BACTERIOLOGICAL PROFILE OF SURGICAL SITE INFECTIONS AND THEIR ANTIBIOGRAM: A STUDY FROM TERTIARY CARE HOSPITAL IN KOSHI REGION (NORTHERN BIHAR)

Varun Kumar1, Randhir Kumar2, Chandan Kumar Poddar3, Ram Nagina Sinha4, Suman Kumar5, S. K. Shah6, R. N. Pappu7, M. N. Singh8

1Assistant Professor, Department of Orthopaedics, Lord Buddha Koshi Medical College and Hospital, Saharsa, Bihar, India.
2Tutor, Department of Anatomy, Anugrah Narayan Magadh Medical College and Hospital, Gaya, Bihar, India.
3Assistant Professor, Department of Microbiology, Lord Buddha Koshi Medical College and Hospital, Gaya, Bihar, India.
4Medical Director and Consultant Surgery, Lord Buddha Koshi Medical College and Hospital, Saharsa, Bihar, India.
5Assistant Professor, Department of Microbiology, Shri Krishna Medical College, Muzaffarpur, Bihar, India.
6Professor, Department of Microbiology, Indira Gandhi Institute of Medical Sciences, Patna, Bihar.
7Associate Professor, Department of Microbiology, Lord Buddha Koshi Medical College and Hospital, Saharsa, Bihar, India.
8Professor, Department of Microbiology, Lord Buddha Koshi Medical College and Hospital, Saharsa, Bihar, India.

ABSTRACT

BACKGROUND
Surgical site infection (SSI) is one of the most common postoperative complications and causes significant postoperative morbidity and mortality. We wanted to determine the incidence of surgical site infections in clean, clean contaminated and contaminated surgeries in our setting along with the factors related to patient and surgical procedures influencing the rate of postoperative surgical wound infections and determine bacteriological profile with antimicrobial susceptibility patterns of the isolates.

METHODS
260 clinical samples received from the Department of Orthopaedic Surgery, in the Department of Microbiology at Lord Buddha Koshi Medical College and Hospital, Saharsa, Bihar were studied. The study period was from September 2018 to April 2019. A total of 260 Staphylococcus aureus isolated was identified by standard biochemical methods. Antibiotic susceptibility testing was performed in our Department by Kirby Bauer Disc Diffusion method. Methicillin resistance was detected in the Microbiology Department by using cefoxitin (30 μg) disc diffusion method as per CLSI guidelines 2016.

RESULTS
Out of 260 S. aureus isolates of the MRSA 94 (36.2%) were derived from pus samples 39 (34.33%), the S. aureus isolates derived from wound samples were MRSA 17 (52.38%), the S. aureus isolates derived from blood samples were MRSA 21 (31.78%), the S. aureus isolates derived from miscellaneous samples were MRSA 13 (41.67%) and the S. aureus isolates derived from urine samples were MRSA 5 (36.36%).

CONCLUSIONS
Surgical site infections are one of the most common hospital acquired infections and are an important cause of morbidity and mortality. Depending on the site of wound infection and clinical symptoms, the role of microbiology laboratory is to determine the clinically significant isolates, perform antimicrobial susceptibility testing, and subsequently provide guidance on the most appropriate treatment. This will help in successful wound management and will also assist in the control of antibiotic usage and hence curtail the spread of antibiotic-resistant bacteria.

KEYWORDS
Surgical Site Infection, Methicillin Resistant Staphylococcus Aureus (MRSA), Prevalence, Antibiotics.

Surgical site infections (SSI), one of the most common causes of nosocomial infections are a common complication associated with surgery, with a reported incidence rates of 2-20%. They are accountable for increasing the treatment cost, length of hospital stay and significant morbidity and mortality. Despite the technical advances in infection control and surgical practices, SSI still continue to be a major problem, even in hospitals with most modern facilities. These infections are usually caused by exogenous and/ or endogenous micro-organisms that enter the operative wound either during the surgery (primary infection) or after the surgery (secondary infection). Primary infections are usually more serious, appearing within five to seven days of surgery. Majority of SSIs are uncomplicated involving only skin and subcutaneous tissue but sometimes can progress to necrotizing infections. The usual presentation of infected surgical wound can be characterized by pain, tenderness, warmth, erythema, swelling and pus formation. A number of patient related factors (old age, nutritional status, pre-existing infection, co-morbid illness) and procedure related factors (poor surgical technique, prolonged duration of surgery, pre-operative part preparation, inadequate sterilization of surgical instruments) can influence the risk of SSIs significantly. In adding together to these risk factors, the virulence and the invasiveness of the organism involved, the physiological state of the wound tissue and the immunological reliability of the host are also the important factors that conclude whether infection occurs or not. Surgery has made great advances in last few decades, but postoperative wound infection is the most common complication faced by surgeons since the advent of surgery. Infection causes delayed recovery and may leave permanent disability. Despite efforts to have a better understanding of sepsis, wound infection is still a clinical problem and some infections in clean wounds remain unexplained. Wound access to bacteria can be prevented by aseptic surgical techniques. The main objective of the present study was to find the incidence of surgical site infections in clean, clean contaminated and contaminated surgeries in our setting along with the factors related to patient and surgical procedures influencing the rate of postoperative surgical wound infections and to determine bacteriological profile with antimicrobial susceptibility patterns of the isolates.

