DISCUSSION
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The epidemiology of antimicrobial resistance began in the modern era with penicillin resistant *Staphylococcus aureus*. Resistance actually began several million years ago, as bacteria were exposed to naturally occurring antimicrobial agents and thus developed resistance mechanisms. Many of these resistance mechanisms lie dormant awaiting the introduction of new antimicrobials. Introduction and successful development of therapeutic class of agents represents a significant medical achievement, this success had also led to complacency within both society at large and the scientific community with regard to the development of bacterial resistance. In spite of the increased understanding of the factors contributing to the development of resistance over the last 60 years, the extent of this problem has not decreased with time and is currently among the strongest global threats to the treatment of infectious diseases. The degree to which this problem has progressed is demonstrated by the fact that resistance has developed against all available classes of antibiotics which results in arising number of antibiotic resistant strains in every ecological niche. This has, therefore, become a great public health problem worldwide.⁴⁶⁵,⁴⁶⁶

Among the resistant pathogens, methicillin- resistance *Staphylococcus aureus* (MRSA) is of great concern because of the predominance of this organism that causes various clinical infections, including those acquired in the community or hospitals ⁶,⁷,⁴⁶⁷,⁴⁶⁸,⁴⁶⁹,⁴⁷⁰. It has also been established in veterinary science ¹³,⁴⁷¹,⁴⁷², foods and food initiated outbreaks ¹⁴,⁴⁷³,⁴⁷⁴, air ¹⁶,⁴⁷⁵,⁴⁷⁶ and water ecosystem.¹⁸

In view of the emergence of alarming antibiotic-resistance among microbial population worldwide specially among MRSA ⁴⁷⁷,⁴⁷⁸,⁴⁷⁹,⁴⁸⁰ the present study was undertaken with the aim of determining the spread of most prevalent antibiotic resistant *Staphylococcus aureus* strains from different ecosystems to human beings in health as well as disease conditions.
These studies have been particularly focused on *S. aureus* especially MRSAs spread and thus, a total of 4885 samples were initially obtained which yielded as many as 1160 *S. aureus* strains from diverse sources including various ecosystems and clinical environment. The strains, under study, were examined by the standard methods (Table-8) and most popular antibiotics in use.

**EPIDEMIOLOGICAL APPROACHES:**

**Incidence/Spread of *Staphylococcus aureus*, especially Methicillin-Resistant *Staphylococcus aureus* (MRSA) in different environments:**

**Clinical specimen:**

A considerable number 284 (24.4%) of *S. aureus* strains were recovered from clinical source. Out of examined *S. aureus* isolates from clinical specimen, a frequency of 31.3% exhibited resistance to methicillin. Among these MRSA isolates the highest number was found in pus (34.5%) followed by blood (33.3%) and respiratory tract infection (32.3%). The incidence of MRSA isolates being drastically high in wound infections appeared to be alarming. The open wounds and the frequent dressings often necessitate a dressing team or multiple persons coming in contact to the patients, immunosupression of such wound patients might lead to MRSA colonization/spread from the wounds. The present study further confirmed that frequency of MRSA occurrence from wound remained consistently higher from this tertiary hospital care as reported from our laboratory (35.5%) earlier.

**Ocular environment:**

The characterization of bacterial ocular flora and its susceptibility pattern is highly justified as it gives powerful tool to help in the prophylactic surgery treatment. Recent reports, of catastrophic eye infections caused by MRSA have led us to pay attention separately from other clinical specimens in order to know their molecular epidemiology. The proportion of *S. aureus* infections resistant to methicillin in ocular environment varied from 3% to 30% in earlier reports. The overall resistance to methicillin in our ocular *S. aureus* was found to be about 11.2% which is in agreement with these reports. Interestingly, the
prevalence of *S. aureus* in the present study was found higher in cases of keratitis (60.9%) than conjunctivitis (30.4%) but MRSA involvement in keratitis patients was less (9.3%) than conjunctivitis (12.5%). Similar observation was made by Freildin et al., where they have reported a total of 78.0% patients with MRSA had blephariconjunctivitis and 14.8% had keratitis. As expected, people of older ages were found more prone to the MRSA infections. The ratio of eye involvement in male was higher than female in this study, but was not clinically significant.

Our data have shown that rapidly growing proportion of ocular isolates of *S. aureus* was resistant to ß-lactam antibiotics tested. The MRSA isolates showed low ciprofloxacin and high tetracycline resistance in contrast to earlier study, which reported high ciprofloxacin and low tetracycline resistance. The susceptibility profile further suggests vancomycin to be the best choice to treat MRSA infection in ocular environment as no case resistant to this was found.

**Hospital Environment:**

The relevant nosocomial pathogens can persist for months on dry inmate surfaces. In addition to this, factors like low temperature, high humidity also helps in longer persistence of *S. aureus*. In hospitals, surfaces with hand contact are often contaminated with nosocomial pathogens and may, therefore, serve as vectors for cross transmission. Compliance rates of healthcare workers in hand hygiene are known to be around 50% and leads to the transfer of infection to patients or environmental surfaces. During outbreaks, the environment is known to play a significant role in transmission of MRSA. The presence of MRSA in wards from various inanimate objects (9.6%) and settle plates (22%) indicated the potential source of airborne transmission as well as spread through direct contact. Since MRSA isolates have been recovered from many sites, including floors, linens, medical equipments and hospital furnishing, thus the transmission via inanimate environments poses significant risk to the personnel. Shiomic et al. mentioned that MRSA counts remained elevated for upto 15 min after bed making was complete. The contribution of bed making to the shedding of MRSA from colonized or infected patients may also be exacerbated by inadequate ward or patient's space.
The anterior nares are said to be the ecological niche for *S. aureus* microorganisms from where the bacteria can spread to other parts of the body\textsuperscript{1155,169}. When preventive strategies were applied to eliminate nasal *S. aureus* by topical treatment with antibiotics, the organism disappeared from other sites of the body in most cases\textsuperscript{1156}. In view of this fact, there is overall agreement that sensitivity of nose swabs in detecting MRSA carriage was reasonably high\textsuperscript{497,498,499} thus our study is confined to nasal specimens for reason of accessibility, compliance and consistency along with other investigations. Patients with only MRSA colonization can be significant source for the spread of MRSA in hospitals\textsuperscript{500,501,502,503}.

