In the celebrated movie Crouching Tiger, Hidden Dragon, two warriors face each other in a closed courtyard whose walls are lined with a fantastic array of martial-arts weaponry, including iron rods, knives, spears and swords. The older, more experienced warrior grabs one instrument after another from the arsenal and battles energetically and fluidly with them. But one after another, the weapons prove useless, each, in turn, is broken or thrown aside, the shards of an era that can hold little contest against a young, triumphant, upstart warrior who has learned not only the old ways but some that are new.

One of the foundations of the modern medical system is being similarly overcome. Healthcare workers are increasingly finding that nearly every weapon in their arsenal of more than 150 antibiotics is becoming useless. Bacteria that have survived attack by antibiotics have learned from the enemy and have grown stronger; some that have not had skirmishes themselves have learned from others that have. The result is a rising number of antibiotic resistant strains. Infections - including tuberculosis, meningitis and pneumonia - that would once have been easily treated with an antibiotic are no longer so readily thwarted. More and more bacterial infections are proving deadly.

The present study was undertaken with the aim of determining epidemiology of clinical and carrier staphylococci and biochemical studies of their acquisition and dissemination of resistance. The rationale of this study was to assess the prevalence of methicillin-resistant staphylococci from different clinical sites of patients, and nose of the hospital workers. We were particularly interested in analyzing the MRSA nasal colonization rate in patients admitted in an Orthopaedic Surgical Ward and the medical personnel and its relation to frequency of MRSA isolates in wound infections. We also observed the prevalence of MRSE in ocular infections and its relationship with nasal colonization of patients and ocular colonization of
hospital workers. The epidemiology of MRSA was analyzed by SDS – PAGE protein profiles and PFGE DNA finger printing patterns of the isolated strains. The results would be useful for medical personnel who need to eradicate the troublesome organisms from the hospital environment. In addition to the reservoir(s) searched, the susceptibility of both MRSA and MRSE isolates to 17 antimicrobial agents were also performed in order to provide update information on the use of appropriate drug(s) in the treatment of MRS infections and to determine the antimicrobial susceptibility pattern (antibiogram) of these strains to be used as a tool in further epidemiological study. Furthermore, the plasmid profiles of staphylococcal isolates and subsequent transformation and conjugation studies were performed in the present study. The isolation of β-lactamase enzyme and its activity against different classes of antibiotics was studied together with the activity of new antimicrobials against MRSA using standard procedures.

Staphylococci are important causes of human infections but are also found as non-pathogenic microorganisms in human samples. The spectrum of S. aureus infections includes toxic shock syndrome, food poisoning, meningitis, as well as dermatological disorders ranging from minor infections and eczema to blisters and scalded skin syndrome. Recent reports have begun to document infections caused by Staphylococcus epidermidis, such as bacterial endocarditis prosthetic heart valve endocarditis, bacteraemia, surgical wound infections, intravascular catheters, post-operative endophthalmitis, conjunctivitis and keratitis. Several other coagulase-negative staphylococci (CNS) species have been implicated at low incidence in a variety of infections. The CNS species Staphylococcus saprophyticus was often regarded as a more important opportunistic pathogen than S. epidermidis in human UTIs, especially in young sexually active females. It was considered to be the second most common cause of acute cystitis or pyelonephritis in these patients.

In the present study highest prevalence of S. aureus was found in pus followed by urine and blood. Likewise the highest number of coagulase negative staphylococcal isolates was found in urine followed by conjunctiva
and pus. Besides, a significant number of staphylococcal isolates were observed from the clinical conditions like post operative infections, bacteraemia, pneumonia, corneal ulcer, prostatitis, septicaemia, mastatitis and endophthalmitis.

Methicillin-resistant strains of staphylococci were identified immediately upon the introduction of methicillin into clinical practice. Methicillin-resistant *S. aureus* (MRSA) was initially identified for the first time in 1961 by Jevons.\(^{39,235}\) Since then strains of methicillin-resistant *Staphylococcus aureus* and methicillin-resistant coagulase-negative Staphylococci have spread worldwide\(^{251,316}\) and have become established inside and outside of the hospital environment.\(^{22,325}\) There are reports of emergence and high occurrence of strains resistant to methicillin from various parts of the world.\(^{480}\) Recent studies have documented the increased costs associated with MRSA infection, as well as the importance of colonization pressure.\(^{147,459}\) Already multiresistant to different classes of antibiotics, MRSA had been reported to acquire resistance to gentamicin and related aminoglycosides,\(^{406,420}\) therefore the treatment of infections due to these organisms and their eradication is very difficult. Constant monitoring of these strains is essential in order to control their spread in the hospital environment and transmission to the community.

