Antimicrobial Susceptibility of Organisms Isolated from Surgical Site Infection in a Tertiary Care Hospital, Bettiah (West Champaran) Bihar, India

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ABSTRACT

Introduction: Surgical site infections are most common hospitals acquired infections and are an important cause of morbidity and mortality. The objective of our study is to conclude the causative bacteria and antimicrobial sensitivity of surgical site infections.

Material and methods: A total of 275 various clinical samples received in Microbiology Laboratory, Government Medical College, Bettiah (West Champaran) Bihar, and Associated Hospital, from March 2018 to April 2019. A total 101 Staphylococcus aureus isolates, were identified by standard biochemical methods. Antibiotic susceptibility testing was performed by Kirby Bauer Disc Diffusion method. Methicillin resistance was detected by using cefoxitin (30µg) disc diffusion method as per CLSI guidelines 2016.

Result: Out of the 275 aerobic bacteria which were isolated, 144 were gram positive cocci (52.37%) and 131 were gram negative bacilli (47.63%). The most common pathogen followed by Staphylococcus aureus 101 (36.36%). Other organisms were Escherichia, Pseudomonas, Klebsiella, Citrobacter, Proteus, and Enterococcus. The Antimicrobial profile of 101 Staphylococcus aureus isolates among MRSA, resistance those they were 100% sensitive to linezolid and vancomycin, with moderate sensitivity (71.14%) to cefuroxime, gentamicin and least sensitivity to (23.81%) doxycycline, (20.95%) ciprofloxacin.

Conclusion: Isolation of MRSA patients and carriers in the hospitals, regular surveillance, and monitoring of antibiotic susceptibility pattern of the hospital and community of that region regularly and formulation of antibiotic policy may help in reducing the treatment failures.

Keywords: Surgical Site Infections (SSI), Methicillin Resistant Staphylococcus Aureus, Antimicrobial Sensitivity.

INTRODUCTION

Surgical site infections (SSI), one of the most common causes of healthcare associated infections are a common complication associated with surgery, with a reported incidence rates of 2-20%.

Surgical site infections (SSI) are a vital cause by the exogenous and endogenous microorganisms that enter the operative wound during the surgery. The incidence of surgical site infection differs widely between surgical procedures, hospitals, patients and between surgeons.

The most frequently isolated bacterial pathogens are Staphylococcus aureus (S.aureus), Streptococci, Enterococci, E.coli, Klebsiella, Enterobacter, Citrobacter, Acinetobacter, Proteus, etc. S.aureus form a part of the normal flora and can be isolated from the noses of up to 60% of the healthy individuals. It is readily transmitted from individual to individual onto the hands and clothes of the health care staff, onto objects and into the air. S.aureus is the common reason of SSI and other nosocomial infections. S.aureus was once susceptible to Penicillin but largely resistant organisms soon emerged. The introduction of Methicillin primarily solves the problem, but later, the strains which were resistant to Methicillin developed. Thus, an increased number of resistant strains have been seen internationally.

The clinical consequence of Methicillin-resistant Staphylococcus Aureus (MRSA) is delicate by the fact that these isolates are frequently resistant to other anti-staphylococcal agents (Clindamycin, Erythromycin, Tetracycline, sometimes Gentamicin and Trimethoprim/Sulphomethoxazole), with the exception of Vancomycin. Sometimes, Methicillin-resistant-Staphylococcus aureus appear to be susceptible in vitro to other β-lactam agents such as Cephalosporins; however, they are clinically unsuccessful. Since MRSA are resistant to all the β-lactam antibiotics, the therapeutic options are extensively limited. The incidence of MRSA in India range from 30-70%.

The prevalence of MRSA in SSI is increasing more in developing countries because of lack of general hygiene. The present study was undertaken to establish the bacteriological resistance of S.aureus and other Enterobacteriaceae.

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profile and antibiogram of surgical site infections.

