5. DISCUSSION
DISCUSSION

*Staphylococcus aureus* has long been recognized as an important pathogen in human disease. Staphylococcal infections occur regularly in hospitalized patients and have severe consequences, despite antibiotic therapy. Due to an increasing number of infections caused by methicillin-resistant *S. aureus* (MRSA) strains, which are now most often multiresistant, therapy has become problematic.

*Staphylococcus aureus* is known to be one of the major causes of infections in humans occurring in the community as well as in the hospital. This microorganism, one of the well-studied of all bacteria, continues to surprise us with new pathogenic potentials and new mechanisms of resistance to antimicrobial agents. Up to now one of the most serious problems with respect to treatment is its resistance to methicillin, indicating resistance to all β-lactam antibiotics (Martin, 1994). The recent emergence of intermediate resistance to vancomycin in methicillin-resistant *S. aureus* (MRSA) is potentially even more serious and may result in full resistance to what has often been the only available antibiotic, thus reducing our arsenal in the fight against MRSA infections and creating a major challenge for therapy (Hiramatsu *et al.*, 1997; Smith *et al.*, 1999)

originated from health care setting-related cases or by spread through horizontal transmission within the community may influence how this problem will be addressed. In this study, individuals whose activities involved contact with a health care facility had a significantly higher rate of MRSA colonization than community individuals, even though the health care-facility related subjects had a lower overall \textit{S. aureus} colonization rate than their community counterparts. Age was found to be the most significant factor for \textit{S. aureus} colonization in this study.

5.1 Isolation and characterization of staphylococcus

In the routine microbiology laboratory prompt identification of the Staphylococcal strains up to species level were done by catalase, coagulase and other standard biochemical test. However, during routine screening by slide coagulase test many strains of \textit{S. aureus} are missed due to their poor sensitivity and falsely reported as coagulase negative staphylococci during routine screening process. In our study we performed tube coagulase tests of all 965 staphylococcal strains. Therefore, the main criterion used for the \textit{S. aureus} identification was tube coagulase test. The over all distribution rate of Staphylococcus was 91.90% among the samples collected from blood, pus, sputum, CSF and urine. The distribution rate of Staphylococcus in clinical samples, hospital personnel and healthy individuals were 54.92%, 22.79%, and 22.27% respectively.

The work of Pohload \textit{et al.}, (1987), is supportive of the present study, when a large number of Gram positive cocci were isolated from various blood, urine and
miscellaneous samples at Henry Ford hospital, Michigan USA. Anderson et al., (2000) and Saleao et al., (1999) carried out similar studies in Norwegian and Portugal Pediatric hospitals on hospital acquired infections for three years and found high prevalence of *Staphylococcus aureus* from clinical specimens of blood, urine and pus. Similarly Espersan, (1995) also isolated high number of *Staphylococcus aureus* in samples from blood followed by wounds, lungs, bone joints and heart walls. During the two years of present study the occurrence of Gram positive cocci was that of blood 35% and 36% followed by 36% and 42% for urine 68% in each year, for pus 46%, 42% respectively in 1998 and 1999. High prevalence rate of *Staphylococcus aureus* has been reported from pus samples at National Institute of Health Islamabad, Pakistan by Siddiqi et al., (2002). Whereas, 75 pus samples yielded a 44% incidence *Staphylococcus aureus* is one of the leading causes of blood stream infection and its incidence has increased during recent decades, in hospitals and in older patients (Espersan, 1995). Mirrett et al., (2001) found coagulase negative *Staphylococci*, in blood cultures which were the frequent causes of infection. In the present study, there was no statistically significant increase in the second year among Gram positive cocci as well as among coagulase positive and coagulase negative strains isolated from blood and urine samples. Fridkin et al., (1997, 1999a, 1999b), and Sahm et al., (1999) found that more than 57% blood stream infections occurred due to Gram positive pathogens.

