

Occurrence and Antimicrobial Susceptibility Pattern of Community and Hospital associated Methicillin resistant *Staphylococcus aureus* strains in Sikkim.

O. Kunsang Bhutia, T.S.K. Singh

Department of Microbiology, Sikkim Manipal Institute of Medical Sciences, East Sikkim, India

Abstract: The objective of this study was to determine the prevalence and antimicrobial susceptibility pattern of the community-associated (CA) and hospital-associated (HA) methicillin resistant *Staphylococcus aureus* (MRSA) strains in Sikkim. A total of 119 clinical strains of *S. aureus* were studied. Detection of MRSA and antimicrobial susceptibility testing were performed by multiplex PCR and Kirby-Bauer disc diffusion methods respectively. The interpretation of the results was done according to the CLSI guidelines 2008. Reference strains ATCC 29213 (MSSA) and 43300 (MRSA) were used as control strains. Out of 119 *S. aureus* strains, 46 (38.65%) were found to be *mec-A* positive (MRSA). Among 46 MRSA isolates, 38 (82.60%) and 8 (17.39%) were categorized as CA- and HA-MRSA respectively. All MRSA were found to be harboring *pvl* gene. The high and equivalent percentage of CA- and HA-MRSA isolates were found to be resistance to penicillin and co-trimoxazole, whereas resistant pattern to other antibiotics had varied. CA-MRSA strains were also found to be resistance to some of the newer drugs such as rifampicin (2.63%), fusidic acid (2.63%), and linezolid (10.52%), however, none of the HA-MRSA showed resistance to these antibiotics except linezolid (25%). Seven CA-MRSA and four HA-MRSA were found to be multidrug resistant (MDR). The study revealed that the prevalence of CA-MRSA infections is higher than HA-MRSA in Sikkim, which is in contrast to the findings of others elsewhere in India. Moreover, the high prevalence of MDR *S. aureus* strains in this small state of India with inadequate health facility is of major concern.

INTRODUCTION

Penicillin was the most effective antibiotic to treat *S. aureus* infections since 1940s, however the first case of penicillin resistant *S. aureus* strains was reported in 1944¹. Subsequently, penicillinase-stable penicillins such as methicillin and cephalosporins became available in the late 1950s². Soon after the introduction of these drugs, *S. aureus* strains resistance to methicillin was first reported in year 1961, termed as Methicillin-resistant *S. aureus* (MRSA)³. Based on the circumstances of acquiring disease, the organism has been often classified as community-associated MRSA (CA-MRSA) and hospital-associated MRSA (HA-MRSA) and current data suggest these are distinct strains of the bacterial species⁴. In the recent years, CA-MRSA has been increasingly reported as an important pathogen in India⁵, and wide spread in the world^{6,7}. Although both CA- and HA-MRSA are resistant to commonly used anti-staphylococcal beta-lactam antibiotics, the former is usually susceptible to a wider spectrum of antimicrobial agents such as sulphonamides, trimethoprim, tetracycline and clindamycin. However, HA-MRSA is resistant to these drugs and susceptible only to vancomycin⁸. The present study was undertaken to determine the prevalence of MRSA in Sikkim, categorizing it depending upon the settings of acquiring infections and to determine the antimicrobial susceptibility patterns of these MRSA isolates. The result of the study will help us to understand the problem of MRSA in Sikkim and to formulate antibiotic policy in the hospitals and measures for the prevention of further spread of MRSA in the hospital as well as in the community.

MATERIALS AND METHODS

Design and Settings of the study

A point prevalence study conducted in 119 *S. aureus* strains isolated from the various clinical specimens during the period from August 2009 to "10" in teaching hospitals.

Case definition and source of data

Hospital-associated MRSA is defined as one cultured from a clinical specimens obtained \leq 72 hrs after patient's hospital admission or whose sources of isolation were associated with risk factors for HA-MRSA infection (e.g. recent hospitalization, recent surgery, residence in a long-term care facility, drug use)^{9,10}, within one year of MRSA isolation date.