The overall prevalence of MRSA (18.5\%) from this study is in agreement with earlier reports, where the reported incidence of MRSA in India was found between 6.9 to 51.6\%.\textsuperscript{504} A significant difference was observed in the nasal carriage between hospital inpatients and health care workers. Perhaps this result can be attributed to the patient’s longer stays in hospital, age, underline risk factors, drug dependency, infection and close contact with the surroundings. We could not detect major difference in resistance profile of MRSA carriage in patients and health care worker. The MRSAs from hospital environment were known to be resistant to multiple antimicrobial agents and our finding is in agreement to previous studies\textsuperscript{505}. In general elevated rates of multi-drug resistance may emerge from diverse isolates of *S. aureus* under antimicrobial pressure or as a result of widespread person-to-person transmission of multidrug-resistant isolates\textsuperscript{124}.

**Community Settings:**

Traditionally, MRSA has been considered a major nosocomial pathogens in health care facilities, but in the past decade it has been observed emerging in the community as well \textsuperscript{6,7,506}. The hospital is the principal source of medical care and the place where people receive expanded spectrum antibiotics. But, scenario is changed nowadays because the patients are receiving potent antibiotics at homes too, both intravenously and orally. Besides, some patients undergo surgery in out-patient centers where they receive antibiotics. Similarly, the physician’s office and other
care environments are important places where patients are given many antimicrobial
drugs.

Infections caused by community associated MRSA (CA-MRSA) in patients
generally lacking traditional MRSA risk factors with onset outside healthcare setting
had been increased globally\(^\text{17}\). Clinical syndromes caused by these MRSA range
from skin and soft tissue infections to necrotizing pneumonia\(^\text{264,507,508,509}\) and
sepsis\(^\text{264,507}\)

The reason for the infection with CA-MRSA typically known to occur in
settings is multiple and can be due to prolonged close contacts between individuals,
such as families, schools, hospital nurseries and sport teams and in situation where
hygiene is poor such as facilities for homeless persons\(^\text{254,261,510,511,512}\). Henceforth, in
our studies, the prevalence of \(S.\) \textit{aureus} nasal colonization in community
environment was recorded to be 22.9% and the rate of MRSA strains among the \(S.
\) \textit{aureus} isolates was 10.2% which is in agreement to the findings of Lu et al\(^\text{513}\).
However, contrary to this, higher prevalence rate upto 48.6% of MRSA has been
reported in community from Turkey\(^\text{514}\).

Children were known as heavy colonizer of CA-MRSA strains with or
without risks factors than adults\(^\text{246,248,253}\). Similarly, age is found to play a significant
role for \(S.\) \textit{aureus} colonization in subjects under 15 years and old age in this study.
The carriage rates of \(S.\) \textit{aureus} varied among different ethnic groups and are
dependent on age\(^\text{1,256,515,516,517}\). The rate of resistance, to all the antimicrobials tested
except vancomycin among our MRSA isolates, was higher than that in a previous
study\(^\text{518}\). The rates of multiple drug resistance among MRSA were also higher than
those presented in other reports\(^\text{150,263,519}\). The prevalence results were similar to the
finding of Lu et al\(^\text{513}\) and Boyle-Vavra et al\(^\text{520}\). Much of our current understanding
of MRSA in rural communities comes from reports of outbreaks or smaller case
series\(^\text{255,257}\). In some rural areas the MRSA incidence approaches or exceeds what
has been reported from larger hospitals in urban areas. Stevenson et al\(^\text{521}\) have
reported 44% of CA-MRSA incidence in rural communities, similar trend was
observed here too, where, the prevalence of MRSA in rural population (9.2%) was
higher than the urban population (6.8%). A mere speculation could be drawn that the
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poor health hygiene in rural community may contribute to higher MRSA colonization/carriage. We observed that the rate of MRSA colonization (13.8%) in Out Patient Door unit (OPD) of tertiary care hospital was higher than the rural and urban community population. The reason could be the more antibiotic within the hospital settings and possible exchange of clones through contact with the health care workers.

Veterinary Environment:-

It's an established fact that animals may serve as reservoirs for infections of human beings \(^{12,471}\) and the transfer of resistant bacteria from farm animals to farmers has been demonstrated in several studies.\(^{13,522}\)

The goal of this study was, therefore, to determine the proportion of \(S.\ aureus\) isolates from dairy herds that were methicillin resistant and to look into the clonal spread of MRSA isolates, if any, between the dairy herds and personnel coming in contact with them.

The present investigation, considered bovine mastitis which is the single most common cause for the antibacterial use in lactating dairy cattle.\(^{523}\) Therapy treating this disease is also the most common source of illegal antibacterial residues in market milk.\(^{524}\) Antibacterial therapy of bacteria induced diseases in cattle has been incriminated as a catalyst for the resistance in bacteria isolated from treated animals, other animals within the herd and food derived from cattle for human consumption.

A recent comparison of pig farmers and non-farming controls had postulated that farmers were at significantly greater risk for colonization.\(^{522}\) Our study, however, demonstrated a frequency up to 16.8% of \(S.\ aureus\) colonization in dairy personnel. Comparison of MRSA carriage rates reported in different studies is difficult since various sampling strategies and isolation methods are used for assessing staphylococcal carriage.

