International Journal of Life science & Pharma Research

# PREVALENCE AND DRUG SENSITIVITY PATTERN OF STAPHYLOCOCCUS AUREUS IN POST-OPERATIVE SURGICAL ORAL & MAXILLOFACIAL INFECTIONS

**BISWAJIT BATABYAL, [1*] SHIBENDU BISWAS, [2] SUKANTA CHAKRABORTY [3] PRITI D. DESAI [4] AND NAVONIL DE SARKAR [5]**

**[1,2]Department of Microbiology, Gurunanak Institute of Dental Science & Research, Panihati, Kolkata-700114, West Bengal, India.**
**[3] Department of Pathology, BJMC, Ahmedabad.**
**[4]Department of Conservative Dentistry & Endodontics, Gurunanak Institute of Dental Science & Research, Panihati, Kolkata-700114, West Bengal, India.**
**[5]Indian Statistical Institute, Human Genetics Unit, Kolkata-700108, West Bengal, India.**

## ABSTRACT

The problem of infection has been persistent in the surgical world even after the introduction of antibiotics. Pathogens that infect surgical site can be acquired from the hospital environment or other infected patients. A total of 66 pus samples from post-operative oral & maxillofacial surgical infections were received in the Department of Microbiology, Gurunanak Institute of Dental Science & Research, Panihati, Kolkata, over a period of one year. The isolates were identified using standard laboratory procedures. All the isolates were tested for susceptibility to various commonly used antibiotics and screened for oxacillin susceptibility according to CLSI guidelines. Out of 66 pus samples received, 34 (51.5%) were culture positive for *Staph. aureus*. Methicillin resistance was documented in 14 (41.2%) of the *Staph. aureus* isolates. Highest efficacy was observed with linezolid (97.0%).All MRSA isolates were 100% sensitive to linezolid. The hospital acquired surgical site infection is alarming. Hospital disinfection and treatment protocols should be practiced.

**Keywords:** *Staphylococcus aureus,* MDR, Surgical post-operative oral & maxillofacial infections.

## INTRODUCTION

Post-operative infections have been a problem in the field of surgery for a long time. Advances in control of infections have not completely eradicated the problem because of development of resistance [Thomas KH. 1981]. Antimicrobial resistance can increase complications and costs associated with procedures and treatment. An infected site complicates the post-operative course and results in prolonged stay in the hospital and delayed recovery [Marjorie B and Susan D, 1977]. Most bacteria live on our skin, in the nasopharynx, gastrointestinal tract and other parts of the body with little potential for causing disease because of first line defenses within the body. Surgical operation, trauma, burns, diseases, nutrition and other factors affect these defenses. The skin barrier is disrupted by every skin incision, and microbial contamination is inevitable despite the best skin preparation [Howard RJ et al. 1980].

The widespread uses of antibiotics, together with the length of time over which they have been available have led to major problems of resistant organisms, contributing to morbidity and mortality. Pathogens that infect surgical site can be part of the patient's normal flora (endogenous source) or acquired from the hospital environment or other infected patients (exogenous source). The skin bacteria comprise commensals, transients and pathogens.The transient organisms include *Staph. aureus*, the hospital acquired methicillin-resistant forms (MRSA) also. Identification of a microbe that has been recovered from a clinical specimen is beneficial to the patient and assists in selection of chemotherapy [Elmer WK et al. 1997]. The present study was conducted to see the prevalence of *Staph. aureus* in post-operative oral & maxillofacial surgical infections and to analyze the antimicrobial susceptibility pattern of the isolates.

## MATERIALS AND METHODS

This was a prospective study conducted during 18 months from March 2011 to August 2012.

### *STUDY AREA AND STUDY POPULATION*

The study was conducted on samples from patients in Gurunanak Institute of Dental Science and Research, Panihati, Kolkata-700114, North 24 parganas, West Bengal, India. A total of 66 specimens obtained from patients who had undergone surgical operations were analyzed.

### *SAMPLE SIZE AND SELECTION CRITERIA*

The patients' samples were collected from department of Oral & Maxillofacial surgery in Gurunanak Institute of Dental Science and Research, Panihati, Kolkata. All patients who had post-operative oral & maxillofacial surgical infections during the 18 months study period were included in the study. Patients were enrolled after obtaining informed consent from them or their guardians.