**MATERIAL AND METHODS**

This study was conduct in the Department of Microbiology, Department of Pharmacology, Government Medical College, Bettiah (West Champaran) Bihar and Associated Hospital. From March 2018 to April 2019. The study population included two hundred seventy five patients suffering from surgical site infections in the surgical and orthopaedic departments of Government Medical College, Bettiah (West Champaran), the Obstetrics and Gynaecology department of Government Medical College, Bettiah (West Champaran) Bihar and Associated Hospital. These patients were selected randomly and they belonged to the age group of five to sixty five years.

**Inclusion criteria**

A surgical site infection with wound discharge, pus discharge and negative cultures, but with symbols of sepsis present concurrently (warmth, erythema, induration and pain) and the physician diagnosis was considered as surgical site infection.6

**Exclusion criteria**

Wounds with cellulites, no drainage and suture abscesses were not included in the study.

**Relevant history**

A small clinical history concerning the age, sex, type of illness, diagnosis, the type of procedure achieves, antibiotics given and the incidence of related diseases like diabetes and peripheral vascular disease was obtained.

**Specimen collection**

Pus samples were assembling from each patient with the help of two sterile swabs used for smear preparation and the other was used for culture.

**Specimen Transport**

The swabs were brought to the Department of Microbiology, Department of Pharmacology, Government Medical College, Bettiah (West Champaran) Bihar and Associated Hospital. Bihar, immediately and processed within thirty minutes of collection.

**Sample Processing:** The pus samples were inoculated onto the media immediately and were incubated at 37°C for 24 hours in 7-10% CO2 concentration. After 24 hours of incubation, the isolated organisms were recognized by standard methods.8 Preliminary detection of bacteria was based on colony characteristics of the organisms. Such as haemolysis on blood agar, changes in physical appearance. Gram-negative rods were recognized by performing a sequence of biochemical tests. Namely: Indole, urea, Simon’s citrate agar and motility. Gram-positive cocci were recognized based on their gram reaction, catalase and coagulase test results.

**Antimicrobial susceptibility testing**

Antibiotic susceptibility testing was used the disc diffusion test which described by the Kirby Bauer method. The antimicrobial containing discs was located on the agar plate within 15 minutes of inoculation by using sterile forceps and these were pressed firmly beside the plate. The plates were inverted and incubated for 18-24 hours at 35°C, at a CO2 concentration of 7-10%.9 The drugs were selected, based on the antibiotic guidelines of our hospitals. Six discs were used on a 9 cm diameter plate. The antimicrobial discs for Staphylococcus aureus were: Penicillin (10 units), Ciprofloxacin (5mcg), Erythromycin (15mcg), Cefoperazone (30mcg), Oxacillin (1 mcg) and Co-trimoxazole (25mcg). These were identified as first line antibiotics. Those which were resistant to the first line antibiotics were identified with second line drugs like Vancomycin (30mcg), Rifampicin (30mcg), Teicoplanin (30mcg.), Cephalotaxine (30mcg) and Amoxiclav(30mcg). The antibiotic discs 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 MRSA recognition was done using oxacillin disc (1 μg) and Mueller Hinton agar with 2% NaCl. The plates were incubated for 24 hours at 35°C and zone diameter was measured. If zone diameter was ≥13mm, it was measured as Methicillin sensitive Staphylococcus aureus (MSSA) and if it was ≤10 mm then it was measured as MRSA.10 All MRSA and MSSA strains were identified for their susceptibility to all antibiotics include ciprofloxacin, co-trimoxazole, amikacin, clindamycin, erythromycin, chloramphenicol, cephalaxin, gentamicin, vancomycin, penicillin, linezolid, amoxicillin, amoxyclyc, cefuroxime, and cefotaxime by Kirby-Bauer disc diffusion technique.11 All tests were perform on Mueller-Hinton agar and were interpret after 24 hours of incubation at 35°C. The inhibition zone diameters were measured around each disc and were interpret according to the Clinical Laboratory Standards Institute guidelines.12 S. aureus ATCC 25923 was used as antimicrobial susceptibility testing.