The \(S.\ aureus\) isolates of clinical herds displayed significantly higher degree of antimicrobial resistance than isolates from normal dairy herds against the drugs tested. This finding infers the use of antibiotic therapy in clinical dairy herds, the
following inference can be justified by previous study.\textsuperscript{525} The MRSA prevalence in dairy cattle were known to range between 2.2\% to 22.7\%.\textsuperscript{525,526} We, therefore, found 8.3\% MRSA prevalence in clinical herds and one MRSA strain from dairy personal which is in broad agreement with earlier reports.\textsuperscript{525,526} There was no statistical difference in the resistance profile exhibited by MRSA isolates from mastitis herds and personnel. Kaszanyitzky et al.\textsuperscript{13} were also able to demonstrate a similar ratio of MRSA colonization between dairy herds and personnel where they had found a total 27 bovine and one human MRSA isolate only and all tested strains showed the same susceptibility pattern.

Three possible explanations may be given for the presence of resistant bacteria in farmers than in non farmers. Firstly, farmers may come in contact with more antimicrobial-resistant bacteria from farm animals; these bacteria are then transferred to the farmers if a few/no precautions are taken during contact with animal feces. Second, farmers may be in frequent contact with antimicrobial agents themselves or antimicrobial residues that are given to the animals in the workplace. The third possibility is that farmers receive more antimicrobial agents for other medical reasons.

\textit{Foods:}

Despite its ubiquity as health care acquired pathogens and its increasing reports of community acquired infections, MRSAs have not been previously reported as a cause of outbreaks of gastroenteritis until 2002.\textsuperscript{473} Food has also been implicated as a source of spread of MRSA in one reported outbreak in hospitalized immunocompromised patients.\textsuperscript{474} Gorman et al.\textsuperscript{527} have shown the ability of food borne disease microorganism to become disseminated from naturally contaminated foods, such as fresh chickens to various hands and food contact surfaces in the domestic kitchen. Consumer awareness is of paramount importance in the handling, preparation, cooking and storage of foods.

Our finding could not demonstrate the presence of any MRSA from sample size taken from raw vegetables, fruits chats, milk products and milk, but the prevalence of MRSA (10.2\%) from cattle meat and (9.0\%) from chicken meat was
observed. In congruence of our observation, Lee et al.\textsuperscript{14} have documented the occurrence of MRSA in dairy cattle and chicken. All MRSA isolates were resistant to \( \beta \)-lactam antibiotics and exhibited less susceptibility to erythromycin (87.5\%), and gentamicin (87.5\%). Such profiles of antibiotic resistance occurred rather frequently in many of the MRSA\textsuperscript{272,528}. Surprisingly, higher resistance to Co-trimoxazole (49.1\%) has been shown by these MRSA isolates. The apparent reason may be the use of drug in this geographical region during the time of our sampling. Interestingly, similar antibiogram “PGMETCl” were observed in organism isolated from butcher and cattle meat samples tested. This is a very strong indication showing a possible spread of multiresistant bacteria including MRSA between human and animals. Another strong evidence comes from the history of the use of antibiotics for growth promotion in Europe\textsuperscript{529}. At first Denmark and then the European Union banned the use of antibiotics for growth promotion, thereby, prevalence of resistant bacteria declined in farm animals, in retail meat and poultry, and within the general human population\textsuperscript{530}.

\textit{Water Ecosystem:-

The \textit{S. aureus} and other staphylococci have been considered better organisms for evaluating the hygienic quality of drinking water supply, community pond water and other water resources for several reasons. They are consistently shed from the skin, mouths, nose, and throat of bathers and are opportunistic pathogens that cause a variety of infections, including boils, carbuncle, skin rashes and eye infections as well. They are 5 to 20 times more resistant to chlorine than coli forms and also are more resistant to other halogens disinfectants which results in a longer survival time\textsuperscript{531,532,533}. The prevalence of antibiotic resistant \textit{S. aureus} in water environment has been reported earlier.\textsuperscript{534,535,536}

In the present study conducted on variety of water samples, the incidence of antibiotic resistant \textit{S. aureus} in pond water (33.3\%) was higher in comparison to drinking water (20.2\%). The higher percentage of occurrence might be due to the surrounding environment of the ponds since these ponds were located in different
residential community, and were receiving domestic waste continuously besides being these used for washing purposes.

In the present investigation, we could not detect any MRSA strains in water sources as well as in nasal samples of washermen. Although, community pond water yielded methicillin sensitive *S. aureus* (MSSA) isolates which showed similar antibiotic pattern with nasal specimen. Crystal violet test for these isolates revealed their host specificity to biotype A (human origin) suggesting their similar origin. Bacteriophage results depicted the typable isolates were of same phage type; group III- phage 6 common in MSSA isolates. These results suggested the possible clonal spread of MSSA between pond water and washermen.

As regards the location of the site of pond, the source is situated near the University Medical College and is used for washing hospital clothes. Although, there is no direct proof of the origin of these strains; they might have traveled through hospital clothes to water during washing and to washermen. Some investigations have also detected no nasal colonization with MRSA isolates\(^{537,538}\). Similar to this finding, MSSA strains were isolated in communal whirlpool water associated with professional football team which is again an example of cross transmission of infection\(^{254}\). The contaminated whirlpool water was implicated as the route of transmission of MRSA in a college football team as reported by Begier et al\(^{18}\).

**Air Environment:**

The ambient airborne transmission of this organism in non- hospital environments was demonstrated by the increasing prevalence of methicillin-resistant *S. aureus* infections in the community\(^{6,17,539}\). In view of these facts, we, therefore, air sampled *S. aureus* from different sites including hospital wards, dinning halls, meat shops, dairy farms, homes and offices, to assess the possible role of airway transmission of drug resistant *S. aureus* between person to person and from persons to associated environment and vice versa. In the present study it was found that the frequency of *S. aureus* is higher in indoor environment than outdoor. The recovery of bioaerosols *S. aureus* in higher frequency from indoor environment
than outdoor, it is assumed that elevated concentrations of *S. aureus* remained indoors compared to outdoors due to human activities. The health risks associated with occupant exposure are difficult to calculate because of the few studies that have reported bacterial aerosol concentrations in indoor air and the resultant lack of epidemiologic data available for typical indoor environment.