### *COLLECTION AND PROCESSING OF SAMPLES*

The specimen was collected on sterile cotton swab without contaminating them with skin commensals. The samples were transported to the laboratory soon after being obtained. In the laboratory, the specimens were registered and cultured aerobically in Mannitol salt agar media (Himedia Laboratories Pvt. Ltd.; Mumbai). The plates were incubated aerobically at $37^{\circ}C$ for 24 hrs. Streak plate technique was used to obtain pure culture of each isolate prior to identification.

### *IDENTIFICATION OF ISOLATES*

The isolates were identified using colony morphology with Mannitol fermentation (used of Mannitol salt agar), Gram staining, Catalase, Coagulase test (slide & tube method) and DNase test as described by Cheesbrough, 2002.

Antibiotic sensitivity tests were carried out on isolated and identified colonies of *Staph. aureus* using commercially prepared antibiotic sensitivity disc using Kirby-Bauer method [Bauer et al. (1966)].

The following concentration of antibiotic per disc as recommended by Clinical Laboratory Standards Institute (CLSI) [Himedia Laboratories Pvt.Ltd.; Mumbai]:
Amoxycillin(20 mcg), Amoxycillin+Clavulanic acid (20+10 mcg), Ampicillin (10 mcg), Ampicillin+Sulbactam(10+10 mcg), Cefpodoxime(10 mcg), Ciprofloxacin (5 mcg), Clindamycin (2 mcg), Erythromycin (15 mcg), Rifampicin (5 mcg), Imipenem (10 mcg), Linezolid (30 mcg),Ofloxacin (5 mcg),Piperacillin (100 mcg),Piperacillin+Tazobactam (100+10 mcg),Ticarcillin (75 mcg),Ticacillin+Clavulanic acid (75+10 mcg),Meropenem (10 mcg),Vancomycin (30 mcg),Oxacillin (1 mcg).
Resistance or Susceptibility was reported based on the CLSI guideline.Two hours Tryptone Soya Broth (Himedia Laboratories Pvt.Ltd.; Mumbai) (3ml) cultures at 37°C of each isolate were adjusted to McFarland turbidity (0.5), and the disc sensitivity screening conducted as described by Cheesbrough, 2002. Sterile swabs were used to inoculate the test organism onto the sensitivity agar (Mueller Hinton agar media) (Himedia Laboratories Pvt.Ltd.; Mumbai). Sterile forceps were used to carefully distribute the antibiotic discs evenly on the inoculated plates. After allowing for about 30 minutes on the bench for proper diffusion, the plates were inverted and incubated aerobically at 35°C for 18 hours. The inhibition zone diameters were measured in millimeters using meter rule.

### *Methicillin Resistant Staph.aureus detection (MRSA)*

Methicillin-resistance was verified by the CLSI (formerly NCCLS) Oxacillin screening test [NCCLS, 2000].
Oxacillin sensitivity was performed on Mueller Hinton agar media with 4% sodium chloride. The strains were reported as sensitive, or resistant, to Oxacillin (1 mcg) with inhibition zone diameter equal or more than 13 mm sand less than or 10 mm respectively. Disk diffusion testing was performed as recommended by the National Committee for Clinical Standards; briefly, a broth culture suspension of the isolate to be tested was prepared in Trypticase soya broth and turbidity adjusted to a 0.5 McFarland standard. The zone sizes were read after 24 hours of incubation in ambient air at 35°C. Isolates were classified as either susceptible Bauer et al. (1966). American Typing Collection (ATCC 25923) of *Staph. aureus* was used as a control strain in antibacterial susceptibility testing.

## RESULTS

Out of 66 pus samples received, 34(51.5%) were culture positive for *Staph. aureus*. Highest efficacy was observed with linezolid (97.0%) followed by imipenem (70.5%), rifampicin (58.8%), ampicillin/sulbactam and clindamycin (47.0%), piperacillin/tazobactam (41.1%) and meropenem (35.2%). Methicillin resistance was documented in 14 (41.2%) of the *Staph. aureus* isolates. All MRSA isolates were 100% sensitive to linezolid.

***Table I:*** shows the occurrence of *Staph. aureus* in post-operative oral & maxillofacial surgical infection in relation to age. The age groups were divided into seven categories: 10-20, 21-30, 31-40, 41-50, 51-60, 61-70 and 71 and above.