Community-associated MRSA isolate is defined as one cultured < 72 hours of a patient's hospital admission, or from patients whose sources of isolation

were not associated with risk factors for HA-MRSA infection as mentioned above. MRSA isolates which are resistant to e" three non-beta lactam antibiotics were classified as multidrug-resistant MRSA (MDR-MRSA)¹¹. The data of the patients were obtained from the laboratory investigation register and medical record file.

Identification of *S. aureus* isolates

All the isolates were identified as *S. aureus* using standard techniques, including slide and tube coagulase, DNase, Phosphatase and Modified OF (Hugh-Leifson) tests. The *S. aureus* strains were inoculated into semi-solid nutrient agar in the screwed capped vials and stored at -20°C for further molecular analysis.

DETECTION OF MRSA

1. DNA isolation : The test inoculum was prepared by inoculating two to three isolated colonies of *S. aureus* into 3 to 4 ml of BHI broth (Hi-Media) and incubated overnight at ambient temperature of 35-37°C. The DNA was extracted by using the HiPurA™ Bacterial and Yeast Genomic DNA Miniprep Purification Spin kit (Hi-Media).
2. Multiplex PCR for the detection of *mec-A* and *pvl* gene: The primers for the amplification of *mec-A* (Gen Bank accession no. Y00688) and *pvl* gene (accession no. -X72700) were MECAP4 (5'-TCCAGATTACAACCTCCACCAGG-3'), and MECAP7 (5'-CCACTTCATATCTGTGTAACG-3') as described by Oliveria *et al*¹², and luk-PV-1 (5'-ATCATTAGGTAATAATGTCTGGACATGATCCA-3') and luk-PV-2 (5'-GCATCAA GTGTATTGGATAGCAAAGC-3') as described by Mclure *et al*¹³ respectively. PCR was performed by using Qiagen Multiplex PCR kit with slight modification. A 25- μ l final reaction volume consisting of 12.5 μ l mastermix, 2.5 μ l primer mix (0.2 μ M of each primer) and 3 μ l of DNA template and 7 μ l of RNase free water was prepared. DNA samples were subjected to thermocycling conditions with initial inactivation step (95°C, 15 min) with three step cycling condition of denaturation (94°C, 30 sec), annealing (60°C, 90 sec) and extension (72°C, 90 sec) for 35 cycles with final extension (72°C, 10 min) and soak at 4°C. Then 5 μ l of amplified products were mixed with 2 μ l of ethidium bromide (Fermentas) and loaded on a 2% agarose gel (Amresco) along with GeneRuler™ 100bp Plus DNA Ladder (Fermentas) and electrophoresis at 100 volt for 50-60 min and visualized under UV transilluminator (Bio-Doc analyzer, Biometra). Reference strains ATCC 29213 (MSSA) and 43300 (MRSA) were used as control strains.

Antibiotic susceptibility testing of MRSA isolates

Kirby-Bauer disc diffusion method was performed with following antibiotics discs

(Hi-Media); penicillin-G (10 units), co-trimoxazole (25µg), erythromycin (15µg), ofloxacin (5µg), gentamicin (10µg), linezolid (30µg/ml), rifampicin (5µg), chloramphenicol (30µg), fusidic acid (30µg). Five discs in one and four in another agar plate were tested. The testing conditions and interpretation of the test was done as per CLSI criteria¹⁴.

RESULTS

A total of 119 *S. aureus* isolates tested, 46 (38.65%) were found to be *mec-A* positive (Fig 1). Thirty-eight (82.60%) met the definition of CA-MRSA, and 8(17.39%) of HA-MRSA and all MRSA isolates were found to be harboring *pvl* gene (Fig 1). All MRSA isolates were from skin and soft-tissue infections, except one (HA-MRSA) which was isolated from blood culture. The majority of CA (24 or 63.15%) and HA-MRSA (5 or 62.5%) were isolated from male patients in comparison to females. All HA-MRSA strains were isolated from the clinical specimens obtained e⁷² hours of patient's hospital admission.