The antimicrobial susceptibility patterns suggested that the prevalence of MRSA was restricted to hospital environment in our finding. The resistance to penicillin-G was as high as 72.3% in overall isolates which is in agreement with the previous findings. The presence of both biotype A and C of human and animal origin in settle plates, suggesting the possible shedding of the *S. aureus* from body parts by both human and animals in their respective environment. In the light of this finding, Gibbs et al. drawn a conclusion that resistant bacterial forms found inside and downwind of the animal (eg; swine) confinement facilities indicated that resistant organism were being produced and released from these facilities which, could cause a potential human health hazard. The two MRSA isolates, however, were confined to biotype-A (human origin). The mechanism of dispersal of these MRSA into air is not clear from this investigation, but it can be speculated that the probably nose, clothes and the skin might have played a role in its dispersion into the environment. Therefore, MRSA in the form of bioaerosol can contaminate the sir and cause airborne infectious diseases.

In order to differentiate the real methicillin-resistant staphylococcal strains from those showing only phenotypic methicillin resistance, strains presumably having *mecA* were examined for MIC. The MIC value of oxacillin for MRSA (n=176) isolates was found to range between 4-256μl/ml, henceforth broadly distributing into borderline resistant strains (32.2%) and methicillin resistant strains (76.6%) in accordance to the NCCLS recommendations. Both these results were characteristically significant for the determination of MRSA.
PHENOTYPIC APPROACHES:

Biotyping:-

The *S. aureus* biotyping has been a useful tool in tracing or estimating the origin of this organism\(^{300,396,542}\) and also in epidemiological investigation of food-poisoning outbreaks\(^{543}\). In order to explore the possibility of spread of organism and consequently genes, from various environments studied we were able to detect overall 7.1% of MSSA isolates and only 0.6% of MRSA isolates as crystal violet positive biotype-C (animal origin) from personnel, which provided insight to our results, indicating a possible transmission of the organism from dairy herds to human beings. Though, high percentage of MSSA (79.5%) and MRSA (80.0%) isolates remained host specific. The host specificity to higher extent has also been shown by Hennekinne et al\(^{544}\).

The biotyping scheme for MRSA in epidemiological monitoring within hospital group was proposed by Coia et al\(^{397}\). Surveillance study carried out by Gupta et al\(^{391}\) showed that all MRSA strains belonged to the same biotype B. Vedhani et al\(^{394}\) reported typeability of MRSA by biotyping to be 91.2%, maximum number of isolates belonged to biotype B (49.5%) followed by biotype D (34.2%). These scientists had isolated MRSA only from hospital environment, while we have extended our search to many other sources including community, veterinary, foods, and air. We too could biotype our 165 MRSA isolates into four groups (A, B, C & D) and observed highest (43.6%) prevalence of MRSA biotype B which indicated that these strains exclusively belonged to orthopaedic and burn unit wards. The occurrence of MRSA strains exhibiting biotype D was viewed as the most frequently encountered strain within the hospital environment. Our studies have also indicated a considerable percentage (30.9%) of such MRSA strains belonging to biotype D, indicating that these were frequently encountered from hospital as well as in external environment.

Bacteriophage Typing:-

For decades, bacteriophage typing was the standard method for typing of *S. aureus*. The phage typing is still widely used today, despite a number of
drawbacks\textsuperscript{124,457,545,546}. Typing of staphylococci is an important approach in epidemiology, in order to find the similarities and differences of the strains obtained from different sources and also to evaluate the significance of these strains for diverse sources in human infection pathology.

In the present investigation, of the 137 MSSA strains and 165 MRSA strains, 51.8\% and 32.1\% respectively were totally typable by international set of phages. The typeability attained by these isolates was, however, slightly lower than reported earlier\textsuperscript{547} wherein 79.1\% of MSSA and 43.8\% MRSA were typable.

The Phage group III has been known to be predominant in Indian isolates as per studies conducted by National Phage Typing Centre, New Delhi\textsuperscript{548}. Similar types of results have also been obtained in our studies, as we could also get as much as 32.1\% of MSSAs and 45.5\% of MRSAs belonging to this phage group III. It may be seen that predominance of particular phage group could vary from country to country\textsuperscript{549,550}.

Normally, a large percentage of MRSA isolates remains non-typable by the conventional set of phages\textsuperscript{551,552}. We also found 13.1\% typeability attained by MRSA isolates. Mathur et al.\textsuperscript{399}, however, have shown that the use of MRSA phages had increased the percentage of typeability of MRSA strains from 17.6\% with conventional set to 45.6\% with MRSA phage set. Upon subjecting our MRSA strains to MRSA phages a comparatively high typeability (21.6\%) could be achieved.

Various predominant phage patterns amongst the individual groups are usually observed in different studies. As indicated by the phage pattern 80/47/54 and 47/54 reported from Belgian epidemic MRSA strains\textsuperscript{553}. Daugeliene at al.\textsuperscript{554} found group III/77 phage type in MRSA strains and MSSA to 6, 42E and 83A were most frequently encountered. In the present study some phage patterns repeatedly observed amongst the isolates from clinical sources, carriers and environment sources, but no particular pattern was predominant. In case of MSSA strains 6/75/84, 6, 6/83A phage patterns were predominant and 6 phage type attained by maximum numbers of MSSA isolates. Similarly, in MRSA strains 30/33/38 complex was found predominant. Moreover, distribution of Phage type III strains and 30/33/38
complex strains among clinical, ocular, nosocomial, community, vet personnel, food handlers, and washermen indicated that these strains of *S. aureus* including both MSSAs and MRSAs were somehow, related to each other and, therefore, we could speculate a possible transmission of these organisms from one source to another source. This belief is supported by the assumption of Morrison et al.\(^555\) where they had indicated on the basis of phage typing that animals may be a source of infection for human population. Further, Boyce et al.\(^404\) reported an outbreak of MRSA in the burns unit, where they showed that all the isolates had identical or similar phage reactions (29/52/52A/80/6/47/53/54/81). Mathur et al.\(^399\) however, had shown a single MRSA phage (phage 622) dominance in MRSA isolates from National capital region indicating the isolates were clonally related to each other.