**STATISTICAL ANALYSIS**

The collected data were statistically analyzed using SPSS Software. The statistical methicillin resistant S. aureus isolate were evaluated using Chi-square test and p < 0.05 was measured as statistically significant.

**RESULT**

Out of the 275 aerobic bacteria which were isolated (Table 1/Fig-1, 2), 144 were gram positive cocci (52.37%) and 131 were gram negative bacilli (47.63%). The most common organism was Staphylococcus aureus, with 101 isolates (36.36%). Other predominant Pseudomonas, Escherichia, Citrobacter, Klebsiella, Proteus, and Enterococcus. Among 101 S. aureus isolates included in our study, 57 (57%) were isolated from pus samples, 26 (25.70%) were isolated from blood, 5 (5.2%) were isolated from Urine, 5 (4.8%) were isolated from sputum, and 8 (7.3%) were isolated from miscellaneous samples as exposed in [Table 2]. Out of 101 S. aureus isolates, 37 (36.6%) were methicillin resistant S. aureus (MRSA) and 64 (63.4%) were methicillin-sensitive S. aureus (MSSA). while, the preponderance of
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Table-1: Aerobic bacteria isolated from infected postoperative wounds Chi-square test: $X^2 = 182.30$; P value $< .05$ statistically significant.

| Types of Sample | Samples Number | Samples Percentage |
|-----------------|----------------|--------------------|
| Pus             | 57             | 57.00%             |
| Blood           | 26             | 25.70%             |
| Urine           | 5              | 05.20%             |
| Sputum          | 5              | 04.80%             |
| Miscellaneous*  | 8              | 07.30%             |
| Total           | 101            | 100                |

Note*: Miscellaneous samples include ear discharge, abdominal drain fluid, throat swab, conjunctival swab and wound discharges etc.

Table-2: Sample-wise distribution of S.aureus isolates [n=101]

| Sample                          | Staphylococcus aureus | Chi-Square (χ²) & p value |
|---------------------------------|-----------------------|---------------------------|
| Resistant to cefoxitin (MRSA)    | 37 (36.5%)            |                          |
| Susceptible to cefoxitin (MSSA) | 64 (63.5%)            |                          |
| Total isolates N = 101 (100%)   |                       |                          |
| Pus due to any other cause, N(%)| 15 (34.23%)           |                          |
| Post operative Wound infection, N (%) | 7 (52.38%)     |                          |
| Blood; and SNCU blood culture, N (%) | 9 (31.78%)     |                          |
| Miscellaneous* Sample, N (%)    | 5 (41.67%)            |                          |
| Urine due to any other cause, N (%) | 02(36.36%)     |                          |

N = Number of isolates; MSSA = Methicillin sensitive Staphylococcus aureus; MRSA = Methicillin resistant Staphylococcus aureus; *p value $< 0.05$ was considered as statistically significant.

Table-3: Distribution of Staphylococcus aureus isolates on the basis of sample and susceptibility to cefoxitin (30 µg) disk.

| Antibiotic tested | Total No. N = 101 (100%) |
|-------------------|---------------------------|
|                   | MARS A NO. (%) | MSS A NO. (%) |
| Vancomycin        | 0(00.00%)      | 101(100%)     |
| Linezolid         | 0(00.00%)      | 101(100%)     |
| Ciprofloxacin     | 80 (79.05%)    | 21(20.95%)    |
| Cefoxitin         | 25(24.76%)     | 75 (75.24%)   |
| Gentamicin        | 23(22.86%)     | 71 (71.14%)   |
| Cefuroxime        | 23(22.86%)     | 71 (71.14%)   |
| Amoxyclov         | 36 (36.19%)    | 64(63.81%)    |
| Amoxicillin       | 1(33.33%)      | 1(67.77%)     |
| Doxycycline       | 76 (76.19%)    | 24 (23.81%)   |
| Levofloxacin      | 40(40%)        | 60(60%)       |