Of the 513 clinical strains of *S. aureus*, 180 (65.1%) were methicillin-resistant *S. aureus* (MRSA) and out of 337 coagulase-negative staphylococcal isolates 76 (22.5%) were methicillin-resistant (MRCNS). Of the 180 MRSA strains the highest number was found in pus followed by urine and blood. Of the 76 MRCNS studied the highest number was obtained from urine followed by conjunctiva and nasal swabs of patients having ocular infections. The number of MRSA isolates being drastically high in wound infections, this might be due to the fact that orthopedic unit is a fertile environment for MRSA. The open wounds and the frequent dressing changes often necessitate a dressing team or multiple persons plus the inherent immunosupression of the wound patients might lead to MRSA colonization.

The present study confirms that MRSA remains a mainly hospital-acquired infection, but a significant proportion may be acquired in community facilities.
like nursing and residential homes. The major reservoir of staphylococci in hospitals are colonized/infected in-patients and colonized hospital workers, with carriers at risk for developing endogenous infection or transmitting infection to health care workers and patients, while transient hand carriage of the organism on the hands of health care workers account for the major mechanism for patient to patient transmission. Low prevalence of MRSA colonization in an adult outpatient population indicated that MRSA carriers most likely acquired the organism through contact with healthcare facilities rather than in the community. These data show that care must be taken when attributing MRSA colonization to the community if detected in outpatients or during the first 24 to 48 hours of hospitalization. The risk to patients in terms of transmission of MRSA seems to be influenced strongly by the proportion of patients with colonization at intensive care unit admission and is associated with severity of illness, length of stay, and exposures to antibiotics and medical devices.

Colonization with *S. aureus* can occur soon after birth and at any given time, the nasal carriage rate in adults is estimated at between 20% and 40%. Healthcare workers have a higher incidence of colonization. The carrier state is clinically important because carriers undergoing surgery will experience more infections than non-carriers. Fierobe et al. has shown a relationship between MRSA postoperative intra-abdominal sepsis and nasal colonization of MRSA. MRSA strains are usually introduced into an institution by an infected or colonized patient or by a colonized healthcare worker, and transfer from one patient to another has led to major epidemics in tertiary care hospitals as well as chronic care facilities. Colonization of anterior nares with MRSA carries a significantly higher risk for infection than does colonization by sensitive strains.

Intranasal 2% mupirocin applied twice daily for five days appears to be the most effective topical agent against MRSA and eliminates 91% of stable carrier states. Rohr et al., have demonstrated significantly reduced MRSA levels from nose, groin and axilla after the treatment of nasal mupirocin combined with octenidine dihydrochloride whole-body wash. By
confining the investigation to nasal carriage in healthy state, colonization at other body sites may have remained undetected and the ‘true’ prevalence in this study may have been underestimated. There is, however, overall agreement that sensitivity of nose swabs in detecting MRSA carriage is reasonably high (>70%).\textsuperscript{10,80,85,100,430} and it was decided to confine the investigation to nasal specimens for reasons of accessibility, compliance and consistency with other investigations. The design of the study does not allow one to distinguish between patients who acquired MRSA during their current episode of hospital stay or patients already colonized on arrival to the hospital. Despite this, most of the carriers appeared to have acquired the organisms in hospital settings, as most of the isolates seem to be of the same types of strains as isolated from nose of the hospital workers.

It is imperative we continue with basic infection control principles like hand washing and contact isolation and barrier nursing. There are conflicting reports regarding control of MRSA in hospital settings. Herwaldt\textsuperscript{202} and Fekety\textsuperscript{145} in their papers justified aggressive measures to counteract this emerging health problem. Our study did not look into the cost effectiveness of MRSA screening, but Papia et al.\textsuperscript{369b} have shown that a screening programme for identification of MRSA colonization may be cost effective. The benefit of such a screening program may need to be confirmed by a randomized controlled trial.