In the present study 436 isolates have showed the resistance to oxacinlin and methicillin. Resistance in pus samples was greater than the other types of samples.
Gentamycin – methicillin resistant *Staphylococcus aureus* strains were initially described by Shanson *et al.*, (1976) and Mc Gowan *et al.*, (1979) to be major pathogens involved in hospital acquired infections in Pitie – Salpetrere Hospital, France. The high incidence of Methicillin resistant strains were found in pus during the present study under discussion (35% - 38%) as compared to an earlier study (Siddiqi *et al.*, 2002) having 12% MRSA in pus. However, Mahmood *et al.*, (2001), reported higher prevalence of MRSA isolates from pus, blood and other sources. While the average prevalence of MRSA strains in Poland Hospital was 2.3% - 59.9% (Hryniewicz *et al.*, 1993; Piechowicz *et al.*, 1993). In European hospitals, Vandenbroucke – Grauls (1994) reported the incidence of MRSA as 60% in ICUs. Leski *et al.*, (1998) pointed out that MRSA was wide spread in carriage sites of patients (health care personnel and hospital environments). Moreover, MRSA has been reported as a frequent cause of Nosocomial and community acquired infections (Gales *et al.*, 2000; Singh *et al.*, 2003). Further, in United States, a retrospective analysis showed that the prevalence of MRSA in 33 hospitals participating from 1996 to 2000 increased from 30.1% to 45.7% in 2000 in inpatient isolates; among outpatient isolates, the prevalence of MRSA increased from 17.3% to 28.6% (Mark, 2002). The prevalence of MRSA has also been determined in different European countries. The highest prevalence of MRSA isolates was noted in hospitals in Portugal (54%) and Italy (43 - 58%), while the prevalence of MRSA was only 2% in participating hospitals from Switzerland and the Netherlands (Fluit *et al.*, 2001a). The major factors associated with MRSA colonization are prolonged
hospitalization, burns, surgery that necessitates intensive care and the use of multiple antibiotics, especially during a prolonged course of treatment (Franklin, 2003; Hidron et al., 2005).

In the present study it was found that the receipt of medical services was the major factor associated with MRSA colonization as well as the high level of multiple-drug resistance in MRSA nasal isolates. These findings may be explained by the high rate of antibiotic use in the country. Another study found that the proportion of patient visits resulting in antimicrobial therapy in primary care units was 13.4% and 31.3% of patients with a diagnosis of the common cold received antibiotic treatment (Chang et al., 2001). These findings are indicative of the presence of strong selective pressure from antimicrobial use in the community.

The present study revealed that highest distribution rate was observed in anterior nares (19.79%) followed by that in forearm (3.62%) and dorsum of palm (2.69%). The distribution of nasal carriage of Staphylococcus aureus was varied among clinical samples, hospital personnel and healthy individuals. The highest rate of Staphylococcus aureus was seen among the adults, followed by children of the clinical samples. The distribution of nasal carriage of Staphylococcus aureus among hospital personnel was the highest among the nurses followed by attenders and doctors. The highest distribution rate of Staphylococcus aureus nasal carriage among the healthy individuals was found in the adults followed by the children.

Similar reports have been documented that the nasal carriage of S. aureus plays a key role in the development of S. aureus infections. It has been clearly
established that it is a major risk factor for the development of infection in certain groups of patients. The community colonization study was performed by Nakamura et al. (2002) the study was conducted to ascertain the prevalence of nasal carriage of MRSA in Nashville. The nasal swabs were collected and a questionnaire was administered to collect demographic data and risk factors. Of the 500 enrolled patients, 29% were colonized with Staphylococcus aureus and of those colonized patients, four were colonized with MRSA. Sattler et al., 2002 conducted a prospective observational study to compare the presence of risk factors for methicillin resistance between CA-MRSA and Community-Acquired Methicillin-Sensitive Staphylococcus aureus patients and household contacts, as well as the demographic and clinical characteristics between patients. Further, methicillin-Sensitive Staphylococcus aureus infections tended to be deep-seated when compared to MRSA infections. This recall the bias of the presence of risk factors was decreased by excluding patients if the investigator or patient’s guardian were aware of the antibiotic susceptibility test results (Nakamura et al., 2002).