N = Number of isolates

Table-4: Resistance to individual antimicrobials in MRSA and MSSA isolated in Government Medical College, Bettiah (West Champaran) Bihar and Associated Hospital. Bihar.
Conducted in Tamilnadu, out of 906 strains, Northern Bihar had reported a higher MRSA prevalence. It was shown in another study conducted in Tamilnadu, out of 906 strains of S. aureus isolated from clinical samples, 250 (31.1%) were found to be methicillin resistant. Our study had MRSA prevalence of 36.5%. This variation in predominance may be because of several factors like healthcare facilities available in the particular hospital, implementation and monitoring of infection control committee, rationale antibiotic usage which varies from hospital to hospital.

In our study, we have included 101 S. aureus isolates derived from pus samples (44.40%), post-operative wound infection (12.60%), blood samples were 25 (25.70%), Miscellaneous Samples were 13 (12.10%) and urine sample 6 (5.20%) from both outpatients and inpatients of Orthopaedic Department of our Institution. The prevalence of Methicillin resistance amongst all S. aureus isolates was found to be 36.5%. This difference could be due to prolonged hospital stay, instrumentation and other invasive procedures. A comparable prevalence rate of 24, 34.6%, and 36.6% were also reported from Northern Bihar, and West Champaran Bihar.

Although MRSA from clinical specimens showed higher susceptibility to individual antibiotics when compared with others, we obtained high percentage of multdrug resistant MRSA from these specimens from Bihar had reported 24% of the MRSA isolated from clinical specimens to be multdrug resistant. Northern Bihar had reported a higher percentage of multdrug resistant MRSA. Bihar reported even a higher percentage of multdrug MRSA but from high risk patients admitted in burns and orthopedic units.

In our study we also looked forward for for Methicillin resistant S. aureus isolates by detecting their antimicrobial susceptibility to various other antibiotics. It was found that all isolates with Methicillin resistant S. aureus isolates were uniformly susceptible to linezolid and vancomycin. In another study, 13% of S. aureus isolates were resistant to methicillin. In other studies, we obtained high percentage of multidrug resistant S. aureus isolates were uniformly susceptible to linezolid and vancomycin, as shown in [Table 4].

**DISCUSSION**

There is a growing concern in India, the importance of MRSA as a problem has been recognized comparatively late. The prevalence of MRSA varies in different parts of India and is not uniform. Reports from a Delhi hospital show a prevalence rate of 51.6% in 2001, whereas it was reported as 38.44% in the same hospital in 2008. A recent study found the predominance to be 42% in 2008 and 40% in 2009. In a study at Aligarh, India it was shown that 35.1% of S. aureus and 22.5% of coagulates-negative staphylococcal isolates were resistant to methicillin. In another study conducted in Tamilnadu, out of 906 strains of S. aureus isolated from clinical samples, 250 (31.1%) were found to be methicillin resistant. Our study had MRSA prevalence of 36.5%. This variation in predominance may be because of several factors like healthcare facilities available in the particular hospital, implementation and monitoring of infection control committee, rationale antibiotic usage which varies from hospital to hospital.

In conclusion, the degree of resistance or sensitivity of MRSA towards commonly used antibiotics is recognized to be diverse from region to region and vancomycin was the prevalent antibiotic resistant. In conclusion, the degree of resistance or sensitivity of MRSA towards commonly used antibiotics is recognized to be diverse from region to region and vancomycin was the prevalent antibiotic resistant.
References