**METHODS**

The present prospective study was conducted in the Department of orthopaedic Surgery, Department of Microbiology at Lord Buddha Koshi Medical College and Hospital, Saharsa, Bihar. The study period was from September 2018 to April 2019 Institutional ethics committee approval was obtained prior to the start of the study.

**Study Design**

This study was based on retrospective data of samples sent from different wards and OPDs of Orthopaedics surgery Department, Lord Buddha Koshi Medical College and Hospital, Saharsa, Bihar and Associated Hospital. Total strains of 300 S. aureus were isolated from pus, urine, sputum, wound swab, aural swab, blood, throat swab and urethral swab during March 2017 to April 2019. Staphylococcus aureus strains isolated from cultures of specimens from patients who have been hospitalized for >48 hours were included in the study. Staphylococcus aureus were characterised by their morphology on Gram staining, growth characteristics and coagulase production.

**Inclusion Criteria**

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

**Exclusion Criteria**

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

**Relevant History**

A little clinical history concerning the age, sex, type of illness, diagnosis, the type of operation performed, antibiotics given and the presence of related diseases like diabetes and peripheral vascular disease was obtained.

**Specimen Collection**

Pus samples were brought together from each patient with the help of two sterile swabs under aseptic precautions, of which one was the use of smear preparation and the other use of culture.

**Specimen Transport**

The swabs were brought to the Department of Microbiology, of Lord Buddha Koshi Medical College and Hospital, Saharsa, Bihar and Associated Hospital. Bihar, immediately and processed within thirty minutes of collection.

**Sample Processing**

The pus samples were inoculated on 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 identified by standard methods. Initial identification of bacteria was based on colony characteristics of the organisms. Such as haemolysis on blood agar, change in physical manifestation in different media and enzyme performance of the organisms. Biochemical tests were performed on colonies from primary cultures for detection of the isolates. Gram-negative Bacci were identified by performing a series of biochemical tests. Namely: Indole, Simon’s citrate agar, urea and motility. Gram-positive cocci were recognized based on their gram reaction, catalase and coagulase test results.

**Antimicrobial Susceptibility Testing**

Antibiotic susceptibility testing was done by the disc diffusion test which was described by the modified Kirby Bauer method. The antimicrobial contain discs were located 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%. The drugs were chosen, based on their action on a particular organism and also on the antibiotic policy of the hospitals. Six discs were used on a 9 cm diameter plate. The antimicrobial discs for Staphylococcus aureus were: Penicillin (10 units), Erythromycin (15 mcg), Ciprofloxacin (5 mcg), Cefoperazone (30 mcg), Oxacillin (1 mcg), and Co-trimoxazole (25 mcg). These were tested as first line antibiotics. Those which were resistant to the first line antibiotics in our department were tested with second line drugs like Vancomycin (30 mcg), Teicoplanin (30 mcg), Rifampicin (30 mcg). Cefotaxime (30 mcg) and Amoxiclav (30 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 millimetre scale. The zone size around each antimicrobial disc was interpreted as sensitive, intermediate or resistant, according to Clinical and Laboratory Standards Institute (CLSI) criteria. Isolated colonies were subjected to identification by colony morphology, gram staining and standard biochemical tests. 8 MRSA detection 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 ≥13 mm, it was considered as Methicillin sensitive Staphylococcus aureus (MSSA) and if it was ≤10 mm then it was considered as MRSA. All MRSA and MSSA strains were tested for their susceptibility to all antibiotics including ciprofloxacin, co-trimoxazole, gentamicin, amikacin, clindamycin, erythromycin, chloramphenicol, cephalixin, vancomycin, linezolid, penicillin, amoxicillin, amoxacil, cefuroxime, and cefotaxime by Kirby-Bauer disc diffusion technique. All tests were performed on Mueller-Hinton agar and were interpreted after 24 hours of incubation at 35°C. The inhibition zone diameters were measured around each disc and were interpreted according to the Clinical Laboratory Standards Institute guidelines. S. aureus ATCC 25923 was used as a control strain for the standardization of antimicrobial susceptibility testing.

Statistical Analysis
The collected data was statistically analysed using SPSS Data Editor Software, Chicago, version 20. The statistical methicillin resistant S. aureus isolates were evaluated using Chi-square test and p < 0.05 was considered as statistically significant.