**Biochemical and Molecular Approaches:**

*Staphylococcal Cassette Chromosome mec (SCCmec) Typing*:

Determining the SCCmec cassette in MRSA isolates is of great value to understand the epidemiology of these strains. The SCCmec cassette typing allowed us to better identify the clonal nature of the MRSA strains circulating in the particular geographical region during the present study. The origin of SCCmec cassette is still unknown and there has been no report of SCCmec in bacteria other than staphylococci.\(^556\) The mechanism(s) responsible for mecA transfer is still obscure but evidence supports horizontal transfer of mec DNA between staphylococcal species and of the mecA gene between different Gram positive genera.\(^557\) It was assumed that the ccr and mec genes were brought together in coagulase negative staphylococci (CoNS) from unknown source,\(^556,558,559,560\) where deletion in the mec regulatory genes occured, before the genes were transferred into *S. aureus* to finally generate MRSA.\(^140,558,559,561\) So far five different types of SCCmec (I-V) have been defined by particular combination of the ccr complex and mec complex,\(^148,149,556\) beside, several subtypes II A to E and subtypes IVA to IVg.\(^150,152,562,563\) The SCCmec types I, II, and III are predominantly found in hospital-acquired MRSA (HA-MRSA) strains, whereas the SCCmec types IV and V are mainly associated with community-acquired MRSA (CA-MRSA) throughout the
The SCCmec typing and genotyping have provided strong evidence for knowing the independent origins of health care associated MRSA and community acquired MRSA \(^{7,23,56}\). Further, the numerous epidemiological studies have demonstrated that most HA-MRSA infections are due to a relatively small number of epidemic MRSA clones spread worldwide, namely, the Iberian (SCCmec I), the New York/Japan (SCCmec II), the Brazilian/ Hungarian (SCCmec IIIA/III) and the Pediatric (SCCmec IV) \(^{151,566,567}\). Upon analysis of 117 CA-MRSA isolates from three continents suggested the spread of a limited number of clones, which are associated with a particular geographic origin.\(^{266,568}\)

Infact, little work has been done about the SCCmec cassette of Indian isolates until recently only few MRSA strains are typed\(^{569,570}\), this further generated interests to explore the epidemiological aspects of MRSA clones from Indian origin. In the present study, a selected group of MRSA (n=93) isolates were screened for the determination of the occurrence of SCCmec cassette, which revealed three major SCCmec cassette type II, III and IV.

The significant finding of the present investigation was the detection of SCCmec cassette in a total of six ocular MRSA isolates revealing the presence of SCCmec type II cassette in one strain (OCMR-9). To the best of our knowledge the presence of SCCmec type II cassette in MRSA from the eye specimen has not been observed earlier from Indian MRSA isolates and therefore, possibly this is the first study in India, atleast as far as ocular MRSA are concerned. However, SCCmec type II cassette has been more frequently isolated from Japanese and Korean MRSA strains\(^{570}\). The New York/Japan (type II) clone was found to be a predominant clone in the United States\(^{464,571,572}\), Canada\(^{573}\), as well as in Finland, Ireland and United Kingdom\(^{567}\).

The SCCmec Type III cassette with III-A [pt181- a tet' gene absent] variant was found prevalent in nosocomial isolates and clinical specimens in our study. The presence of SCCmec type III cassette both in air MRSA isolates (AMR-1,2) suggested that the strains are linked to nosocomial environment. The presence of two MRSA strains (CA-2,10) with SCCmec type III cassette in community personnel indicates that the origin of nosocomial strains into community had taken
Now the question is whether hospital-origin clones been transmitted into community environment? Simor et al.\textsuperscript{573,574} in this context had evidently revealed that MRSA harboring \textit{SCCmec} type II cassette which was related to the New York/Japan clone having a hospital- origin was the most common cause of community- onset infection among the people in Canada. Thereby, reflecting the movement of CA-MRSA-II from hospital into the community. Moreover, the presence of \textit{SCCmec} type III cassette clones (VMR4) in dairy herds and major food animals (FMR2,6,7) suggested that, either the animals may have acquired \textit{SCCmec} type III cassette endogenously when put on drug previously or might have acquired from some other source.

Further, the investigation of community MRSA isolates, revealed the presence of \textit{SCCmec} type IV\textsubscript{a} which remained confined to personnel in urban and rural residential community. The presence of \textit{SCCmec} type IV\textsubscript{a} cassette has also been reported from ethnic community of Malay, Chinese, Indian, and Filipino from Asian continent\textsuperscript{575}. It was also known to be quite prevalent in France\textsuperscript{576} and Australia\textsuperscript{577}. The CA-MRSA strains were remarkably uniform in their \textit{SCCmec}. Of the vast majority of CA-MRSA strains studied so far, most harbored \textit{SCCmec} type IV\textsubscript{a}, suggesting that in the Indian community environment, this is the most-transmissible and best adapted type of \textit{SCCmec} cassette. However, how, and from where the \textit{SCCmec} has been acquired is yet to be determined. From epidemiological prospective, recovery of \textit{SCCmec} type IV\textsubscript{a} cassette (CA-4,5,6,7) in Out Patient Door unit (OPD) indicates the spread of clones to hospital environment as well in this particular geographical region. Because, the patients infected or colonized with MRSA return to the hospital, this gives much less clear cut distinction between hospital acquired infections and community- acquired infections\textsuperscript{578}. Once introduced into a hospital, the \textit{SCCmec} type IV strains may present a competitive advantage over the predominant endemic multiresistant MRSA clones. It has been suggested that their multiplication and transmission rates are superior to MRSA strains with \textit{SCCmec} type I, II and III cassette\textsuperscript{7}. The term "community-acquired" however, may need to be modified, since MRSA strains carrying \textit{SCCmec} type IV or V are now
introduced from their community site of origin into the hospital setting with the potential to cause nosocomial spread.\textsuperscript{579,580,581}

Surprisingly, the presence of common SCC\textit{mec} type IV\textsubscript{b} cassette among dairy herds (VMR-1,2,3) dairy personnel (VMR-5), major food animals and food handlers (FMR-3,5,1,8,4) indicated the prevalence of same clonal spread of this organism. Similar to our finding, Weese et al.\textsuperscript{582} showed that all tested isolates from horses with clinical infection contained SCC\textit{mec} type IV and similar MRSA clones were predominant in colonized horse personnel in North America, thereby, indicating the relatively widespread transmission of similar clones. While, in their another finding\textsuperscript{583} these workers have identified a concurrent colonization with MRSA harboring SCC\textit{mec} type II cassette between domestic pets and human from six different case studies at different time interval, strongly suggesting a possible clonal exchange between interspecies and also a predominance of similar clones (CA-MRSA-II) in a particular Canadian geographical region.