From this study we suggest that all patients having history of previous hospital admission and patients admitted directly from nursing homes should be screened for MRSA prior to any elective surgical or orthopaedic operative procedures. We believe that patients for routine elective procedures should have a negative result before undergoing the procedure. If surgery cannot be delayed due to medical reason then prophylaxis against MRSA should be given prior to the operation, though we appreciate this may not have much benefit in case of emergency admissions. However we would suggest that in patients with any of the above risk factors admitted for emergency surgery which may involve prosthetic implant, should be screened as well as given MRSA prophylaxis prior to their operations.
It is well documented that MRSA is likely to be multiple resistant (Figure 4.10). Although methicillin-resistant staphylococci are clinically thought to be resistant to all β-lactams and cephalosporins but our in vitro studies showed that 1.1%, 5.6% 3.9% and 30% of MRSA strains showed susceptibilities to ampicillin, cefazolin, cephotaxime and imipenem, respectively. No MRCNS strain showed susceptibility to any of the β-lactam or cephalosporin antibiotics assayed except imipenem. Methicillin resistance gene mecA is associated with transposons and insertion sequences, including Tn554 and IS431. IS431 is a common insertion sequence in staphylococcal chromosome and plasmids and can be associated with host of resistance determinants. The ability of IS431 elements through homologous recombination to trap and cluster resistance determinants with similar IS elements explain the multiple drug resistance phenotype that is characteristic of methicillin resistant staphylococci.\textsuperscript{15,46}

Broad-spectrum insusceptibility of all 180 MRSA and 76 MRCNS isolates to common antimicrobial agents were observed. Among all 17 antimicrobial agents used only vancomycin was shown to be consistently effective against all MRSA and MRCNS. We did not find any glycopeptide resistant S. aureus in our study, and vancomycin and teicoplanin remain the drugs of choice, although decreased susceptibility as well as resistance to vancomycin has been reported recently.\textsuperscript{87,203,410,434}

Of the 850 staphylococcal isolates, surprisingly about 90%, 80% and 50% strains were resistant to Pencilllin G, ampicillin and co-trimoxazole, respectively. This might be due to the fact that at present time these agents are tremendously used in the treatment of general infections. On the contrary to the multiple resistance of MRSA, MSSA seemed to be much more susceptible to all tested antimicrobial agents except for pencillin, ampicillin and co-trimoxozable. As far as the clinical versus carrier staphylococcal isolates are concerned the general observation was that resistance in carrier isolates was lesser than clinical strains in case of all antibiotics tested. In the present study it is documented that resistance to chloramphenicol and ciprofloxacin was comparatively higher in ocular isolates as compared to staphylococcal strain isolated from other clinical
sources. This might be due to more pronounced use of these antibiotics for treatment of ocular infections.

The high rates of multiple resistance is a direct consequence of hospital and community use of antibiotics and its concurrent selection of resistant strains. In India, as in many other developing countries, antibiotics are readily available over the counter. A person can buy the antibiotic of their choice or the pharmacist will prescribe one just by describing their symptoms. Patients, therefore, treat themselves with the wrong antibiotic or wrong dosage. Thus, patients may then come to the hospital already harbouring resistant strains. These strains may be the cause of endogenous infections or exogenous infections in other patients. Another important cause of resistance is excessive or inappropriate use of antibiotics in hospitals. The magnitude of the problem of multi-resistance is such that clinicians must be familiar with the causes of antibiotic resistance and the measures for preventing or minimizing the emergence of resistance. This study underscores the need for hospital clinicians to be aware of the common bacterial isolates in their unit and their usual antibiotic susceptibility. This is imperative in order to make rational decisions for the prudent use of antibiotics, particularly for empirical therapy.