Risk factors for acquisition of MRSA include the administration of multiple antibiotics. The nasal bacterial flora is modified when systemic antibiotics are given. Interestingly, older data indicate that increased environmental contamination with penicillin was an important risk factor for colonization of the nares of hospitalized patients with penicillin-resistant staphylococci and for the transmission of penicillin-resistant S. aureus to other patients. It has also been revealed that administration of tetracycline to patients colonized with a tetracycline-resistant
strain of *S. aureus* induced the dispersal of this organism in the environment, thus contributing to further spread. MRSA strains are usually resistant to several groups of broad-spectrum antibiotics that are used on a large scale in the hospital. This mechanism of increased spreading under antibiotic pressure may have contributed to the worldwide increase in the prevalence of MRSA in hospitals (Sahm *et al.*, 1999). In the present study the major risk factor was found to be the age of the individuals. Whereas, among 31-40 year age group the 66.66% isolates were procured.

Asensio *et al.*, (1996) identified six factors that were independently associated with MRSA infection and colonization, namely increasing age, ward type (particularly intensive care units), coma, previous hospitalization, invasive procedures and length of hospitalization. In the present study the length of the hospitalization and age group plays an important role in colonization of *S. aureus*. The prevalence of MRSA varied among the three hospitals included in the study and the differences were statistically significant. This could be attributed to variations related to the rapid identification and strict policies of isolation of patients with MRSA colonization or infection, combined with the restricted use of antibiotics and the hygiene practices employed in each hospital (Fluit *et al.*, 2001). In Shifa Hospital, especially in the intensive care unit where the rate of MRSA was 45%, the situation suggests that some patients may have a greater chance of becoming colonized or infected (Fluit *et al.*, 2001).
It has been reported that over the last decade, methicillin resistant Staphylococcus aureus (MRSA) strains have become endemic in hospitals worldwide. In addition, it is now incipient community pathogen in many geographical regions. The emergence of high levels of penicillin resistance followed by development and spread of strains resistant to the semi synthetic penicillins (methicillin, nafcillin and oxacillin), macrolides, tetracyclins, and amino glycosides has made therapy of Staphylococcal disease a global challenge. By the 1990s, resistance to semi synthetic penicillins had spread throughout the world, compromising the use of these drugs for empiric therapy for Staphylococcal infections in a number of regions. This had lead to increased reliance on vancomycin for treatment on documented MRSA infections. As a consequence, selective pressure was established that eventually lead to the emergence of strains of S. aureus and other species of staphylococci with decreased susceptibility to vancomycin and other glycopeptides. Hiramatsu et al., 1997, have studied and reported the first clinical isolate of Staphylococcus aureus with reduced susceptibility to vancomycin from Japan. This report was quickly followed by similar ones from other countries, including United States (Smith et al., 1999), Belgium (Denis et al., 2002), Germany (Birebaum, 1999), and India (Assadullah et al, 2003). The first clinical infection with vancomycin resistant Staphylococcus aureus was reported in July 2002 from Michigan (Smith et al.,) with second case in Pennsylvania. Further, the second confirmed VRSA from Pennsylvania (Fred et al., 2002) was reported which represents the VRSA isolate from patient in United States. The strains of S aureus with reduced susceptibility to vancomycin have been
recognized in the United States and abroad (Tenover et al., 2001). Such isolates are inhibited by vancomycin concentrations of 8 to 16 μg/mL, which falls into the range of intermediate susceptibility by National Committee for Clinical Laboratory Standards (NCCLS) criteria (Tenover et al., 2001). However, infections caused by these “vancomycin-intermediate S. aureus” have not responded well to vancomycin (Andrade-Baiocchi et al., 2003 and Fridkin et al., 2003). It has been reported that handful of true vancomycin-intermediate S. aureus isolates (which were also MRSA) have been encountered in the United States (Tenover et al., 2001 and Fridkin et al., 2003). The mechanisms of resistance have not been completely defined, but appear to involve non-productive binding of vancomycin to a thickened, poorly cross-linked cell wall (Sieradzki et al., 1999 and Finan et al., 2001). In 2002, 2 isolates of MRSA were encountered in the United States that were fully resistant to vancomycin, with minimum inhibitory concentrations of 32 to 64 μg/mL and >1000 μg/mL. Vancomycin resistance in these isolates arose by yet another mechanism: acquisition of the van A genes, which cause vancomycin resistance in enterococci (CDC., 2002).