Detection of the \textit{mec} complex and \textit{ccr} complex were essentially important for the classification and characterization of structural types SCC\textit{mec} in MRSA strains. As different SCC\textit{mec} cassettes carry distinct combinations of \textit{mec} and \textit{ccr} gene complexes, the type of SCC\textit{mec} was determined with these two elements\textsuperscript{556}. All the MRSA isolates under study except six \textit{mecA} negative MRSA strains were, therefore studied for the presence of \textit{mec} complex and \textit{ccr} complex. It was found that the tested strains stayed to their specificity with respective cassette ie; type II SCC\textit{mec} (type 2\textit{ccr} and class A \textit{mec}), type III/III-A SCC\textit{mec} (type 3 \textit{ccr} and class A \textit{mec}) and type IV SCC\textit{mec} (type 2 \textit{ccr} and class B \textit{mec}) as mentioned in literature.\textsuperscript{153,462,584}

Further, we observed that six of the MRSA strains with borderline resistance failed to produce \textit{mecA} gene were subjected to PCR. The probability of this finding can be understood that these low MIC bearing isolates probably did not actually harbor \textit{mecA} gene but the resistance to oxacillin was conferred by the overexpression of the PBP2\textsubscript{a} (penicillin binding protein 2a) protein which lead to the intrinsic resistance\textsuperscript{585,586}. In agreement to this assumption, \textit{mecA} negative strains at low MIC value have also been reported by Lee et al.\textsuperscript{14} in dairy cattle and food
animal isolates where, only 15 out of 28 specimens were found positive by PCR for \textit{mecA} gene. Tai et al.\textsuperscript{587} have also found 10 \textit{mecA} negative MRSA strains out of 68 in PCR assay. Japoni et al.\textsuperscript{588} in clinical isolates, showed the absence of \textit{mecA} gene band at low (2\textmu/ml - 4\textmu/ml) MIC values, which further supports our belief that \textit{mecA} gene was not present in such strains at low MIC values.

\textit{Randomly Amplified Polymorphic DNA (RAPD) assay:--}

In view of the heterogeneity in the natural population of \textit{S. aureus}, RAPD-PCR technique constitutes a valuable tool for tracing the sources of \textit{S. aureus} infections of either human or animal origin\textsuperscript{14,589,590,591} and also has been widely used in tracing the outbreaks in nosocomial settings\textsuperscript{463,592}.

In the present study, 15 selected MRSA isolates were grouped into 6 different patterns in single primer based study. It was observed that isolates from clinical (MR-4,18), community (CA-2), ocular(OC-7), nosocomial (HA-42,48), air (AMR-1), and one each from bovine (VMR-5) and food (FMR-3) shared single pattern (pattern1). Further, isolates with the same RAPD type (pattern1) were within the same antibiotype categories, whereas variation in the antibiogram generated different pattern. In support of this finding, Lee at al.\textsuperscript{14} had documented the RAPD patterns of six of the isolates from animals which were identical to the patterns of clinical isolates from humans. The antibiotypes of the six animal isolates were similar to those of the human isolates.

One community isolates (CA-12) shared a same cluster of group ‘1’ with bovine (VMR-4) and food (FMR-7) isolates at a homogeneity of above 75%, genetic similarity suggested that human and bovine isolates were genetically linked. The genetic similarity of human and bovine isolates between 30% to 80% was observed by Reinoso et al\textsuperscript{589}. A study conducted by Pereira et al.\textsuperscript{590} showed two strains of human origin and one of bovine origin sharing the same amplification profile in RAPD.

Despite of harboring SCC\textit{mec} type IV cassette but with similar antibiotype “PGMETCl” by two MRSA isolates one each from bovine (VMR-5) and food (FMR-3) were found clustered in pattern ‘1’ with human isolates. To achieve higher
discrimination of such strains, a combined DNA fingerprints with 3 primers or primer pairs may be required which is a limitation of this study. Our data at-least in VMR-5 and FMR-3 strains suggested that genome of the bovine and food isolates were identical to the patterns of certain human MRSA isolates, and were a possible source of human infection caused by consuming contaminated food products made from these animals. Once interspecies transfer has occurred, such isolates might become widespread in the environment posing a potential threat to human health.

*Pulsed Field Gel Electrophoresis (PFGE):* -

Pulsed Field Gel Electrophoresis has been known for its great discriminatory power and high degree of specimen typeability, thus it has been accepted as the gold standard for the molecular typing of S. aureus isolates.\(^4\)\(^2\)\(^3\)\(^4\)\(^2\)\(^5\)\(^9\)\(^3\)\(^6\)\(^9\)\(^4\). It has also been successfully used to study the epidemiology of S.aureus nosocomial infection and prevalence of methicillin resistance.\(^5\)\(^9\)\(^5\)\(^6\)\(^9\)\(^7\)\(^5\)\(^9\)\(^8\)

In the present study, the total of 71 strains of MRSA (possessing type II, III cassette) were undertaken for PFGE typing, and a total of 9 PFGE patterns of these strains could be obtained. It was observed that PFGE pattern (A to H) comprised of similar type of MRSA strains with indiscriminate banding pattern. This suggests that most of the MRSA have been acquired by personnel during their current contact to its respective surroundings. PFGE pattern I of OC-9 (harboring type II) had shown unique banding pattern.