***Table II:*** shows the relationship between postoperative oral & maxillofacial surgical infections and sex. There was no significant difference in the occurrence of hospital infection between males and females.

***Table III:*** shows Sensitivity pattern of *Staph. aureus* isolated from patients in post-operative oral & maxillofacial surgical infections, the organism was sensitive to linezolid, imipenem, rifampicin, ampicillin/sulbactam, clindamycin, piperacillin/tazobactam and meropenem, with linezolid showing the highest percentage sensitivity.

**Table 1**

***Relationship between post-operative oral & maxillofacial surgical infections in relation to age***

| Factors | No. of sample | MSSA | MRSA |
|---|---|---|---|
| 10-20 | 08 | 04 | 02 |
| 21-30 | 12 | 04 | 00 |
| 31-40 | 12 | 02 | 02 |
| 41-50 | 12 | 01 | 04 |
| 51-60 | 12 | 04 | 04 |
| 61-70 | 08 | 05 | 02 |
| 71-above | 02 | 00 | 00 |
| Total | 66 | 20 | 14 |
| Percentage (%) | | 30.3 | 21.2 |

**** MSSA: Methicillin-sensitive Staph. aureus.***

**** MRSA: Methicillin-resistant Staph. aureus.***

**Table 2**

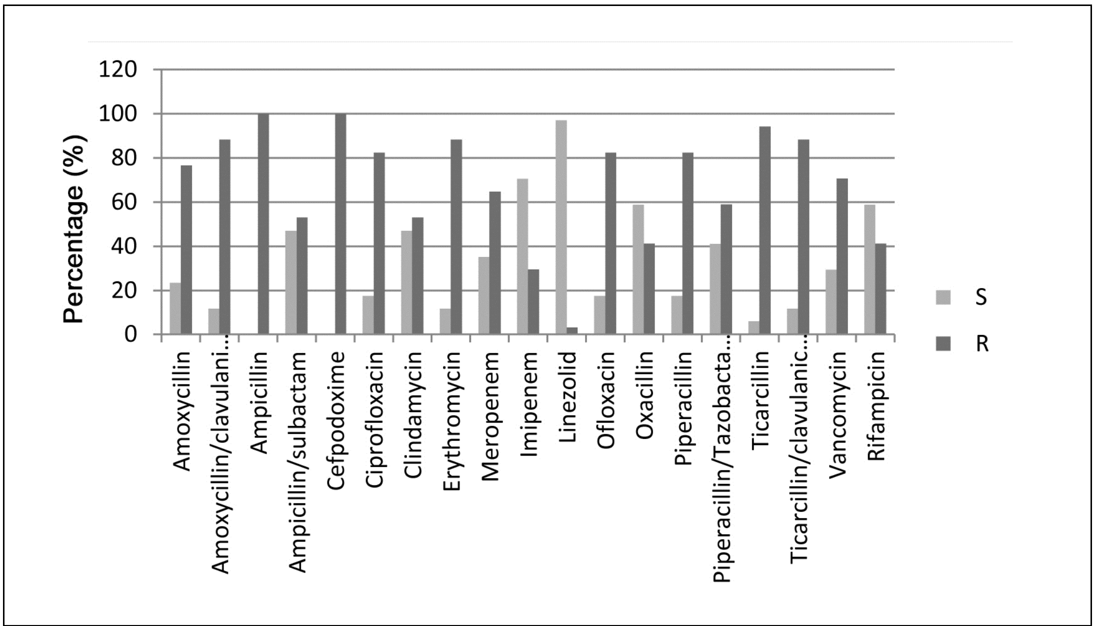
***Relationship between postoperative oral & maxillofacial surgical infections and sex***

| Sex | Total No. of sample | MSSA | MSSA% | MRSA | MRSA% |
|---|---|---|---|---|---|
| **Male** | 46 | 15 | 32.6 | 07 | 15.2 |
| **Female** | 20 | 05 | 25.0 | 07 | 35.0 |
| **Total** | 66 | 20 | 30.3 | 14 | 21.2 |