The results of *in-vitro* susceptibility testing of MRSA are given in (Table1). The very high percentage of CA-MRSA (92.10%) and all isolates of HA-MRSA were resistance to penicillin-G and co-trimoxazole. The resistant pattern of CA-MRSA strains to other antibiotics were not so significant; ofloxacin (23.68%), followed by erythromycin (21.05%), gentamicin (13.15%), linezolid (10.52%) and least (2.3%) with rifampicin, fusidic acid and chloramphenicol. HA-MRSA strains showed 37.5% resistance to erythromycin and 25% to gentamicin, ofloxacin and linezolid, and least with chloramphenicol (12.5%). However, none of the HA-MRSA strains was found to be resistant to rifampicin and fusidic-acid. Among CA- and HA-MRSA, 18.42% (7/38) and 50% (4/8) were found to be multi-drug resistant (MDR) respectively. MDR-CAMRSA isolates were grouped into three resistance profiles (RPs) according to the resistant pattern to the panel of nine antibiotics: RP-I (resistance to P, Co, Lz, E), RP-II (resistance to P, Co, G, Of) and RP-III (resistance to P, Co, G, Of, E, C). Two (28.57%), four (57.14%) and one (14.28%) isolates were fell under RP- I, II and III respectively. Whereas, all four MDR-HAMRSA isolates had different RPs, RP-I (P,Co,Fc,Lz,R,C), II (P,Co,G,E), III (P,Co,G,Of,E,C) and IV (P,Co,Of,Lz).

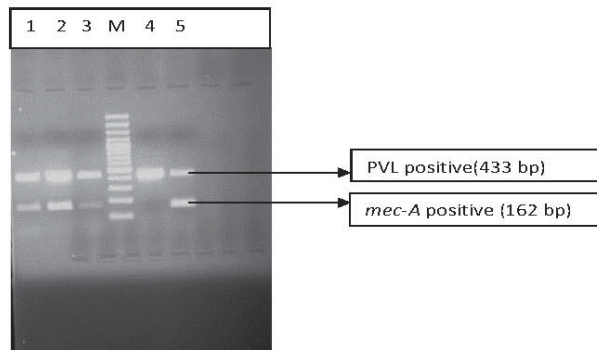


Figure 1: Multiplex PCR (*mec-A* and *pvl* gene). Lane 1,2,3,5 = Positive *mec-A* (162bp) and *PVL* (433bp), M= Marker (100bp DNA ladder), Lane 4 =Negative *mec-A* (162bp) and positive *PVL* (433 bp).

Table 1: Antibiotic resistance pattern of CA-MRSA and HAMRSA isolates.

Antibiotic (µg/ml)	CA-MRSA (n=38)	HA-MRSA (n=8)
Penicillin (10)	35 (92.10%)	8 (100%)
Co-trimoxazole (25)	35(92.10%)	8 (100%)
Erythromycin (15)	8(21.05%)	3 (37.5%)
Ofloxacin (30)	9 (23.68%)	2 (25%)
Gentamicin (10)	5(13.15%)	2(25%)
Linezolid (30)	4(10.52%)	2 (25%)
Chloramphenicol (30)	1 (2.63%)	1 (12.5%)
Fusidic acid (30)	1(2.63%)	0
Rifampicin (5)	1(2.63%)	0

