A Study on Surgical Site Infections Caused by *Staphylococcus Aureus* with a Special Search for Methicillin-Resistant Isolates in Oral & Maxillofacial infections

BISWAJIT BATABYAL[1*], SHIBENDU BISWAS[1], GOUTAM KR. KUNDU [2], SUKANTA CHAKRABORTY [3]

1. Lecturer in Department of Microbiology, Gurunanak Institute of Dental Science & Research, Panihati, Kolkata-700114, North 24 parganas, West Bengal, India.

2. Professor & HOD, Dept. of Pedodontics & Preventive Dentistry, Gurunanak Institute of Dental Science & Research, Panihati, Kolkata-700114, North 24 parganas, West Bengal, India.

3. Consultant pathologist, Theism ultrasound centre, Department of pathology, Dum Dum, Kolkata, West Bengal, India.

***Corresponding author** biswajit.batabyal@gmail.com

Abstract

With the Objective, to study the prevalence of Staphylococcus aureus and Methicillin-Resistant Staphylococcus aureus (MRSA) in surgical site infections (SSIs) at the Dental hospital in Kolkata. This study was conducted on 66 patients who underwent surgery in the department of Oral & Maxillofacial, Gurunanak Institute of Dental science & Research, Kolkata. Pus samples were collected with two sterile swabs and processed in the Microbiology department. Result of the 66 pus samples, the most common organism which was isolated was Staphylococcus aureus, with 34(51.5%) isolates. Of these, 14 (41.2%) were Methicillin Resistant Staphylococcus aureus (MRSA). All MRSA isolates showed multiple drug resistance (MDR), except linezolid. Indian clinicians and infectious disease specialists are facing formidable challenges from Methicillin Resistant Staphylococcus aureus. Despite the best surgical practices, nearly a tenth of all the SSIs cases could be caused by MRSA. Routine screening for these multidrug resistant organisms in the hospital staff, especially in the surgical departments and pre-surgical screening of the patients could help in reducing the incidence of MRSA.

Keywords: *Surgical Site Infections, Methicillin resistant Staphylococcus aureus (MRSA), Multidrug Resistant (MDR).*

INTRODUCTION

Surgical infections which are acquired in the hospital are recognized to be associated with an extended length of hospital stay, pain, discomfort and sometimes prolonged or permanent disability [1]. Surgical site infections (SSIs) are common complications that follow all types of operative procedures [1]. These infections are usually caused by the exogenous and endogenous microorganisms that enter the operative wound during the course of the surgery [1]. The incidence of the infected surgical wounds may be influenced by factors such as pre-operative care, the theatre environment, post operative care and the type of surgery. The aim of these guidelines [2] has been to reduce surgical site infections by ensuring that the hospital personnel practice optimal preoperative, intraoperative and postoperative care. The common pathogenic bacteria in SSIs include Staphylococci, Pseudomonas, Streptococci, Enterococci, E.coli, Klebsiella, Enterobacter, Citrobacter, Acinetobacter, Proteus, etc. *Staph. aureus* can be acquired from the hospital environment or other infected patients It is readily transmitted from person to person onto the hands and clothes of the health care staff, onto objects and into the air [1], [2]. *Staph. aureus* is the commonest cause of SSI and other nosocomial infections. *Staph. aureus* was once susceptible to Penicillin but widely resistant organisms soon emerged. The introduction of Methicillin initially solved the problem, but later, strains which were resistant to Methicillin developed. Thus, an increased number of resistant strains have been seen worldwide. [2], [3]

The clinical significance of Methicillin-resistant *Staphylococcus aureus* (MRSA) is heightened by the fact that these isolates are usually resistant to other anti-staphylococcal agents (Clindamycin Erythromycin, Tetracycline, sometimes Gentamicin and Trimethoprim/Sulphomethoxazole), with the exception of Vancomycin. Sometimes, Methicillin-resistant-*Staphylococcus aureus* appears to be susceptible in vitro to other β-lactam agents such as Cephalosporins; however, they are clinically ineffective [3]. Since MRSAs are resistant to all the β-lactam antibiotics, the therapeutic options are significantly limited. The incidence of MRSA in India ranges from 30-70% [4], [5]. The incidence of nosocomial infections which are caused by MRSA continues to increase; therefore, the importance of their detection, especially for treatment and epidemiological purposes, arises [6], [7], [8]. The present study was done in a Dental hospital in Kolkata to determine the incidence of MRSA in surgical site infections.

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 analysed.

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 by colour change of the medium around each colony from red to yellow (used of Mannitol salt agar), Gram staining, Catalase, Coagulase test (slide & tube method) and DNase test as described by Cheesbrough, 2002 [9].