**Table 3**

***Sensitivity pattern of Staph. aureus isolated from patients in Post-operative oral & maxillofacial surgical infections***

| | Total Isolates : 34 | | | |
|---|---|---|---|---|
| **Antibiotics** | **S(No.)** | **R(No.)** | **%S** | **%R** |
| Amoxycillin | 08 | 26 | 23.5 | 76.5 |
| Amoxycillin/clavulanic acid | 04 | 30 | 11.8 | 88.2 |
| Ampicillin | 00 | 34 | 00 | 100 |
| Ampicillin/sulbactam | 16 | 18 | 47.0 | 53.0 |
| Cefpodoxime | 00 | 34 | 00 | 100 |
| Ciprofloxacin | 06 | 28 | 17.6 | 82.4 |
| Clindamycin | 16 | 18 | 47.0 | 53.0 |
| Erythromycin | 04 | 30 | 11.8 | 88.2 |
| Meropenem | 12 | 22 | 35.2 | 64.8 |
| Imipenem | 24 | 10 | 70.5 | 29.5 |
| Linezolid | 33 | 01 | 97.0 | 3.0 |
| Ofloxacin | 06 | 28 | 17.6 | 82.4 |
| **Oxacillin** | **20** | **14** | **58.8** | **41.2** |
| Piperacillin | 06 | 28 | 17.6 | 82.4 |
| Piperacillin/Tazobactam | 14 | 20 | 41.1 | 58.9 |
| Ticarcillin | 02 | 32 | 5.9 | 94.1 |
| Ticarcillin/clavulanic acid | 04 | 30 | 11.8 | 88.2 |
| Vancomycin | 10 | 24 | 29.4 | 70.6 |
| Rifampicin | 20 | 14 | 58.8 | 41.2 |




- ***S: Sensitive.***
- ***R: Resistant.***

**Figure1**

***Pattern of Staphylococcus aureus susceptibility***

## STATISTICAL ANALYSIS

We have performed binary logistic regression analysis to find out the effect of age and sex on the MRSA positive or negative status/ methicillin resistance pattern among the study group. It is found to be no significant in each case ($p_{age}$= 0.40556, $p_{sex}$= 0.13329). So, age and sex has no effect on the status of *Staphylococcus aureus* methecilin resistance pattern for our study group. We tried to understand the correlation pattern of MRSA resistance pattern and sensitivity-resistance pattern of different studied antibiotics. Ampicillin and cefpodoxime were not considered for this correlation since it has a cell value 0. As a matter of fact all of the cultures from the study cases are observed to be resistant. So, definitely these two antibiotics cannot be the drug of choice rather may be the opposite. It is observed that after Benjamini Hochbergh correction at 0.05 levels, Amoxycillin, Ampicillin/Sulbactam, Clindamycin, Rifampicin, Imipenem, Piperacillin/Tazobactam and Meropenem are showing significantly correlated resistance pattern to that of Oxacillin. This itself implies that these antibiotic are as effective as the Oxacillin. So, may be these antibiotic could be good choice of drug to treat MRSA negative strains. Although this comment is based on statistical analysis, and if a single case was found to show antibiotic resistance even for oxacillin sensitive case, it should not be considered for MRSA negative strain's drug of choice. For obvious reason count wise, only Imipenem and Rifampicin should be considered as drug of choice for only MRSA negative strains.

| Correlations | MRSA | | |
|---|---|---|---|
| | Pearson Correlation | Sig. (2-tailed) | N |
| AMOXYCILLIN | .502 | .002 | 34 |
| AMOXYCILLIN/CLAVULANIC ACID | .306 | .079 | 34 |
| AMPICILLIN / SULBACTAM | .789 | .000 | 34 |
| CIPROFLOXACIN | .387 | .024 | 34 |
| CLINDAMYCIN | .549 | .001 | 34 |
| ERYTHROMYCIN | .306 | .079 | 34 |
| RIFAMPICIN | .757 | .000 | 34 |
| IMIPENEM | **.772** | .000 | 34 |
| LINEZOLID | -.146 | .411 | 34 |
| OFLOXACIN | .387 | .024 | 34 |
| PIPERACILLIN | .387 | .024 | 34 |
| PIPERACILLIN/TAZOBACTAM | .700 | .000 | 34 |
| TICARCILLIN | .209 | .235 | 34 |
| TICARCILLIN/CLAVULANIC ACID | .306 | .079 | 34 |
| VANCOMYCIN | .015 | .931 | 34 |
| MEROPENEM | .618 | .000 | 34 |

