compared to the latter. Both were sensitive to linezoid and vancomycin. The study suggested de-escalation of high end microbials once sensivity profile is established to prevent reduce antimicrobial pressure on the patient and to also have aggressive screening measures to identify and isolate carriers and environmental sources which may contain these organisms [39].

In 2009, a study was carried on MRSA in skin and soft tissue isolates in three major hospitals in Delhi providing tertiary care. Of 709 samples, 221 samples were confirmed to be MRSA by antimicrobial testing; thus showing a prevalence rate of 31.2%. Low levels of rifampicin and chloramphenicol resistance was noted in all strains; however, all strains had high levels of muciprocin resistance. PFGE typing of 220 samples revealed presence of clones I, III and IV in all the samples. Clone III was the most widespread in all the samples (40%) followed by Clone I (20.5%). Clone III showed close similarity to UK-EMRSA-1 strain (74% similarity). Clone III had higher resistance to the following antibiotics – erythromycin, cotrimoxazole, tetracycline and ciprofloxacin (>80% resistance) as compared to other clones. MLST types 293 and 277 were found and these showed similar results as the clone III. The study concluded that MRSA subtypes varied between regions and institutions as well. Muciprocin resistance also poses more problems in treating MRSA infections. Prevalence of clones III and I showed that they are more widespread and clone III is gaining more footage in North India. The study recommended that more regions be surveyed to monitor and control MRSA spread [40].

Between2009-2010, a study was conducted to study the molecular epidemiology of *S.aureus* of pharyngitis patients of a hospital in Madurai, Tamil Nadu. Throat swabs were used to acquire samples. Of 265 swabs, 165 *S.aureus* samples were obtained. 63 were determined to be MRSA and 102 were MSSA. All 63 strains were resistant to oxacillin, penicillin and piperacillin. Maximum resistance was toward kanamycin (61 strains) and minimum resistance was towards rifampicin (8 strains). In MSSA, pvl positive MSSA strains showed more resistance to antibiotics as compared to pvl negative MSSA strains. 44 of 63 MRSA strains and 57 of 102 MSSA strains contained pvl gene. Predominant SCCmec types were Type V (32 samples), Type III (28 samples) and one sample which contained SCCmec Type I, II and IVa. Predominant MLST type was ST722 which was pvl positive and SCCmec V and antibiotic resistant whereas studies worldwide showed this type as antibiotic sensitive 10 of 63 strains were found to be Vancomycin Intermediate (MIC: 4µg/ml). The study suggests that MRSA may play major role in pharyngitis and future diagnostic and therapeutic measures in hospital must take into account the presence of MRSA [41].

From 2010-2011, a study carried out on bacteremia caused by *S.aureus* in a tertiary care hospital. Of 70 cases, 54% were found to be methicillin resistant. 74% of the cases were Community Acquired *S.aureus* bacteremia. 16% of these cases were confirmed to have PVL gene, predominantly from the CA-*S.aureus* (82%). The overall fatality rate was 27%. Early diagnosis, carrier screening and control of antibiotic use was recommended for controlling MRSA infections and reducing mortality rate [42].

From 2010-2012, a retrospective study was carried out in a teaching hospital in Nepal using various samples from inpatients and out patients. 306 *S.aureus* isolates were recovered. 43.1% were found to be MRSA by antibiogram typing and 12.4% were found to have inducible clindamycin resistance by D-test. All strains were sensitive to vancomycin and teicoplanin. The study concluded that the prevalence of MRSA was high and rigorous surveillance and control measures needed to be implemented to reduce prevalence and spread of MRSA [43].

In 2011, a study was conducted in a teaching hospital in Odisha to investigate the infection of hospital and community acquired 'erythromycin induced clindamycin resistant' strains (D-test) positives of clinical isolates of *S.aureus* with and without methicillin resistant. Of a total of 278 isolates, 140 were D-test positives. 117 samples of the 140 were methicillin resistant (84%). 91 (65%) and 49 (35%) samples were hospital and community acquired samples respectively. 118 of the 140 showed other medical conditions existing independently and simultaneously with MRSA infection (Comorbidities). 108 of the 140 had history of prior antibiotic use. Comorbidities and prior antibiotic use were the determinative factors for D-test positivity. All 278 samples were resistant to 17 antibiotics; minimum resistance of 28% to vancomycin and maximum resistance of 97% to gentamicin was recorded. D-test was recommended for assessment and treatment of suppurative infections caused by *S.aureus* [44].

From 2011-2012, a study was conducted in two tertiary care hospitals in West Bengal to assess the etiology, precipitating factors, treatment and outcomes of Disseminated Staphylococcal Disease (DSD) in children. Three inclusion factors for the study were: 1-12 years of age, involvement of two distant organs with gram positive cocci in clusters and/or *S.aureus* growth in one sterile body fluid (and) persistent bacteremia despite antibiotic treatment and involvement of two or more separate tissue sites. 36 cases were obtained under the inclusion criteria with 1-5 year age group being more vulnerable to disease. MRSA was found to be the causative agent for all cases with VRSA being found in 88.9% of cases. All the strains were sensitive to linezolid [45].