DISCUSSION

The prevalence rate of MRSA in Sikkim was found to be 38.65 %, which is comparable to the prevalence rate reported from different parts of India: Tamil Nadu (35 %)¹⁵, New Delhi (38.56%)¹⁶, Maharashtra (39.1%)¹⁷ except Assam (23.6%)¹⁸. Similarly, MRSA prevalence rate of 26.14% and 35% were reported from Nepal¹⁹ and China²⁰ respectively. On the contrary, an alarmingly high prevalence of MRSA infections (54.85 %) was reported from Uttar-Pradesh²¹. MRSA once regarded as almost exclusively a hospital-associated pathogen has been increasingly identified as a cause of community-associated infections in the recent years^{21, 22}. Several reports from Asia including India have highlighted the prevalence of MRSA in the community and community-acquired pyoderma^{23, 5}.²⁴ The present study has revealed that the prevalence of MRSA in the CA-infections (82.60%) was much higher than the HA-infections (17.39%) in Sikkim. The majority of CA-MRSA strains were isolated from patients with skin and soft-tissue infections, which is in agreement with the finding of Fergie *et al* (2001)²⁵. In contrast high prevalence of HA-MRSA was reported from Maharashtra, India (77%)²⁶, Korea (94.7%)²⁷, and USA (85%)⁷. The low prevalence of MRSA in the community-associated infections in the studies (stated above) might be due to differences in case definitions of CA and HA-MRSA with ours²⁶ and inclusion of patients with bloodstream infections only²⁷, as CA-MRSA is mainly associated with skin and soft-tissue infections²⁵. However, over the years since mid 1990s the prevalence of CA-MRSA has been on the rise⁹.

Antibiogram analysis has been a good epidemiological marker for MRSA. Most contrasting finding in the present study, was very high percentage (92.10%) of CA-MRSA isolates were resistant to co-trimoxazole. On the contrary, Benoit *et al*²⁸ and Gorwitz *et al*²⁹ reported that CA-MRSA strains were susceptible to multiple antimicrobial agents, most importantly co-trimoxazole. On the basis of their findings, co-trimoxazole may be a viable, cost-effective treatment option for many CA-MRSA infections. A study from eastern UP²¹ also reported high percentage of MRSA isolates were resistant to co-trimoxazole. The increased incidence of co-trimoxazole resistant MRSA in India may be due to the misuse of the drug as it is cheap and easily available drug alternative to penicillin, similarly in Sikkim co-trimoxazole is most frequently used antibiotics for the treatment of Staphylococcal infection, in alternative to penicillin suggest possible abuse of this drug in our region. In our study too the number of HA-MRSA isolates resistance to different antibiotics comparatively higher than CA-MRSA, however, very little insignificant number of isolates of CA-MRSA was also found to be resistance to newer drugs like fusidic acid and rifampicin.

Besides time based criteria, in microbiology MRSA has been often categorized based on susceptibility pattern to various antibiotics²⁸. Based on this definition, CA-MRSA has wider spectrum of susceptibility to antibiotics compare to HA-MRSA. In support of this a study has reported 33% of MRSA isolates as MDR-MRSA, where CA-MRSA isolates were less likely to be resistance to antibiotics than HA-MRSA isolates¹¹. Similarly, Fey *et al*³⁰ in their study, reported 87.5% of HA-MRSA was MDR, whereas no MDR was found among the CA-MRSA isolates. Our study in agreement to them, that occurrence of MDR-MRSA strains is more prevalent in HA-MRSA (50%) than CA-MRSA (18.42%), but contrasting at the same time indicating that HA-MRSA strains may be the important reservoirs of MDR strains, but now it is being slowly acquired by CA-MRSA strains. Therefore, our finding proposed that microbiological definition would be unreliable in the near future for the proper categorization of MRSA isolates due to emergence of MDR resistance strains among CA-MRSA as well. Antibiogram pattern of MRSA varies in different geographical areas. Therefore, the choice of antibiotic for the treatment of infections caused by CA-MRSA and HA-MRSA should be guided by the antibiotic susceptibility test of the isolate and or current antibiotic policy whenever possible not based on the type of MRSA infections. The data on the antibiotic susceptible pattern of common bacterial pathogens should be made available to the clinicians.