As much as seven MRSA (type III) isolates of pulsotype-B showed indistinguishable banding pattern to Hungarian (HUSA 304) epidemic clone. The clonal similarity of type III to Hungarian clone (HUSA 304) had been also reported from two hospitals of South India.\(^6\)\(^0\). Although we have looked at a limited number of strains from Aligarh city (North India), but on the basis of PFGE it was clear that the isolates related to Hungarian epidemic clones had made their appearance in India. Furthermore, the present findings suggested that MRSA isolates of pulsotype-B from the hospitalized patients and medical staff (HA-2,39), the air (AMR-2) and MRSA isolates of pulsotype-E constituted with the nosocomial (HA-9,10,47), and the air (AMR-1) might share a common origin, thereby, indicating that such strains
recirculated among these sources. Henceforth, we presumed firmly that MRSA could be acquired by medical staff and patients through air.

The close association of clinical specimens (n=36) with hospital acquired MRSAs (n=22) constituted in PFGE pattern A to H indicated that nosocomial infections can originate from clinically ill patients. Moreover, the PFGE pattern D constitute almost every source undertaken in the present study, including clinical, ocular, nococomial, community, bovine and foods which further suggested that these MRSA strains representing pulsotype-D were widely spread in this particular geographical region.

Moreover, PFGE pattern of 6 MRSA isolates (type IVa and IVb cassette) from community (CA-12,3), bovine (VMR-5,2) and food (FMR1,3) sources generated 3 pulsotype with one subtype each by one band difference, suggesting the close clonal relatedness of clones within the source and possible transmission of clones had taken place between them at some point of time. Similarly, the indistinguishable but unique banding pattern of the human and three bovine isolates from the same farm, combined with epidemiological temporal-spatial link between the disease in farmer and his cows, provided evidence of a common clonal, aetiology in one episode of disease in both^599. Similarly, Kaszanyitzky et al.13 found indistinguishable PFGE pattern in 7 mecA-positive bovine strains and one human mecA-positive isolate in the same manner.

In the light of PFGE pattern we can say that persons in close contact with MRSA-infected catties including veterinarians, farmers, milkman and persons working in slaughterhouses may become colonized from the bovine source in due course of time.

Phylogenetic Analysis:

In order to further know the homology between our test MRSA strains from different environment a phylogenetic analysis of 16S rRNA gene was undertaken. Interestingly, the sequence of 16S rRNA of one of our ocular isolate MRSA OC-9 (SCCmec Type II) was phylogenetically linked to show more than 99% homology to referral strain (N315, Japan). Similar observation was reported when
whole genome sequences carried out on two health-care-associated MRSA strains, N315 (type II) and Mu50 (Vancomycin resistant *S. aureus*) and one community-acquired MRSA strain MW2 (MW standing for mid-west USA)\textsuperscript{135,136} showed that there were 99.7% nucleotide sequence identity between N315 and Mu50, 94.8% between MW2 and N315, and 94.7% between MW2 and Mu50.

Some of the selected MRSA strains corresponded with minor variation (>99% homology) between clinical (MRS), bovine (VMR3), nosocomial (HA2), community (CA12), food (FMR3) sources. Hanssen et al\textsuperscript{600} however, have obtained 99% homology between staphylococcal species.

The percent homology has further suggested that inspite of different antibiotic pressure, the isolates were able to maintain their conserved sequences.

The following conclusions can be drawn from the present study:

- The resistance undoubtedly emerged in men and animals probably due to indiscriminate use of antibiotics in most of Indian settings against normal as well as pathogenic *S. aureus*.
- An alarming increase in the resistance patterns against β-lactam antibiotics, especially the penicillin-G was recorded among *S. aureus* strains isolated almost from all sources undertaken. High incidence of prevailing resistance among such *S. aureus* isolates, including MRSAs from hospital and other human associated environments clearly indicated the existence of corresponding genes mediating such resistance and thereby exploring their possibility of spread between them.
- Environmental sources such as air, water appeared to act as potential reservoir for the transmission of antibiotic resistant *S. aureus* including MRSA strains to human beings, and being re-circulated among the patients, the air and the inanimate objects in hospital environment.
- Biotyping of the test *S. aureus* isolates of diverse origin exhibited a high percentage of both MSSA and MRSA strains to be host specific, probably revealing the origin from their corresponding source. Further, the MRSA
biotyping scheme by and large, explored the prevailing clonal similarity within their cluster group.

- Use of conventional set of phages and MRSA phage set, though, rendered most of the *S. aureus* population untypable, yet, the phage grouping of the typable strains could clearly establish their clonal similarity existing in their sources of origin.

- The emergence and spread of MRSA strains in hospital and community settings appeared to be at an alarming state. Molecular/biochemical approaches including SCC*mec* complex evidently established a trend to and fro dissemination of hospital and community evolved MRSA strains between these environments.

- The approach of SCC*mec* cassette determination among MRSAs of eye origin yielded the presence of SCC*mec* type II which might be a *first report* of its kind to the best of our knowledge. This finding further increased our thrust to explore new cassette, if any, existing in the ocular environment at genetic level. The incidence of variants of SCC*mec* type IV (a & b) cassette in our MRSA collection further indicated that Northern Indian region might probably be their best adapted environment for existence.

- During PFGE studies, the indiscriminate band patterns obtained in some of selected MRSA isolates with a standard Hungarian epidemic clone further strengthened our view about the existing fact that the spread of a relatively small number of epidemic MRSA clones usually takes place world wide and thus in India too. Moreover, the appearance of similar patterns among MRSA isolates from hospital alongwith most of the human associated environments studied suggested strong dissemination of such clones between infected/colonized human beings and the surroundings, thus, showing a possible exchange of genes again. Thus, virtually patients with MRSA infection or colonization appeared to acquire their strains from external sources.