In 2012, a study to determine the prevalence and antibiotic profile of CA-MRSA in a rural area of Andhra was conducted. Of 119 Community Acquired – *S.aureus* (CA-SA) and 89 Healthcare Acquired – *S.aureus* (HA-SA), 64.7% and 70.7% were found to be MRSA respectively. CA-MRSA had higher resistances towards ciprofloxacin, erythromycin, gentamicin and cotrimoxazole than CA-SA. HA-MRSA had more resistance to clindamycin and doxycycline than CA-SA. The study indicates that the spread of CA-MRSA is replacing CA-SA in the area and can lead to further spread to other areas [46].

In 2012, a surveillance study in a tertiary care centre in Pondicherry on 172 patients showed the presence of *S.aureus* in 72 isolates of which 51 were confirmed to be MRSA and 21 MSSA, All isolates showed absolute resistance pattern to beta-lactam antibiotics and were sensitive to macrolide and lincosamide antibiotics. Gene distribution of genes femA, mecA and lukS was determined using quadriplex PCR, which showed presence in 100, 94,4 and 69.4% of total MRSA isolates respectively. The study also confirmed the increased presence of CA-MRSA in the local population [47].

In 2012, a variant of Epidemic MRSA 15 (EMRSA 15) was isolated and genotyped from infected tissue of a necrotizing fasciitis patient in Manipal. The strain was resistant to all beta-lactam antibiotics, gentamin, erythromycin and ciprofloxacin. It was sensitive to several non-beta lactam antibiotics such as amikacin, cotrimoxazole, tetracycline, clindamycin, linezolid, rifampcin, teicoplanin and vancomycin. Genotyping revealed SCCmec type IV with presence of pvl gene and enterotoxin gene cluster (egc) by multiplex PCR. PFGE typing revealed the strain to be sequence type 22 which is a closely related variant of EMRSA 15 whose variations may be the reason for increased virulence. Presence of pvl gene indicated that the strain is CA-MRSA type. Epidemiological studies to detect and characterize such strains were emphasized for preventing morbidity and for quicker treatment methods [48].

In2013. a study was carried out in Chennai, Tamil Nadu to assess the prevalence of MRSA in carriers among healthy individuals from various communities. 352 nasal swabs were collected from 352 individuals with no history of hospitalization or antibiotic treatment for one year. 103/352 of individuals had *S.aureus* in their nasal carriage. Of these, 13 were determined to be MRSA by SCCmec screening. PVL gene screening revealed 25/103 to be CA-SA type of which 4 were MRSA. Exotoxin production by the strains was done by agr gene screening revealing 21 strains (12.6%) having these genes. The study stressed the need for screening of pvl, MRSA and exotoxin producing genes in carrier isolates of aysmptomatic individuals in closed communities thus preventing spread of CA-MRSA infections [49].

In 2013, a study was carried out in a tertiary care centre in Odisha for surveillance of drug resistant uropathogens in hospitalized patients for a period of 18 months. Samples were collected at six month intervals. Of 1245 samples, 996 showed presence of pathogens of which 152 were confirmed to be *S.aureus*. 75-85% of 152 samples showed resistance to aminoglycoside antibiotics; 74-85% of samples were resistant to beta-lactams; 68-86% were resistant to cephalosporins; 45-83% were resistant to fluoroquinolones; 26% were resistant to vancomycin. Suitable control measures such as personal hygiene, proper antiseptic measures and public awareness is necessary to prevent spread of MDR pathogens in hospitals and community [50].

CONCLUSION

Phenotypic data by antibiogram testing and D-test shows high levels of resistance in MRSA to beta-lactam class of antibiotics as well as cephalosporins with lower levels of resistance towards vancomycin and increasing inducible resistance, particularly towards clindamycin. Genotypic data shows lower prevalence of VRSA as compared to MRSA in both community and health care settings. Also, the prevalence of CA-MRSA in the community being carried by healthy individuals is increasing as observed in certain studies. The prevalence

of enterotoxin genes and virulence genes also point to the increased spread. Predominant SCCmec types found in the studies are types I, III and V. PFGE pulsotype data reveals that Indian strains are closely related to UK, Brazilian and Hungarian clones.

All of the studies, including global surveys and reviews, insist on the need for increased intensive survey for MRSA on small scale on a regular basis that is necessary for correct assessment of MRSA threat by determining its antibiogram patterns and genetic character that will allow determination of dispersion patterns for prevention of further spread of the organism. In addition, the studies also recommend modification of health practices to spread awareness of MRSA and to plan and implement preventive measures to restrict the further spread of MRSA in the populace.

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