PFGE, because of its great discriminatory power and high degree of specimen typeability is accepted as the gold standard for the molecular typing of *S. aureus* isolates. It has successfully been used to study the epidemiology of *S. aureus* nosocomial infection and methicillin resistance. Nevertheless, PFGE is time-consuming and labor intensive, in this study PFGE exhibited superiority as a technique for analyzing epidemiology of *S. aureus*. In the present study it was observed that the 61 strains of MRSA isolated in an Orthopaedic Surgical Ward displayed 42 patterns of antibiogram. These 61 MRSA strains were subjected to PFGE analysis whereby 14 PFGE patterns were observed. These results suggest that most of the MRSA appeared to have been acquired by patients during their current episode of hospital stay. Moreover, similarity of PFGE patterns of MRSA isolates from pus, skin and nasal cavity suggest that most MRSA types isolated from pus were derived from the
nasal cavity but some types were derived from the nearby skin and that these microorganisms occasionally cause wound infections.

The association between the administration of antibiotics and the occurrence of MRSA is complex. Since the MRSA status of our sample populations on admission is unclear, it becomes difficult to decide if the antibiotics had (i) selected for resistant strains that were already colonizing on admission, or (ii) supported persistent colonization by excluding competing organisms or by enhancing the adhesion and colonizing capacity of the carriage strains, or (iii) increased the susceptibility of the patients to colonization with MRSA. An association between the use of antibiotics and MRSA has been repeatedly observed in different studies and ciprofloxacin has been identified as a risk factor in outbreak situations.

MRSA acquisition depends on 2 major and independent determinants: colonization pressure and antimicrobial selective pressure. In case of colonization with distinct multiple clones of MRSA, antimicrobial pressure plays a major role; in the case of colonization with a single dominant clone of MRSA, colonization pressure plays a major role. McGown proposed a biological model to explain the relationship between antimicrobial use and the emergence of resistance. At the level of individual patient, antimicrobial treatment leads to a large modification in the endogenous flora. The usual result is that susceptible strains are replaced by resistant ones. At the collective level, antimicrobial use in a hospital unit tends to maintain the presence of multidrug-resistant organisms in inpatients, healthcare workers, and the environment. In cases in which basic infection-control practices are inconsistently applied, these pathogens are implicated in the majority of infections. Antimicrobials such as β-lactams and fluoroquinolones, which are ineffective against MRSA and have excellent tissue diffusion, could promote the acquisition of MRSA by increasing the 'receptiveness' of the patients and thereby allowing the progression towards colonization and infection.

Our observations in Orthopedic Surgical Ward through antibiograms and PFGE patterns indicated that most MRSA infections resulted from colonization in hospital settings, although the role of community-MRSA has
not been taken into consideration. Therefore the prevention and control of drug–resistant infections in hospital settings requires measures to promote the appropriate use of antimicrobial drugs and prevent the transmission of infections. At present, control policies primarily consist of both infected and colonized (asymptomatic) patients and increased staff hygiene measures (principally handwashing compliance). Both of these are intended to reduce patient-to-patient transmission mediated by transiently colonized health workers. In the strongest form of isolation, patients carrying MRSA are placed in dedicated isolation units (IUs). If operated appropriately with designated staff, such units should be effective at preventing almost all transmission to patients elsewhere in a hospital.

The spread of resistance to antimicrobial agents in *S. aureus* is largely due to the acquisition of plasmids and/or transposons. Although transfer of resistance between staphylococcal strains in the laboratory has been shown to occur via transformation, transduction, and conjugation, only conjugative transfer appears to be significant in vivo. In staphylococci, the conjugative transfer of resistant determinants is usually mediated by conjugative plasmids but has also been shown to occur in the absence of detectable conjugative plasmids. Conjugative plasmids, usually 35 to 50 kb, spread resistance determinants between species and genera. Besides transferring the resistance determinants, they can mobilize non-conjugative plasmids, recombine with nonconjugative plasmids to form new plasmids, or acquire and transfer resistance transposons. Studies with human staphylococcal strains indicate that *Staphylococcus epidermidis* is a reservoir of antibiotic resistance genes that can be transferred to *S. aureus* under *in vitro* and *in vivo* conditions.

*In vitro* studies of drug resistance transfer between clinical and carrier staphylococcal strains, was done in the present study. The results indicated that tetracycline resistance was transferred from clinical to carrier isolates. This reflects that the use of antibiotics in humans to treat infections can promote resistance in normal flora.
Plasmid transfer among bacteria provides a means for dissemination of resistance to multiple antibiotics. Resistance to multiple antibiotics is frequently found with S. aureus clinical isolates and is often plasmid mediated. Transduction as a mechanism of gene transfer was first described in S. aureus in 1958 by Ritz. In contrast to the Enterobacteriaceae, transduction is believed to be virtually the only means of transfer of genetic material between staphylococci cells. Although transformation in staphylococci in vitro has been described, practically all cultures of S. aureus contain high levels of nuclease, which can be expected to prevent transformation under natural conditions.