So for obvious reason, we would not consider the rest of antibiotics other than Imipenem, Rifampicin and Linezolid for farther analysis of drug of choice. To find out the drug of choice pair wise one way Fisher's Exact test was performed (since the one of the cell frequency of Linezolid is less than 5). As a matter of fact, for the studied data set it needs no statistical test to find the best drug of choice, which is Imipenem (since only one of the 34 studied culture from 34 different study subjects are resistant, and none other antibiotic's counts are way close to it). We performed one tail Fisher's Exact test for the 3 best performing antibiotic's resistance pattern to that of the Oxacillin, which is a bench mark of Methicillin resistant group. Here we tested whether a given antibiotic performed better than Oxacillin. (P- Values are given in the Table follows). R package was used for this analysis. Bonferroni correction was performed and then checked again whether the p value is still significant. Only Linezolid was found to be significant even after Bonferroni correction whereas other two antibiotics were not significant even before correction. Thus Linezolid is significantly better drug than Oxacillin in treating *Staphylococcus aureus* than all other study drugs. To be more sure we performed a second level of statistical test.

| Greater tail p- Value | |
|---|---|
| Linezolid | 0.0001 |
| Imipenem | 0.2235 |
| Rifampicin | 0.4016 |

Considering the ideal situation that is all of the cultures are susceptible as the comparable sets, we performed a second round of Fisher's exact test. And we found only Linezolid is statistically not significant from the ideal condition; that implies any *Staphylococcus aureus* case can be treated with this antibiotic and not Imipenem and Rifampicin. Automatically, this implies that the performance of other antibiotics would be even worse.

| Greater tail p- Value | |
|---|---|
| Linezolid | 0.49 |
| Imipenem | 0.0005 |
| Rifampicin | <0.0001 |

Nb. During analysis all the susceptible counts were coded as 1 the resistant counts were coded as 2 and again MRSA negative strain cultures from different cases and males are coded as 1 for analysis, whereas MRSA positive culture and Females are also coded as 2.

## DISCUSSION

The problem of infection has been persistent in the surgical world even after the introduction of antibiotics. Ongoing researchers on antibiotic development signal the fact that numerous drug resistant organisms still continue to bother us and render the management of surgical wound infections a continue challenge. Inspite of proper application of the basic principles in surgical wound care, a number of patients develop infection needing proper identification of the organisms for appropriate management.

Although aerobic wound infections have been known to cause devastating postoperative complications, most anaerobicidal agents are very effective in neutralizing this problem. It is in the treatment of aerobic bacterial infections where variable antimicrobial responses exist. With the influx of voluminous number of antibiotics, the clinician often finds himself overwhelmed by the variety of options. Determination of the etiologic agent is vital in the final choice antibiotics. Most especially in a situation where empirical treatment has to be started without the benefit of a gram-stain or culture and sensitivity results, a working knowledge of the most likely causative organism and the prevailing antibiotic sensitivity/resistance pattern will be of great help.

The high prevalence of *Staphylococcus aureus* infection may be because it is an endogenous source of infection. Nasal carriage of *Staph. aureus* is an important risk factor for infection of surgical site as the organism is a normal flora in the nostrils. Infection with this organism may also be due to contamination from the environment e.g. contamination of surgical instruments. With the disruption of natural skin barrier *Staph. aureus*, which is a common bacterium on surfaces, easily find their way into surgical sites [ Brown AD. 1990].

The primary aim of this study was to determine the occurrence of *Staph. aureus* in post-operative oral & maxillofacial infections and its sensitivity pattern to commonly used antibiotics. The incidence of *Staph. aureus* in our study was 51.5%.It is in concordance with various other reports [Massadeh HA et al. 2009 & Dhar S et al. 2007]. High incidence has been reported by others [Anguzu JR & Olila D, 2007 & Tyagi A et al. 2008]. This could be attributed to differences in geographical location and hygienic measures.