CONCLUSION

The CA-MRSA has indeed emerged in Sikkim as an important cause of skin and soft tissue infections. The high prevalence of MDR strains among CA-MRSA suggests the significant change in the microbial characteristics and epidemiology of MRSA in the community and hospitals. The possible factors that contribute the increased prevalence of CA-MRSA infections are patients who have acquired the MRSA infections in the hospitals and returned to community without complete cure or asymptomatic carriers and complete treatment and cure of MRSA infected

patients before discharging from the hospitals and proper awareness in use of anti biotics may significantly reduce the further spread of MRSA in the community and as well as in the hospitals.

FUTURE WORK PROPOSED

Future molecular typing (SCC-mec and MLST) of such discrepant isolates, will be helpful to know the genetic basis of MRSA and also to establish the proposed finding that MRSA circulating in our health-care settings are mainly of community origin. It will be helpful in taking appropriate measures in control and prevention of further spread of MRSA.

REFERENCES

- Boyle-Vavra S, Daum RS. Community-acquired methicillin resistant *Staphylococcus aureus*: the role of Pantone-Valentine leukocidin. *Lab Invest* 2007; 87(1): 3-9.
- Sampathkumar P. Methicillin resistant *Staphylococcus aureus*: The Latest Health Scare. *Mayo clinic Proc* 2007; 82(12): 1463-7.
- Jevons MP. Celbenin-resistant *Staphylococcus*. *Br.Med.J* 1961; 1: 124-5.
- Okuma K, Iwakawa K, Turnidge JD, Grubb WB, Bell JM, O'Brien FG et al. Dissemination of new methicillin-resistant *Staphylococcus aureus* clones in the community. *J Clin Microbiol* 2002; 40(11): 4289-94.
- Nagaraju U, Bhat G, Kuruvila M, Pai GS, Jayalakshmi, Babu RP. Methicillin-resistant *Staphylococcus aureus* in community-acquired pyoderma. *Int J Dermatol* 2004; 43(6): 412-4.
- Naimi TS, Ledell KH, Como-Sabetti K, Borchardt SM, Boxrud DJ, Etienne J et al. Comparison of community- and healthcare-associated methicillin-resistant *Staphylococcus aureus* infection. *JAMA* 2003; 290(22): 2976-84.
- Moran GJ, Krishnadasan A, Gorwitz RJ, Fosheim GE, McDougal LK, Carey RB et al. Methicillin-resistant *S.aureus* infections among patients in the emergency department. *N Engl J Med* 2006; 355(7): 666-74.
- http://en.wikipedia.org/wiki/Methicillin_resistant_Staphylococcus_aureus – Categories *Staphylococcaceae / Bacterial disease/Occupational safety and health*. Last modified 2008; 1-9.
- Herold BC, Immergluck LC, Maranan MC, Lauderdale DS, Gaskin RE, Boyle-Vavra S et al. Community-acquired methicillin-resistant *Staphylococcus aureus* in children with no identified predisposing risk. *JAMA* 1998; 279(8): 593-8.
- Garner JS, Jarvis WR, Emori TG, Horan TC, Hughes JM. CDC definitions for nosocomial infections. *Am.J.Infect.Control* 1988; 16(3): 128-40.
- Charlebois ED, Pendreau-Remington F, Kreiswirth B, Bangsberg DR, Ciccarone D, Diep BA et al. Origins of community strains of methicillin-resistant *Staphylococcus aureus*. *Clin Infect Dis* 2004; 39(1): 47-54.
- Oliveira DC, Lencastre H de. Multiplex PCR strategy for rapid identification of structural types and variants of the mec element in methicillin-resistant *Staphylococcus aureus*. *Antimicrob Agents Chemother* 2002; 46(7): 2155-61.
- McClure J, Conly JM, Lau V, Elsayed S, Louie T, Hutchins W et al. Novel multiplex PCR assay for detection of the staphylococcal virulence marker Pantone-Valentine Leukocidin Genes and simultaneous discrimination of methicillin-susceptible from -resistant staphylococci. *J Clin Microbiol* 2006; 44(3): 1141-4.