1. Hohmann C, Eickhoff C, Radziwill R, Schulz M. Adherence to guidelines for antibiotic prophylaxis in surgery patients in German hospitals: a multicentre evaluation involving pharmacy interns. Infection, 2012; 40: 131–37.
2. Nichols RL. Current Strategies for Prevention of Surgical Site Infections: Curr Infect Dis Rep 2004; 6:426-34.
3. Pradhan GB, Agrawal J. Comparative study of post-operative wound infection following emergency lower segment caesarean section with and without the topical use of fusidic acid. Nepal Med Coll J, 2009; 11: 189–91.
4. Mangram AJ, Horan TC, Pearson ML, Silver LC, Jarvis WR. Guidelines for Prevention of SSI, 1999, Criteria for defining SSI. 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.
5. Susan LK. Concepts in Antimicrobial therapy; TB of Diagnostic Microbiology: 3rd edition: WB Saunders. Editors; Mahon C.R, Manuselis G:2007 pg 82.
6. Verma S, Joshi S, Chitnis V, Hemavani N, Chitnis D. Growing problems of Methicillin Resistant Staphylococci-Indian Scenario. Indian J Med Sci 2000; 54:535-40.
7. 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.
8. 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.
9. Oslon M, Connor O, Schwartz ML. A Five year Prospective Study of 20193 Wounds at Minneapolis V a Medical Center. Ann Surg. 1984; 199; 253-9.
10. Larsen HS, Connie RM. Staphylococci: TB of Diagnostic Microbiology 3rd edition; WB Saunders. Editors; Mahon CR, Manuselis G:2007 pg 341.
11. Howard BJ. Nosocomial Infections- an Overview. Text Book of Clinical and Pathogenic Microbiology, 1994:2nd Ed. Howard BJ (Editor MosbyYear Book Inc., St. Louis, MO, USA.
12. Central Laboratory Standards Institute (CLSI). Performance standards for antimicrobial disc susceptibility tests, Approved standards 10th ed (2010). CLSI document M02-A10, vol 29, No.1.
13. Rajaduraipandi K, Mani KR, Panneerselvam K, M, Bhaskar M, Manikandan P. Prevalence and antimicrobial susceptibility pattern of methicillin resistant Staphylococcus aureus: a multicentre study. Indian J Med Microbiol 2006; 24:34-8.
14. Tiwari HK, Sapkota D, Sen MR. High prevalence of multidrug-resistant MRSA in a tertiary care hospital of northern India. Infection and Drug Resistance 2008; 1:5761.
15. Sangeeta Joshi, Pallab Ray, Vikas Manchanda, Jyoti Bajaj, D.S. Chitnis, Vikas Gautam. Methicillin resistant Staphylococcus aureus (MRSA) in India: Prevalence & susceptibility pattern. Indian J Med Res 2013;137:363–369.
16. Dar JA, Thoker MA, Khan JA, Ali A, Khan MA, Rizwan M, et al. Molecular epidemiology of clinical and carrier strains of methicillin resistant Staphylococcus aureus (MRSA) in the hospital settings of north India. Ann Clin Microbiol Antimicrob 2006; 5:22.
17. Poddar CK, Kumar R, Sinha RN, et al. Microbiological surveillance in the intensive care unit: a tertiary hospital experience in Koshi Area (Northern Bihar) India. Journal of Evolution of Medical and Dental Sciences 2014; 3:9050-6.
18. Pappu RK, Poddar CK, Kumar S, et al. Incidence of inducible clindamycin resistance in clinical isolates of staphylococcus aureus isolates from tertiary care hospital; experience in Koshi area (Northern Bihar), India. J. Evid. Based Med. Healhtc. 2019; 6: 71-76.
19. Rajak KC, Poddar CK, Kumar R, et al. Inducible clindamycin resistant staphylococcus aureus isolates from tertiary care hospital, Bettiah, India. J. Evolution Med. Dent. Sci. 2018;7:3984-3990.
20. Gupta V, Datta P, Rani H, et al. Inducible clindamycin resistance in Staphylococcus aureus: a study from north India. J Postgrad Med 2009;55:176-9.
21. Sasirekha B, Usha MS, Amruta JA, et al. Incidence of constitutive and inducible clindamycin resistance among hospital-associated Staphylococcus aureus. 3 Biotech 2014;4:85-9.
22. Pai N, Sharma B, Sharma S, et al. Detection of inducible clindamycin resistance among staphylococcal isolates from different clinical specimens in western India. J Postgrad Med 2010; 56:182-5.