Antimicrobial susceptibility testing

Antibiotic susceptibility testing was done by the disc diffusion test which was described by the modified Kirby Bauer method. The antimicrobial containing discs were placed on the agar plate within 15 minutes of inoculation by using sterile forceps and these were pressed firmly against the plate. The plates were inverted and incubated for 18-24 hours at 35°C, at a CO2 concentration of 7-10%. [10] The drugs were chosen, based on their action on a particular organism and also on the antibiotic policy of the hospitals. Nineteen discs were used on a 9 cm diameter plate. The antimicrobial discs for *Staphylococcus aureus* were: 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) and Oxacillin (1 mcg). The antibiotic discs were obtained from HiMedia Laboratories, Pvt. Ltd. Mumbai. After 18-24 hours of incubation, the diameter of the inhibitory zone was measured by using a millimeter scale. The zone size around each antimicrobial disc was interpreted as sensitive, intermediate or resistant, according to The National Committee for Clinical Laboratory Standards (NCCLS), which is now called the Clinical and Laboratory Standards Institute (CLSI) criteria. The test of diffusion in the agar was applied according to the CLSI recommendations (CLSI, 2007) [11], by using Mueller-Hinton Agar and antibiotic discs.

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

Methicillin-resistance was verified by the CLSI (formerly NCCLS) Oxacillin screening test [NCCLS, 2000] [12]. Oxacillin sensitivitywas 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) [13].

Quality Control

Staphylococcus aureus ATCC 25923 (Oxacillin susceptible) and *Staphylococcus aureus* ATCC 43300 (Oxacillin resistant) were used as the control strains.

RESULTS

Of the 66 pus samples, the most common organism which was isolated was *Staphylococcus aureus*, with 34(51.5%) isolates. Of these, 14 (41.2%) were Methicillin Resistant *Staphylococcus aureus* (MRSA). All MRSA isolates showed multiple drug resistance (MDR), except linezolid.

DISCUSSION

The prevalence rate of surgical site wound infections, though preventable, is high. Several studies all over the world have well established that the early detection of Methicillin resistance is very essential in the prognosis of infections which are caused by *Staph. aureus.* Studies by Agarwal (1972) [14], Rao and Harsha (1975) [15], Kowli et al. (1995) [16] and Anvikar (1999) [17] have shown the surgical site infection rates in India to be between 4 to 30%. *Staph. aureus* has been known to acquire resistance to most antibiotics including the penicillinase resistant ones like Methicillin. A study by Weigelt et al in USA, found an incidence of 20.6% MRSA [18] in SSIs. Still higher incidences of 45% and 58.2% MRSA have been documented by Eagye et al [19] and Keith et al [20]. The incidence of Methicillin–resistant *Staph. aureus* in our study was 14 (41.2%). We found that the MRSA isolates showed multiple drug resistance (MDR), except linezolid. This finding could have relevant clinical use in the antibiotic policy guidelines for hospitals.

It is an undisputed fact that the infection may originate from the patient's normal flora or that it may be derived from the hospital environment. Multidrug resistant *Staph. aureus* is commonly associated with the hospital environment. In the present study, 51.5% of the postoperative wound infections were due to this organism and this indicates that the possible source of infection in these cases may be from the hospital environment. This fact needs to be kept in mind by the surgeons. Also, is well known that surgical site infections are also associated with significant increases in the length of hospital stay, additional costs, morbidity and mortality. The widespread availability and the use of antimicrobial agents for prophylaxis seem to have altered the surgical practice in the past twenty years. Since MRSA strains are highly resistant to many groups of antibiotics, there is an increased rate of morbidity and mortality in the ICU facilities, as well as in immuno-compromised individuals. Staphylococcal resistance to oxacillin/methicillin occurs when an isolate carries an altered Penicillin-binding protein, PBP2a, which is encoded for by the mecA gene. This penicillin-binding protein binds beta-lactams with lower avidity, which results in resistance to this class of antimicrobial agents. Recently, Harbarth et al. (2008) [21] have observed that methicillin-resistant *Staph. aureus* (MRSA) alone constituted 5.1% of the surgical site infections.