- Clinical and Laboratory Standards Institute/ NCCLS, 2008. Performance standards for Antimicrobial susceptibility testing; Eighteenth Informational Supplement. Approved standard M100-S18; Vol:28 No1. Clinical and Laboratory Standards Institute, Wayne, PA.
- Prakash M, Rajasekar K, Karmegam N. Prevalence of methicillin-resistant *Staphylococcus aureus* in clinical samples collected from Kanchipuram town, Tamil Nadu, South India. *Journal of Applied Sciences Research* 2007; 3(12): 1705-09.
- Mohanty S, Kapil A, Dhawan B, Das BK. Bacteriological and antimicrobial susceptibility profile of soft tissue infections from Northern India. *Indian J Med Sci* 2004; 58(1): 10-15.
- Kandle SK, Ghatole MP, Takpore AY, Hittinalli VB, Yemul VL. Bacteriophage typing and antibiotic sensitivity pattern of *Staphylococcus aureus* from clinical specimen in and around Solapur (South Maharashtra). *J Commun Dis* 2003; 35: 17-23.
- Majumdar D, Bordoloi JN, Phukan AC, Mahanta J. Antimicrobial susceptibility pattern among methicillin resistant *Staphylococcus aureus* isolates in Assam. *IJMM* 2001; 19(3): 138-40.
- Kunari N, Mohapatra TM, Singh YI. Prevalence of Methicillin-resistant *Staphylococcus aureus* (MRSA) in a Tertiary-Care Hospital in Eastern Nepal. *J Nepal Med Assoc* 2008; 47(170): 53-6.
- Wang H, Liu Y, Sun H, Xu Y, Xie X, Chen M. In vitro activity of ceftobiprole, linezolid, tigecycline, and 23 other antimicrobial agents against *Staphylococcus aureus* isolates in China. *Diagn Microbiol Infect Dis* 2008; 62(2): 226-9.
- Anupurba S, Sen MR, Nath G, Sharma BM, Gulati AK, Mohapatra TM. Prevalence of methicillin resistant *Staphylococcus aureus* in a tertiary referral hospital in eastern Uttar Pradesh. *IJMM* 2003; 21(1): 49-51.
- Moran GJ, Amii RN, Abrahamian FM, Talan DA. Methicillin-resistant *Staphylococcus aureus* in community-acquired skin infections. *Emerg Infect Dis* 2005; 11(6): 928-30.
- Saxena S, Singh K, Talwar V. Methicillin-resistant *Staphylococcus aureus* prevalence in community in the east Delhi area. *Jpn J Infect Dis* 2003; 56(2): 54-6.
- Tan HH, Tay YK, Goh CL. Bacterial skin infections at a tertiary dermatological centre. *Singapore Med J* 1998; 39(8): 353-6.
- Fergie JE, Purcell K. Community-acquired methicillin-resistant *Staphylococcus aureus* infections in south Texas children. *Pediatr Infect Dis J* 2001; 20(9): 860-3.
- Tambekar DH, Dhanorkar DV, Gulhane SR, Dudhane MN. Prevalence and antimicrobial susceptibility pattern of Methicillin Resistant *Staphylococcus aureus* from healthcare and community associated sources. *Afr. J. Infect.Dis.* 2007; 1(1): 52-6.
- Park SH, Park C, Yoo JH, Choi SM, Choi JH, Shin HH et al. Emergence of community-associated methicillin-resistant *Staphylococcus aureus* strains as a cause of healthcare-associated bloodstream infection in Korea. *Infect Control Hosp Epidemiol* 2009; 30(2): 146-55.
- Benoit SR, Estivariz C, Mogdasy C, Pedreira W, Galiana A, Galiana A et al. Community strains of methicillin-resistant *Staphylococcus aureus* as potential cause of healthcare-associated infections, Uruguay, 2002-2004. *Emerg Infect Dis* 2008; 14(8): 1216-23.
- Gorwitz RJ, Jernigan DB, Powers JH, Jernigan JA, and Participants in the CDC Convened Experts' Meeting on Management of MRSA in the Community. Strategies for clinical management of MRSA in the community: summary of an experts' meeting convened by the Centers for Disease Control and Prevention. 2006.
- Fey PD, Said-Salim B, Rupp ME, Hinrichs SH, Boxrud DJ, Davis CC et al. Comparative molecular analysis of community- or hospital-acquired methicillin-resistant *Staphylococcus aureus*. *Antimicrob Agents Chemother* 2003; 47(1): 196-203.