The present study indicated that linezolid had highest sensitivity (97.0%) to the *Staph. aureus,* followed by imipenem (70.5%) and rifampicin (58.8%).MRSA is now one of the commonest bacteria causing nosocomial infections. The present study also revealed high prevalence rate of MRSA in our hospital (41.2%). This may be due to prolonged stay in the hospital, instrumentation and surgical intervention. The MRSA isolates showed multiple drug resistance (MDR), except linezolid. The multiple resistances in MRSA could be due to mecA gene that encodes for protein PBP-2a that binds to available a lactams. Another reason is the production of Staphylococcal penicillinase and other enzymatic deactivators. Presence of other resistance factors e.g. Plasmids could have been involved as well [Elmer WK et al. 1997]. The resistance observed in *Staph. aureus* could also be attributed to irrational use of antibiotics for conditions that may not clinically indicate their use, over the counter sell of antibiotics in pharmacies without prescription by authorized practitioners, some new drug formulations which may be of poor quality and dumping of banned products into the market where the public may get access to them. In view of the resistance observed, infections caused by MRSA can be expensive in terms of costs of treatment, morbidity and prolonged hospitalization [Hiramtsu K et al. 1997].

The overall result of this study emphasizes that the magnitude of post-operative surgical infection problem may be increasing because many of the causative organisms have started to develop some of drug resistance to currently used antibiotics. It is essential to give special attention in reducing surgical site infections. Hospital disinfection and treatment protocols should be practiced vigorously and monitored regularly by hospital infection control committee to keep the incidences in control.

## ACKNOWLEDGEMENTS

We would like to acknowledge the assistance and guidance provided by Dr. ChandraNathMajumder and Prof. (Dr.) T.K. Saha, Director cum Principal of Gurunanak Institute of Dental Science and Research, Panihati, Kolkata-700114, West Bengal for permission to do the work in Gurunanak Institute of Dental Science and Research.

## REFERENCES

1. Anguzu, J.R., and Olila, D. Drug sensitivity patterns of bacterial isolates from septic post-operative wounds in a regional referral hospital in Uganda.African Health Sciences.2007;Vol**7** No 3: 148-154.
2. Beyers Marjorie, Dudas Susan. The clinical practiced of Medical-Surgical Nursing. Boston: Publisher Little, Brown and Company; 1977.
3. Bauer A.W., Kirby W.M., Sherris J.C., Turck M. Antibiotic susceptibility testing by a standardized single disk method. Am. J. Clin. Pathol.1966; **45**(4): 493-496.
4. Brown AD.Bacteriology of wound infections in surgical ward of teaching hospitals.West African Journal of Medicine. 1990; **9**:4285-4290.
5. Cheesbrough M. District Laboratory Practice in Tropical countries. Part-2.Cambridge University Press. 2002: 135-162.
6. Dhar S, Saraf R, Singh K, Raina B. Microbiological profile of chronic burn wounds among patients admitted in burn unit, JK Science. 2007; **9**(4): 182-85.
7. Elmer WK, Stephen DA, William MJ, Schreckenberger Paul C, Winn Washingtone C. Winn Jr Antimicrobial susceptibility testing in, Colour Atlas and text Book of Diagnostic microbiology. $5_{th}$ edition. 227 East Washington Square, Philadelphia:Raven Publisher. 1997: 69-120.
8. Howard RJ,Ravitch MM, Steichen FM. Host against infections.Current problems in surgery.New English Journal of Medicine. 1980; **12:**1823-1830.
9. Hiramtsu K, Hanaki H, Ino T, YabutaK, Oguri T, Tenover F. C. Methicillin-resistant *Staphylococcus aureus* clinical strain with reduced vancomycin susceptibility. Journal of Antimicrobial Chemotherapy. 1997; **40**: 135-136.
10. Massadeh HA, Jaran AS. Incident of P.aeruginosa in post-operative wound infections, Am J Infect Dis. 2009; **5**(1): 1-6.
11. National Committee for Clinical Laboratory Standards.Approved Standards M7-A5, 2000. Test to detect MRS must be incubated for full

24 hours (rather than 16 to 20 hours) at 33°C to 35°C (do not exceed 35°C) $5_{th}$ ed. Approved Standards. NCCLS Wayne, Pa.

12. Performance standard for antimicrobial susceptibility testing. CLSI approved standard M100-S17. Clinical and Laboratory Standards Institute. 2007; Wayne,PA.
13. Thomas KH. Surgical wound infection, an overview. American Journal of Medicine. 1981; **70**:712-718, [PubMed].
14. Tyagi A, Kapil A, Singh P. Incidence of Methicillin resistant *Staphylococcus aureus* in pus samples at a tertiary care hospital, AIIMS, New Delhi, JIACM **9**(1): 33-35, (2008).