Transfer of resistance in mixed broth cultures by S. aureus has been described and is dependent on the presence of calcium chloride. In some reports, investigators have attributed the transfer in mixed-culture transfer of resistance as being phage-mediated conjugation. This process requires calcium chloride and is abolished by citrate, and transferring particles are often not detectable in donor culture supernatants. Lacy proposes that the bacteriophage is cell bound, causing alterations in the cell surface of either the donor or recipient and thereby allowing plasmid transfer. However, the process of transfer here characterized is not dependent on the presence of calcium ions.

In light of these data, we conclude that conjugation is the probable mechanism for transfer. Although the role of bacteriophage cannot be completely excluded, our experimental data are more consistent with a conjugal transfer process. Cell-to-cell contact was essential for transfer since resistance transfer could not be shown when donor and recipient cells were not allowed in contact with one another, analogous to transferable plasmids well characterized in streptococci. The transfer process also required viable donor cells and was energy dependent.

In order to see the effect of lower temperature as found in the eye and nose environment (i.e. 22 °C) upon the conjugation process, it was observed that the application of lower temperature is having a profound effect upon conjugation transfer frequency. At a temperature of 30 °C there was a marginal decrease and at 22 °C there was a substantial reduction in transfer.
frequency. But at 20 °C there was hardly any conjugation observed as compared to control (at 37 °C). These results indicate that the natural conditions prevailing in the eye and nose environment do not favour the resistance transfer mechanism significantly.

The resistance often is transferable at interspecies and intergeneric levels.\textsuperscript{149,176,246,311,355,471} The transfer of plasmids from \textit{S. aureus} to \textit{E. coli} has previously been demonstrated to be possible.\textsuperscript{177b} However in these studies, the staphylococcal plasmids were modified by \textit{in vitro} manipulation before their introduction into \textit{E.coli} cells. In contrast, the transfer reported here was performed without any alteration of the native plasmid structure. Therefore, these experiments strongly support the hypothesis that genetic material could successfully flow from \textit{S. aureus} to \textit{E. coli} by transformation.

Other Gram-negative bacteria of medical importance may also act as receptors for \textit{S. aureus} plasmids (i.e., bacteria of the genus \textit{Klebsiella} or \textit{Serratia}). After transfer of plasmid DNA from Gram-positive to Gram-negative bacteria, illegitimate recombination is a likely step for stabilization of the exogenous resistance genes.

The MICs for A, G and Ak are shown in Table 4.11. As can be seen, the resistance to A and G were lower in \textit{E. coli} transformants than in \textit{S. aureus}. However, the MIC for Ak was higher in \textit{E. coli}. This result indicates that the Ak resistance gene is very efficiently expressed in \textit{E. coli}.

Although the staphylococcal resistance to methicillin is attributed to the altered PBP\textsubscript{S},\textsuperscript{91,136,131,186,194} but virtually all the penicillin resistant strains of staphylococci isolated from hospital infections owe their resistance to the production of β-lactamase.\textsuperscript{1,24,134} The ‘efficiency of β-lactamase depends not only on the maximum velocity, \(V_{max}\), of hydrolysis of the substrate but also on the Michaelis constant, \(K_m\), of the enzyme, and Pollack\textsuperscript{382b} has suggested the term ‘physiological efficiency’ (defined as \(K_m/V_{max}\)) to describe the efficiency of a β-lactamase at destroying penicillin under physiological conditions. In the present study the substrate profile of β-lactamase so extracted from a methicillin-resistant \textit{S. aureus} against different penicillins and caphalosporins indicated that methicillin and
cephotaxime remain the most stable antibiotics to the staphylococcal β-lactamase. The results indicated that methicillin resistance is independent of β-lactamase production in MRSA.