Tolvaptan

DRUG PROFILE

Mechanism of action: Tolvaptan is a selective vasopressin V2- receptor antagonist with an affinity for the human V2-receptor 1.8 times that of native vasopressin. Tolvaptan antagonize the effect of vasopressin and cause an increase in urine water excretion that results in an increase in free water clearance (aquaresis), a decrease in urine osmolality, and a resulting increase in serum sodium. Urinary excretion of sodium and potassium, and plasma potassium concentrations are not significantly changed, serum potassium levels should be monitored in situations of serum potassium >5 mEq/l or hyperkalemia. **Pharmacodynamics/pharmacokinetics:** In healthy subjects receiving a single dose of tolvaptan 60 mg, the onset of the aquaretic and sodium-increasing effects occurs within 2 to 4 hours post-dose. The peak effect of about a 6mEq/l increase in serum sodium and about a 9mL/min increase in the urine excretion rate occurs 4 to 8 hours after the dose; the effects of tolvaptan in the recommended dose range of 15 to 60 mg once daily appear to be limited to aquaresis and the resulting increase in serum sodium concentration. At least 40 % of the dose is absorbed as tolvaptan or metabolites. Peak concentrations of tolvaptan are observed between 2 and 4 hours post -dose. Tolvaptan is eliminated entirely by nonrenal routes and mainly, if not exclusively, metabolized by CYP 3A. Moderate or severe hepatic impairment or congestive heart failure decrease the clearance and increase the volume of distribution of tolvaptan. **Indication and Important Limitations:** (Tolvaptan) is indicated for the treatment of clinically significant hypervolemic and euvolemic hyponatremia (serum sodium <125mEq/L or less marked hyponatremia that is symptomatic and has resisted correction with fluid restriction), including patients with heart failure, cirrhosis, and syndrome of Inappropriate Antidiuretic Hormone (SIADH). **Drug Interaction:** Cocomitant use of tolvaptan is contra indicated with strong inhibitors of CYP3A. **Such as :** Ketoconazole, Clarithromycin, Itraconazole, Telithromycin, Saquinavir, Nelfinavir, Nefazodone, Erythromycin, Fluconazole, Aprepitant, Diltazem, Verapamil, Rifampin, Phenytoin, Carbamazepine. **Co-administration with medications known to raise potassium:** Treatment with Tolvaptan is associated with an acute reduction of extracellular fluid volume which could result in increased serum potassium .Although specific interaction studies were not performed, in clinical studies when used concomitantly with beta-blockers, angiotensin receptor blockers (ARBs), angiotensin-converting enzyme inhibitors (ACEIs), and potassium – sparing diuretics. Adverse reactions of hyperalemia were approximately 1% to 2 % higher tolvaptan was administered with ARBs, ACEIs, and potassium sparing diuretics compared with administered with placebo. Electrocardiogram monitoring should begin immediately and continue until ECG parameters are within normal ranges. Dialysis may not be effective in removing tolvaptan because of its high binding affinity for human plasma protein (<99%). Close medical supervision and monitoring should continue until the patient recovers.