Similarly, in the present study the distribution of VRSA and VISA among Staphylococcus aureus isolates from the various samples showed that the overall isolation rate of VRSA found to be 1.63%. The distribution of VRSA was highest 6 in 4 in blood 1 in CSF and 1 in sputumpus and 1 VRSA was obtained from CSF. However, the incidence of VISA among the collected samples was found to be
highest 4 in pus, 1 in CSF and 1 in blood. The distribution of VISA was highest in pus followed by CSF and blood.

Further, in Indian perspective study related to vancomycin resistant *Staphylococcus aureus* is very limited. This super bug has become an alarming threat of the risk of transmission in the community. Strains of vancomycin intermediate *Staphylococcus aureus* (VISA) with vancomycin MIC 8 μg/ml have been reported from Japan, United States, France, United Kingdom and Germany. Most of these have emerged from preexisting MRSA infections. In India, Assadullah *et al.*, (2003) have reported reduced susceptibility to vancomycin against MRSA and CoNS. Keeping this in view Hare Krishna *et al.*, (2006) performed extensive longitudinal study of current situation of vancomycin resistance and have reported the first incidence of VRSA emergence from Northern part of India. Whereas, the present study reports is the only document from southern part of India.

In the present study the overall multi-drug resistance pattern of isolates revealed that 102 isolates were resistant to maximum 11 antibiotics followed by 78 isolates to 10 antibiotics, 92 isolates to 9 antibiotics and 3 isolates to only 5 antibiotics. The total percent resistance pattern of MRSA showed that 23.39% of the isolates were resistant to 11 antibiotics and the lowest 0.68% of the isolates was resistant to only 5 antibiotics. The percent resistance pattern of MSSA showed 44.07% of the isolates were resistant to 1 antibiotic and the lowest 0.25% of the isolates were resistant to only 5 antibiotics.
In present study the rate of multiple-drug resistance among MRSA isolates were also higher than those presented in other reports (CDC., 1999; Gross-Schulman et al., 1998; Ma et al., 2002), in which most of the clinical C-MRSA isolates were susceptible to various antibiotics except beta-lactams. Further they have reported that the rate of resistance to clindamycin (92.9%) among the C-MRSA isolates from individuals without risk factors in this study was also higher than that in a study from the United States (Herold et al., 1998), in which most C-MRSA isolates from individuals without risk factors were susceptible to clindamycin. The high rate of resistance to clindamycin among community MRSA isolates (90.6%) was similar to the rate of resistance among clinical MRSA isolates in Taiwan (94.2%) (Hsueh et al., 2002; Wang et al., 2002), indicating that clindamycin resistance is quite common among community and health care facility-related MRSA isolates in Taiwan. This study indicates that receipt of medical services was the major factor associated with MRSA colonization as well as the high level of multiple-drug resistance in MRSA nasal isolates. These findings suggests the high rate of antibiotic use in the Taiwan community, as shown in a previous study (Liu et al., 1999).