The recent emergence of MRSA with decreased susceptibility to vancomycin has intensified the search for alternative therapies for the treatment of infections caused by this organism. In this study, the anti-MRSA activity of a synthesized compound in an Inorganic Lab of A.M.U. was determined by in vitro testing. The results indicated that the Nickel-metalo complex of 2-mercaptobenzimidazole has good activity against MRSA (MIC, ≥ 64 μg/ml), and demands to be analyzed for clinical applications in future.

5.1 SUMMARY

The emergence of multidrug-resistant bacteria is a phenomenon of concern to the clinician and the pharmaceutical industry, as it is the major cause of failure in the treatment of infectious diseases. Staphylococci have a record of developing resistance quickly and successfully to antibiotics. This defensive response is a consequence of the acquisition and transfer of antibiotic resistance plasmids and the possession of intrinsic resistance mechanisms. The acquired defense systems by staphylococci may have originated from antibiotic-producing organisms, where they may have been developed and then passed on to other genera. There are reports of emergence and high occurrence of staphylococcus strains resistant to methicillin, especially MRSA, from various parts of the World. These organisms are mostly resistant to multiple antibiotics; therefore the treatment of infections due to these organisms and their eradication is very difficult.

The present study was undertaken with the aim of determining epidemiology of clinical and carrier staphylococci and biochemical studies of their acquisition and dissemination of resistance.

The prevalence of methicillin-resistant staphylococci was determined in 750 subjects infected/colonized with staphylococci providing 850 isolates. Of 850 strains 575 were isolated from clinical specimens, 100 from nasal cultures of hospitalized patients, 125 from nasal and 50 strains were isolated from
ocular swabs of hospital workers. It was shown that 35.1% (180/513) of *Staphylococcus aureus* and 22.5% (76/337) of coagulase-negative staphylococcal isolates were resistant to methicillin. Highest percentage of MRSA (35.5%) was found in pus specimens (n=151) while as urine samples (n=153) contributed 25.0% of methicillin-resistant coagulase-negative staphylococci. 29.7% of methicillin resistant *Staphylococcus aureus* isolates were found in nasal swabs (n=175) while as 10.0% and 16.5% of methicillin-resistant coagulase-negative staphylococci were observed in nasal swabs (n=50) and ocular cultures (n=50) respectively. The results indicated that major reservoir of methicillin resistant staphylococci in hospitals are colonized/infected inpatients and colonized hospital workers, with carriers at risk for developing endogenous infection or transmitting infection to health care workers and patients. Restriction mapping and SDS-PAGE protein profiles of both clinical and carrier methicillin-resistant *Staphylococcus aureus* isolates confirmed the results. The multiple drug resistance of all MRSA (n=180) and MRCNS (n=76) isolates was detected. In case of both methicillin-resistant as well as methicillin-sensitive staphylococcal isolates zero resistance was found to vancomycin followed by fusidic acid while as highest resistance was found to penicillin G followed by ampicillin.

Selective methicillin-resistant staphylococcal isolates were subjected to plasmid isolation and curing treatments to study their mode of resistance. The plasmids were transformed into antibiotic sensitive *Escherichia coli* Strain DH5-α. In one of the experiments plasmid pJMR10 from *Staphylococcus aureus* coding for ampicillin (A'), gentamicin (G') and amikacin (Ak') resistance was transformed into *Escherichia Coli*. Transformation efficiency was about 2 x 10³ transformants/µg of plasmid DNA. The minimal inhibitory concentrations (MICs) for A and G were lower in *E. coli* than in *S. aureus*. However, the MIC for AK was higher in *E. coli* transformants than in *S. aureus*. In one of conjugation studies transfer of erythromycin resistance was observed between clinical and carrier strains of *Staphylococcus aureus*. Furthermore enzyme mediated resistance particularly through β-lactamase was studied in selected strains. The substrate profile of β-lactamase so
extracted was studied against different classes of pencillins and cephalosporins. The results indicated that methicillin remains the most stable penicillin to the staphylococcal β–lactamase.

The anti-MRSA activities of a synthesized compound from an Inorganic Lab of A.M.U; was determined by in vitro testing. The results indicated that the Nickle–metalco, complex of 2-mercaptopenimidazole has good activity against MRSA (MIC > 64 μg/ml) and demands to be analyzed for clinical applications in future.