- The homogeneity attained by RAPD on some of the MRSAs could demonstrate a close relationship of human community as a whole with bovine and its products. The risk for the spread of MRSA from bovine
sources into the human population was, however, low. The persons in close contact with MRSA-infected cattle seemed to disseminate the organisms to farmers, milkmen, and butchers working in meat shops due to colonization.

- A limited number of MRSA isolates one each from five different environments when subjected to the determination of 16S rDNA gene sequencing, revealed them to be homologous to each other suggesting that their gene remained conserved inspite of enormous antibiotic pressures in these environments.

- Extensive future research in this area is required to know much about the reservoirs of resistance genes so as to curb their dissemination from various ecosystems to human beings or viva versa.

Finally, a comprehensive flow chart was proposed to depict a possible transfer of genes governing resistance among *S. aureus* isolates. The spread of these drug resistant *S. aureus* organisms between different environmental sources might be finally reaching to human beings through either routes depicted. The epidemiological studies on the spread of resistant pathogenic/ normal *S. aureus* isolates were indicative of the spread of corresponding genes governing antibiotic resistance especially among MRSA population, posing a potential threat to human being and its surrounding environments.
Selection of resistance due to antibiotic use.

Fig. 1. Possible routes of transmission of antibiotic-susceptible or resistant pathogens between human beings and its surrounding environment.
CONTROL AND PREVENTION OF THE TRANSMISSION OF MRSA:

The spread of infection from patients to patients by contaminated healthcare workers was noted over 150 years ago by Holmes and Semmelweis. Although gloves greatly reduce the risk of hand contamination, they do not obviate the need for hand washing after their removal. Use of a mask may be of some benefit in preventing nasal acquisition of MRSA by healthcare workers. It is difficult in many out-patients practices to identify MRSA. The establishment of electronic medical record systems may aid in this endeavor.

Prescribed treatment regimens should be appropriate in both dose and duration, because inadequate dose or excessive duration of therapy may cause development of resistance. Steps should be taken to decrease the occurrence of new resistant strains by adhering to guidelines advocating against the routine prophylactic use of vancomycin for MRSA infection. Although, vancomycin is certainly the standard for MRSA infection control at present, but wide scale use is risk factor for reduced susceptibility and frank resistance in *S. aureus*. New antibiotic classes with good activity against Gram-positive organisms such as ketolides and oxazolidinones which have shown promise in the treatment of MRSA should be used.

In view of development of resistance against any newly introduced drug over the period of time a physicians/epidemiologists, therefore, need to turn to alternative agents also for the treatment of staphylococcal infections including; (i) Antiseptic benzethonium chloride 0.02%. (ii) Lysostaphin, a zinc metalloproteinase extracted from *S. simulans* that lyses *S.aureus* by disrupting its peptoglycan layer. (iii) An enzyme Las A protease (staphylolysin) a staphylolytic endopeptidase secreted by *P. aeruginosa* which also targets the peptoglycan of *S. aureus* have proved effective in the treatment of *S. aureus* infections.

Traditional antimicrobial susceptibility test methods require at least 24 hrs to perform. Polymerase chain reaction (PCR) analysis has been used to detect contamination with MRSA more rapidly than conventional culture methods. However, PCR is not routinely used by most clinical diagnostic laboratories. Newer detection methods for MRSA including the BBL Crystal MRSA ID system,
Velogene Rapid MRSA Identification Assay, MRSA-Screen, a Slide Latex Agglutination Test, will give promising results within 4hrs or just in 15 min. Therefore, such kits may provide early and accurate detection of colonized individuals which further helps to prevent the spread.

Efforts should be concentrated on minimizing the transmission of all food-borne pathogens regardless of their antibiotic susceptibility, by insistence on good hygiene practices on farms, in abattoirs (poultry), during distribution and marketing of food, in food preparations and finally, by the consumers. Whatever is done, competent surveillance of disease and antibiotic resistance as well as repeated refinement of risk analysis is a necessity.

**FUTURE PERSPECTIVES:**

The Staphylococcal genome is under continuous change, with genetic elements moving in and out. Therefore, new variants of SCCmec will emerge that are not detected by the existing simplex or multiplex PCR methods. This will lead to a revision of PCR methods every time a new variant is discovered. Detailed SCCmec typing is unsuitable for the routine laboratory, but important for epidemiological purposes, especially since SCCmec is part of the nomenclature of international MRSA clones. The significance of SCCmec variants should also be investigated, perhaps by SCCmec-typing from larger collection of isolates. Very little is known about the potential donors, recipients, mechanisms or direction of SCCmec transfer. There is a need to continue the search for the origin of SCCmec and to identify the route and mechanism of transfer as there is a common agreement that SCCmec in MRSA has been transferred from coagulase-negative staphylococci.

PFGE proved to be the most satisfactory method on the basis of its discriminatory ability and reproducibility. The major disadvantage of PFGE and other methods that compare DNA fragments on gels is the difficulty of comparing the results obtained in different laboratories, even when reagents and conditions are standardized. Secondly, it works well for local and short term epidemiological investigation. For this reason, the recent advances in bacterial genomics,
bioinformatics, and DNA sequencing have enriched the molecular tools for better understanding of the origins and spread of MRSA clones

*Spa* typing and multilocus sequence typing (MLST) techniques which has an advantage in terms of speed, interpretations and interlaboratory comparisons can be brought into use for further studies to establish a clonal relationship to nationally and internationally spread clones. The low mutations rate of the seven housekeeping genes of MLST and polymorphic X-region of the protein A gene (*Spa*) requires a long term and global epidemiological study. The data obtained would help in simulation central database on the World Wide Web, along with associated data, which can be accessed via the internet. The determination of Spa types was simplified by software [http://spaserver.ridom.de](http://spaserver.ridom.de). And the seven gene fragments of an *S. aureus* isolates could be submitted to the MLST website [http://mlst.ox.ac.uk](http://mlst.ox.ac.uk), where data can be compared with all of the MRSA and MSSA isolates maintained in the database.