5.2 Phenotypic characterization of MRSA

In present study the minimum inhibitory concentrations (MIC) values were determined for 436 isolates of Staphylococcus aureus from samples collected from the patients, hospital personnel and healthy individuals. MIC values of Staphylococcus aureus were determined against the different groups of antibiotics. The MIC values
of these drugs were evaluated according to the NCCLS interpretive standards. The
Minimum Inhibitory concentrations obtained for each isolates against various
antibiotics. The highest number of isolates from clinical samples had oxacillin MIC
of 24.12% to 64 μg/. The percent of isolates was low at higher and lower MIC values.
From hospital personnel the highest (27.45%) of the isolates have oxacillin MIC
values of 64 and 128 μg/ml each. In healthy individuals the oxacillin MIC values
showed the highest (40%) of the isolates have the MIC value 16 μg/ml and 32 μg/ml
respectively

These findings are similar to those of Odelola et al., (1989) who studied
ampicillin, gentamycin against clinical isolates of Staphylococcus aureus, E. coli and P.
aeruginosa and observed minimum inhibitory and bactericidal activities. Resistance
of these strains was 98% among isolates against ampicillin standard powder and its
four market brands. 63 isolates were inhibited at 2048 μg/ml with standard powder
and 43 isolates were inhibited with standicillin. Maximum number of isolates had
MIC in the range of 128 and 512 μg/ml among rest of three brands (Penbritin,
Ampicillin sodium and Ampicillin). Further, the high resistance in the present
study is supported by other authors. Ravaorinora and Therrien, (1996) reported
MIC of 32-256 μg/ml, for MRSA isolated from Canadian teaching hospital. Only 2%
of isolates had MIC in sensitive range according to NCCLS breakpoints. This higher
rate of Staphylococcus aureus against commonly used antibiotics and their various
brands in isolates under study could be attributed to many factors like misuse/
overuse of antibiotics. Antibiotic use provides selective pressure favoring resistant
bacterial strains. Inappropriate use increases the risk for selection and dissemination of antibiotic-resistant bacteria, which are placed at a competitive advantage. Therefore, the drugs, which are more commonly used, lead to development of bacterial resistance in developing countries, which are generally inexpensive, and popular broad spectrum agent (Calva et al., 1996; Rahal et al., 1997; Sack et al., 1997; Hoge et al., 1998). The emergence and spread of bacterial resistance due to a use of antibiotic, is complex phenomenon. Antibiotic use in clinical practice alone can not explain the high frequency of resistant organism (Col and O’connr, 1987; Kunin et al., 1990) moreover, the excessive use (misuse) is practically responsible for increased rate of resistance, in worldwide in hospital setting. Another factor responsible for development of antibiotic resistance in bacteria could be due to non-access of health workers to health information (Cash, 1996).

Therefore, the minimum inhibitory concentration of the β-lactum antibiotics was lower compared to the above reports.

5.3 Antibiotic susceptibility test

In developing countries the wide spread use of antibiotics leads to increase in resistance profile (Smolinski et al., 2003). S.aureus has always been a stumbling block for antimicrobial chemotherapy and the introduction of new classes of antimicrobial agents is usually followed by the emergence of resistant form of these pathogens (Kim et al., 2004; Hiramatsu et al., 2001). Therefore, surveillance on the antimicrobial susceptibility pattern of s.aureus is of utmost importance in understanding new and
emerging resistance trends and in the management of both hospital and community acquired infection.

In the present study the antibiotic susceptibility testing (AST) was performed for all 856 isolated strains of *Staphylococcus aureus*. Among the MRSA isolates the percent resistance to various antibiotics showed that the highest resistance 79.77% exhibited to β-lactum group of antibiotics followed by 70.87% to aminoglycosides, 65.36% to macrolides, 57.22% to Fluoroquinolones, 39.44% to Cephalosporines and only 3.21% to glycopeptides. These results can be compared with similar reports (Pulimood *et al.*, 1996; Manoharan and Lalitha 1997). Most of the MRSA isolates in this study were resistant to many classes of antibiotics and thus considered as multidrug resistant *Staphylococcus aureus* (MDR-SA). The over all isolation rate was highest among MRSA (50.93%) which was higher than the findings of Mujumdar *et al.*, (2001) from Assam who reported 23.20% of MRSA.