The widespread occurrence and dissemination of β–lactamase and PBP mediated resistance leading to multiple antibiotics ineffective, thus increasing the cost of health care, needs to be tackled logistically by wise and judicious use of existing antibiotics and by developing ideal and cost effective antibiotics having least chances of acquiring resistance. Furthermore the hospital acquired MRSA infections through colonization of patients and hospital workers demands appropriate and timely measure to counteract this health problem.

5.2 LIMITATIONS AND FUTURE CONSIDERATIONS

Knowledge and understanding of the growing problem of antimicrobial resistance is a pre-requisite for a planned and coordinated scientific response to this challenge. The emerging threat of antimicrobial resistance demands sustained research that will lead to an increased understanding broadly of: microbial physiology, ecology, genetics, mechanisms of resistance, host factors, and of the impact of variable antimicrobial use patterns, preventive, therapeutic, and growth promoting agents and environmental residues on the emergence and spread of resistant organisms and resistance factors. In this direction research community needs genomics and other powerful technologies to identify targets in critical areas for the development of new rapid diagnosis methodologies, novel therapeutics and interventions to prevent the emergence and spread of resistant pathogens. Various infection control interventions have been recommended for limiting the
spread of MRSA in hospitals. The recommendations have included laboratory surveillance for MRSA, implementation of a variety of barrier precautions, isolation procedure and cohorting, eradication of MRSA from colonized patients and staff, and disinfection of the inanimate environment of infected individuals.

The present study though aimed at determining epidemiology of clinical and carrier staphylococci and biochemical studies of their acquisition and dissemination of resistance, has few limitations. The epidemiological study of MRSA infections in S.K. Institute of Medical Sciences and Institute of Ophthalmology although depict the clear picture of prevailing antibiotic resistance patterns but the source of infections would have been clear if molecular biological typing of both nosocomial and community acquired MRSA and MSSA could have been done. Studies have reportedly found little evidence for sustained community spread of important nosocomial MRSA strains, and most patients carrying MRSA in community prevalence studies have had recent hospital exposure. For MRSA, as with all infectious diseases, ultimate control depends on keeping the mean number of secondary cases caused by each case below one. However, when carriage can persist for a long time, the secondary cases by each case may be distributed over several hospital admissions. Even limited community transmission of MRSA, although difficult to detect may profoundly alter the dynamics. Quantifying such transmission is an important area for future research.

As far as the epidemiological study of MRSA infections in Orthopedic Surgical Ward is concerned, the SDS-PAGE whole cell protein banding though help in identification of strains but its role in epidemiology is not clear. Although the PFGE typing is clearly showing the role of carrier–MRSA in nosocomial and self infections but the study would have been of substantial importance if the carrier state before admission to Orthopedic Surgical Ward would have been typed as control.

PFGE has proven to be robust enough to type strains with great resolution, is highly reproducible, and is considered the 'gold standard" technique for
typing MRSA. However, PFGE is time consuming and requires both specialized electrophoretic equipment and software. So there is the need of a simple technique just like PCR based typing of MRSA that relies upon the length of polymorphisms of the hypervariable region of the staphylococcal methicillin resistance gene (mec) for strain resolution. The near future will provide techniques for typing and for prediction of the epidemic capacity or pathogenicity of MRSA strains. Both structural and transcriptional differences in genomes can be revealed using DNA microarray assays. The availability of complete genome sequences of several strains will result in identification of new areas important for understanding the adaptive fitness of MRSA strains in different environments.

The curing experiments, transfer of resistance markers through conjugation and transformation together with the β-lactamase assay against selected antibiotics reveals a missing experimental-tag, the emphasis on which we believe will be a safe and cost effective measure to combat antimicrobial resistance: the use of consumable herbs of medicinal importance. The extracts from these herbs can be studied as curing agents and/or the inhibitors of antibiotic gene transfer and resistance enzymes. The metabolites so identified can be used in combination with antibiotics for counteracting this health problem.

The substantial decreases in frequency of conjugation at lower temperature particularly at 22 °C, the temperature prevailing in the anterior nares of human beings depicts a biological significance. Moreover, the clinical importance of regular nose washing as experienced by some communities such as Muslims, five times in a day while they offer prayers, needs to be analyzed based on population studies.