Further, it has been established by Classen et al (1992) [22] that prophylactic antimicrobials are often not administered at the optimal times, to ensure their best presence in effective concentrations peri-operatively. This point influences most surgical outcomes. It is highly desirablethat prophylactic antibiotics should be administered within one hour before the operation and that they should be continued for only 24-72 hours post operatively, as a policy to avoid multidrug resistance. Inappropriate dosage and the duration of antibiotic use not only fail to reduce the infection, but also lead to an increase in multidrug resistant mutants due to selection pressures. Hence, the prevention of SSIs is essential and this poses a major challenge in any healthcare system. Infection control measures such as the active surveillance of SSIs, the implementation of a checklist, compliance observations and instruction/training of healthcare workers, as well as *Staphylococcus aureus* /MRSA screening, clipping instead of shaving, adherence to perioperative antibiotic prophylaxis, maintaining intraoperative normothermia and blood glucose control are essential in order to prevent SSIs. The prevention of nosocomial infections is not only highly desirable for patients, but it is now seen as a major political priority in all the hospitals, irrespective of whether they are private or public. Guidelines and protocols for basic infection control practices such as hand washing and written protocols for the safe insertion and maintenance of devices such as intravascular catheters, should be widely available and adhered to.

Further, we feel that the high incidence of MRSA could be reduced by screening for these organisms routinely in all the hospital staff and that the nation’s celebrated health care facilities should lead to the change here. Relevant policy and protocol changes could usher in a culture of cleanliness and abundant caution in the operation theatres. The Municipal Corporation of the Indian cities can pitch in implementing and monitoring operation theatre protocols. A pre-surgical screening of the patients and treating those who carry these bugs would definitely help in reducing infections, the duration of hospital stay and the economic burdens which are associated with these. It is high time for the Indian hospital managements to ensure a regular, close clinical liaison between the surgical team, the microbiologist, and the infection control team to provide proper quality surgical services. Regular periodic studies could be established by the Indian medical colleges to control and conquer the resistant organisms in surgical site infections. The national governments should formulate national standard operating procedures and infection control standards to face the challenges which are posed by antibiotic resistant organisms.

As Dr. Vincent Cheng explains, the anti-MRSA success of Hong Kong [23] is a consequence of several factors – an aggressive approach, single room isolation, and contact screening. Forvarious reasons, almost every cluster of cases was reported in the press. Nonetheless, in the UK at least, stories of “dirty hospitals” are evocative, with public acceptance, leading to acycle of reinforcement that eventually transforms the history of MRSA control in the country. Since 2003, Hong Kong has leaped a big step forward and there is no reason why we can’t emulate Hong Kong.

Indian governments should act more proactive and emulate anti-community acquired MRSA measures as aggressively as United Kingdom and Hong Kong and make it notifiable. The judicious use of antibiotic prophylaxis and the use of an organized system of wound surveillance and reporting can help in reducing the wound infection rate to an attainable minimum.

The Indian Council of Medical Research has invited research proposals from scientists to generate scientific evidence on antimicrobial resistance. This move indicates that the apex medical research body has finally realized there is no place for jingoism in matters of science, and that the latest findings on antibiotic resistance must be taken seriously and verified scientifically. This exercise will prove to be useful only if the researchers are truly free to report the presence of the superbugs and the extent of their spread. The second important development has been the drafting of the much-needed national policy for the containment of antimicrobial resistance. The policy admits that the use of antibiotics is inappropriate in 20 per cent to 50 per cent of the cases. It targets the indiscriminate use of antibiotics in food animals and intends to curb the practice, since it ultimately causes drug resistance in humans. Most importantly, the access to third generation antibiotics like carbapenems is to be restricted to Indian tertiary hospitals. But even in these hospitals, efforts must be directed towards restricting their use to patients with severe infections. The Indian government should waste no time in creating a national surveillance system for measuring antibiotic resistance if it is serious about getting on top of the problem. By all parameters, our war against antibiotic-resistant bacteria is far from winning. The question is – are we serious? Will the Indian government do its role and save the hospitals and its public?

CONCLUSIONS

Our most common surgical site infection isolate was *Staphylococcus aureus* (51.5%). Methicillin resistant *Staphylococcus aureus* (MRSA) constituted 41.2% of the total SSI isolates. Due to the increased morbidity and mortality which are associated with these drug resistant organisms, an early detection and intervention is a prerequisite in surgical patients. Although surgical wound infections cannot be completely eliminated, a reduction in the infection rate to a minimal level could have significant benefits, by reducing postoperative morbidity and mortality, and the wastage of health care resources. Antimicrobial therapy should be designed to deliver an adequate drug concentration to the site of infection. The governments should take proactive steps in setting up national hospital antibiotic policy guidelines and in instituting a culture of simple anti-MRSA measures like hand washing among the health care personnel. Hospitals should screen for MRSA among their staff and treat the hospital staff who are affected by the bacteria.

ACKNOWLEDGEMENTS

We would like to acknowledge the assistance and guidance provided by Dr. ChandraNath Majumder 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] Nichols RL. Current Strategies for Prevention of Surgical Site Infections: *Curr Infect Dis Rep*.2004 Dec; 6(6):426-434.