Cephalosporin antibiotics those can be given both orally as well as injection (Dollery *et al.*, 1991). Cephalosporins were being used for more than thirty years. Cephalosporins group was used in previous antimicrobial therapies and was prescribed by doctors, which includes Cefaclor, cephradine, cefoperazone, cefotaxine, ceftazidine and ceftriaxone. This excessive use of cephalosporins in chemotherapy of hospitalized and outdoor patients has led to an increase in resistance among *Staphylococcus aureus*. In present study 40% of isolates were resistant to cephalosporins. These potent antimicrobials are used as prophylaxis and for treatment in variety of clinical situations (Lorhtolary *et al.*, 1995).
Gentamycin is an aminoglycoside most often used because of its low cost and reliable activity against Gram positive bacteria. El Solh, (1986) reported MRSA also resistant to gentamycin, kanamycin and related aminoglycosides. The appearance of gentamycin resistant MRSA (GR-MRSA) is of great concern, as *Staphylococcus aureus* continues to show their resistances against antibiotics and gradually with time build resistance against them (Pavillard *et al.*, 1982).

In the present study over 80% of the isolates have showed the increased resistance to aminoglycosides. The first outbreak of GR-MRSA was reported in 1975 to 1976 and wild gentamycin has been used since early 1970s (Mc Gowan *et al.*, 1979; Shanson *et al.*, 1976). Bacterial killing is concentration dependent, greater the concentration greater the killing of bacteria. Aminoglycoside resistance mechanism in different species varies in different geographic regions. this mechanism is a complex and mechanisms and change with time and geographic area. The resistance mechanism in bacteria is also brought about by protein inhibition and decreased translation of mRNA at ribosomal level (Shannon and Phillips, 1982). Siddiqi *et al.*, (2002) reported 20% aminoglycoside resistance among *S. aureus* isolates at National Institute of Health Islamabad, Pakistan. This resistance could be due to clones of Gm MRSA strains. The changes in the use of antibiotics often lead to parallel changes in resistance patterns, some times with a delay of several years (McGowan, 1983).

In the present study the isolates have showed the resistance to quinolone such as ciprofloxacin and gatifloxacin 72.93% and 60.55% respectively. The
Ciprofloxacin is a DNA gyrase inhibitor, which allows the super coiling to be relaxed and reformed. Quinolones penetrate macrophages and neutrophils and inhibit DNA super coiling hence most of the antibiotics are useful in treatment of infection. In present study, the development of resistance with quinolones to *S. aureus* had been created by previous antimicrobial chemotherapy of either patient before hospitalization or surgery. Moreover, a major reason is the use of combinations of antibiotic groups during treatment and chemotherapy of patients either before surgery increasing uses of quinolones in antimicrobial therapy also increases selective pressure of antibiotics. This study provides an in site in quinolone action and resistance mechanism by which these drugs had increased level of DNA cleavage possibly by altered or mutated *S. aureus* (Anderson *et al.*, 2000). Earlier studies have also reported the resistant range of *S. aureus* with oxacillin resistance range of 0.12 to >4 μg/ml (Jones, *et al.*, 1991), 4 to 64 μg/ml (Visser *et al.*, 1991), which is in agreement to the present study. Therefore, the higher rate of resistance may be due to the increased use of quinolones and alteration or mutation in *Staphylococcus aureus*.

In the present study indicates that the development of resistance against vancomycin, which is normally a drug of choice against MRSA in the range, was found to be 1 to 64 μg/ml. Further, most of the isolates were oxacillin resistant. This is in accordance with the study of Maskell *et al.*, (1989) on oxacillin resistant strains isolated at a London hospital in the U.K. Several MRSA strains with similar level of vancomycin resistance have also been isolated in other countries (Noble *et al.*, 1992;
Cui et al., 2000; Cui et al., 2003; Sieradzki et al., 2003 and Griethuysen et al., 2003). Vancomycin is a glycopeptide antibiotic and glycopeptide resistance has emerged in *S. aureus* with inter-species transfer of resistant gene from non pathogenic *Enterococcus faecalis* in vitro (Noble et al., 1992). Moreover, the cellular modification due to prolonged use of vancomycin results in an increased extracellular material which cause thickening of cell wall (Clark et al., 1993 and Shlaes et al., 1993; Sieradzki and Tomasz, 1997; Cui et al., 2003).

Indian literature showed the incidence of MRSA to be on the rise-the value ranges from 5 to 50% in different institutional studies (Mathur et al., 1994). The incidence of hospital acquired MRSA infections in Indian hospitals has been recorded at between 30 to 80% (Manoharan et al., 1997; Anupurbaet al., 2003). The incidence was as low as 6.9% in 1988 and reached to 24 and 32.%, in Vellor (Pulimood et al.,1996) and Locknow (Mathur et al.,1994) in 1994, respectively and was of the same order in Mumbai, Delhi and Bangalore in 1996 and in Rohtak and Mangalore in 1999 (Varma et al.,2000).

Though the rate of MRSA from various samples showed higher susceptibility to different antibiotics when compared with others, the present study reports high percentage of MDR-MRSA.
5.4 Molecular characterization of S. aureus

The examination of the strains with primers for the van A, B and C genes revealed that 4 VRSA strains in this study were vancomycin-resistant because of the presence of the van A gene, only one showed van B gene but van C gene could not be detected in the isolated VRSA strains.

The large scale development and subsequent spread of resistance to vancomycin has been perceived as a fearsome threat to the already challenging therapy of MRSA. The true mechanism of vancomycin resistance in S. aureus is not known. However, it was initially feared that S. aureus would acquire the van gene that code for vancomycin resistance in Enterococcus spp; this phenomenon was successfully accomplished in the laboratory (Noble et al., 1992). Further, Showsh et al, 2001 (Showsh et al., 2001) have demonstrated the presence of sex pheromone in S. aureus that promotes plasmid transfer in Enterococcus spp. Release of these pheromones by S. aureus with proximity to vancomycin-resistant enterococci causes the transfer of plasmids encoding van gene to the S. aureus. In present study among 14 VRSA 5 strains have showed the presence of van genes. In contrast 9 VRSA strains were negative for van A, van B, van C gene by PCR. Therefore the absence of van A/B genes in the present isolates does not rule out that these strains are not VRSA or VISA. There is another hypothesis which says that cell wall thickening is responsible for the development of vancomycin resistance. The mechanism of vancomycin resistance has been extensively studied with the first clinical VRSA strain, Mu50 (Hanaki et al., 1998; Cui L et al., 2000). Biochemical and transmission
electron microscopy (TEM) examination of the Mu50 cell, suggested that it produces increased amounts of peptidoglycan. More murein monomers and more layers (probably 30-40 layers as judged by cell-wall thickness observed with TEM) of peptidoglycan are considered to be present in the cell wall. As a result, more vancomycin molecules are trapped in the peptidoglycan layers before reaching the cytoplasmic membrane where peptidoglycan synthesis occurs. Moreover, a higher concentration of vancomycin would be required to saturate all the murein monomers that are supplied at an increased rate in Mu50. Besides the vancomycin-trapping mechanism, designated "affinity trapping" (Hiramatsu et al., 1998; Rotun et al., 1999; Marchese et al., 2000) Hiramatsu has suggested that dense accumulation of vancomycin molecules within the thickened cell-wall significantly delays the timing of complete inhibition of cell-wall synthesis by not allowing efficient penetration of vancomycin molecules through the thickened cell-wall layers (Hanaki et al., 1998). The thickened cell wall of VRSA strains become thinner with the loss of vancomycin resistance during the drug free passage and again become thick in resistant mutants. Pallazo et al., (2005) have also demonstrated the thickening of cell wall in vancomycin resistant staphylococci. This could be the possible mechanism behind the vancomycin resistant staphylococcal isolates that we have reported though we could not perform the test for the demonstration of cell wall thickening in these isolates. However, further investigation is essential in this regard to confirm the cell wall thickening by using electron microscopy.