[2] Mangram AJ, Horan TC, Pearson ML, Silver LC, Jarvis WR.Guidelines for Prevention of SSI, 1999,Criteria for defining SSIs. The Hospital Infection Control Practices Advisory Committee. *Infection control and Hospital Epidemiology* 1999; 20; 4; 24. Available at http://www.cdc. gov/ncidod/hip/SSI/SSI.pdf.

[3] Susan LK. Concepts in Antimicrobial therapy; TB of Diagnostic Microbiology: 3rd edition: WB Saunders. Editors; Mahon C.R, Manuselis G: 2007 pg 82.
[4] Verma S, Joshi S, Chitnis V, Hemavani N, Chitnis D. Growing problems of Methicillin Resistant Staphylococci-Indian Scenario. *Indian J Med Sci* 2000; 54:535-540.
[5] Bratzler DW, Hunt DR. The surgical infection prevention and surgical care improvement projects: national initiatives to improve outcomes for patients having surgery. *Clin Infect Dis* 2006; 43: 322-330.
[6] Bannerman TL. Staphylococci and other catalase positive cocci that grow aerobically. In: Manual of Clinical Microbiology, 8th Ed. Washington DC: ASM Press In: Murray PR, Baron EJ, Jorgensen JH, editors;2003; p.384-404.
[7] Oslon M, Connor O, Schwartz ML .A Five year Prospective Study of 20193 Wounds at Minneapolis V a Medical Center. *Ann Surg*.1984; 199; 253-259.
[8] Larsen HS, Connie RM. Staphylococci: TB of Diagnostic Microbiology 3rd edition; WB Saunders. Editors; Mahon CR, Manuselis G:2007; pg 341.
[9] Cheesbrough M. District Laboratory Practice in Tropical countries. Part-2. Cambridge University Press; 135-162 (2002).

[10] Howard BJ. Nosocomial Infections- an Overview. Text Book of Clinical and Pathogenic Microbiology, 1994:2nd Ed. Howard BJ (Editor Mosby- Year Book Inc., St. Louis, MO, USA.
[11] Performance standard for antimicrobial susceptibility testing. CLSI approved standard M100-S17. Clinical and Laboratory Standards Institute, CLSI. Wayne,PA, 2007.

[12] 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 to 35°C (do not exceed 35°C) 5_{th} ed. Approved Standards. NCCLS Wayne, Pa.

[13] 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.

[14] Agarwal SL: Study of postoperative wound infections: *Ind.J.Surg*. 1972:314-320.
[15] Rao AS, Harsha M (1975). Postoperative wound infections. *J Indian Med. Assoc*.
64: 90 -93.
[16] Kowli SS, Nayak MH, Mehta AP, Bhalerao RA Hospital infection. *Indian J. Surg*. 1985; 48: 475-486.
[17] Anvikar AR, Deshmukh AB, Karyakarte RP, Damle AS, Patwardhan NS, Malik AK, Bichile LK, Bajaj JK, Baradkar VP, Kulkarni JD, Sachdeo SM . A one year prospective study of 3,280 surgical wounds. *Indian J. Med. Microbiol*. 1999; 17: 129-132.
[18] Weigelt JA, Lipsky BA, Tabak YP, Derby KG, Kim M, Gupta V: Sugical Site Infections: Causative pathogens and associated outcomes; *Am J Infect Control*,2010 Mar;38(20):112-120.Epub
[19] Eagye KJ, Kim A, Laohavaleeson S, Kuti JL, Nicolau DP: Surgical Site Infection: does inadequate antibiotic therapy affect patient outcomes?: *Surgical Infect (Larchmt)*.2009 Aug;10(4):323-331.
[20] Keith SK, Deverick JA, Sloane R, Chen LF, Choi Y, Link K, Sexton DJ, Schmader KE: The impact of Surgical Site Infection on older operative patients: *J Am Geriatr Soc*.2009 January;57(1):46-54.

[21] Harbarth S, Fankhauser C, Schrenzel J, Christenson J, Gervaz P,Bandiera-Clerc C, Renzi G, Vernaz N, Sax H, Pittet D. Universal Screening for Methicillin-Resistant *Staphylococcus aureus* at Hospital Admission and Nosocomial Infection in Surgical Patients. *JAMA*.2008;
299: 1149-1157.
[22] Classen DC, Evans RS, Pestotnik SL, et al. The Timing of Prophylactic administration of antibiotics and risk of *Surgical wound Infections N Engl J Med*. 1992; 326:281–286.
[23] Pak-leung Ho .A Losing Battle against Extensively-resistant Bacteria? *The Hong Kong medical Diary*. In: Editorial.Vol.16 No.4 April 2011.