RESULTS

<table>
<thead>
<tr>
<th>Types of Sample</th>
<th>Samples Number</th>
<th>Samples Percentage</th>
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<tbody>
<tr>
<td>Pus</td>
<td>146</td>
<td>56.10%</td>
</tr>
<tr>
<td>Blood</td>
<td>64</td>
<td>24.60%</td>
</tr>
<tr>
<td>Urine</td>
<td>14</td>
<td>05.30%</td>
</tr>
<tr>
<td>Spuutm</td>
<td>15</td>
<td>05.80%</td>
</tr>
<tr>
<td>Miscellaneous**</td>
<td>21</td>
<td>08.20%</td>
</tr>
<tr>
<td>Total</td>
<td>260</td>
<td>100</td>
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</table>

<table>
<thead>
<tr>
<th>Sample</th>
<th>Staphylococcus Aureus</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Resistant to Cefoxitin (MRSA)</td>
</tr>
<tr>
<td>Pu due to other cause, N (%)</td>
<td>39 (34.33%)</td>
</tr>
<tr>
<td>Post-operative Wound infection, N (%)</td>
<td>17 (52.38%)</td>
</tr>
<tr>
<td>Blood; and ShRU BLOOD CULTURE, N (%)</td>
<td>21 (31.78%)</td>
</tr>
<tr>
<td>Miscellaneous Sample, N (%)</td>
<td>13 (41.67%)</td>
</tr>
<tr>
<td>Urine due to any other cause, N (%)</td>
<td>05 (36.36%)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Antibiotic Tested</th>
<th>MARSA No. (%)</th>
<th>MSSA No. (%)</th>
</tr>
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<tbody>
<tr>
<td>Vancomycin</td>
<td>201 (77.69%)</td>
<td>79 (30%)</td>
</tr>
<tr>
<td>Linezolid</td>
<td>203 (78.05%)</td>
<td>77 (30%)</td>
</tr>
<tr>
<td>Cefoxitin</td>
<td>67 (25.76%)</td>
<td>193 (74.24%)</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>62 (23.86%)</td>
<td>182 (76.14%)</td>
</tr>
<tr>
<td>Cefuroxime</td>
<td>62 (23.86%)</td>
<td>182 (76.14%)</td>
</tr>
<tr>
<td>Amoxiclav</td>
<td>91 (35.19%)</td>
<td>169 (64.81%)</td>
</tr>
<tr>
<td>Amoxicillan</td>
<td>1 (34.33%)</td>
<td>2 (66.67%)</td>
</tr>
<tr>
<td>Doxycycline</td>
<td>201 (77.19%)</td>
<td>59 (22.81%)</td>
</tr>
<tr>
<td>Levofloxacin</td>
<td>109 (42%)</td>
<td>151 (58%)</td>
</tr>
</tbody>
</table>

| Table 3. Resistance to Individual Antimicrobials in MRSA and MSSA Isolated in Koshi Region, and Associated Hospital of Bihar |

N= Number of isolates

- Among 260 S. aureus isolates included in our study, 146 (56.10%) were isolated from pus samples, 64 (24.60%) were isolated from blood, 14 (5.3%) were isolated from Urine, 15 (5.8%) were isolated from sputum, and 21 (8.20%) were isolated from miscellaneous samples as shown in (Table 1).
- Out of 260 S. aureus isolates, 94 (36.2%) were methicillin resistant (MRSA) and 166 (63.8%) were methicillin-sensitive S. aureus (MSSA). Although, majority of the MRSA isolates were derived from pus samples 39, however, the S. aureus isolates derived from post-operative wound infection were mostly MRSA 17. This finding was found to be statistically significant (P=0.0008703) (Table-2).
- Out of 260 S. aureus isolates of the MRSA 94 (36.2%) were derived from respectively Pus samples 39 (34.33%), the S. aureus isolates derived from Wound Samples were MRSA 17 (52.38%), the S. aureus isolates derived from Blood Samples were MRSA 21 (31.78%), the S. aureus isolates derived from Miscellaneous Samples were MRSA 13 (41.67%) and the S. aureus isolates derived from Urine Samples were MRSA 5 (36.36%).This finding was found to be statistically significant (p=0.347891). (Table-2)
- The antimicrobial susceptibility test result of all the 260 S. aureus isolates Among MRSA, resistance that they were 100% sensitive to vancomycin and linezolid, with
moderate sensitivity (70.14%) to gentamicin, cefuroxime and least sensitivity to (22.81%) doxycycline, (21.95%) ciprofloxacin as shown in (Table 3).

DISCUSSION
The problem of postoperative wound infection is seen in both developed and developing countries, despite introduction of meticulous antiseptic regime in surgical practice. It can occur growing concern about the rapid rise in resistance of S. aureus to antimicrobial agents. In India, the importance of MRSA as a problem has been recognized relatively late.13 The prevalence of MRSA varies in different parts of India and is not uniform. Reports from a Delhi hospital showed a prevalence rate of 51.6% in 2001, whereas it was reported as 38.44% in the same hospital in 2008.14 A recent study15 found the prevalence to be 42% in 2008 and 40% in 2009. In a study at Alligarh, India16 it was shown that 35.1% of S. aureus and 22.5% of coagulase-negative staphylococcal isolates were resistant to methicillin. In another study17 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 36.6%. This variation in prevalence 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 260 S. aureus isolates derived from pus 115 (44.20%), post-operative wound infection 33 (12.80%), blood samples were 66 (25.50%), Miscellaneous Samples were 32 (12.20%) and urine sample 14 (5.30%) 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 36.2%. 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 Bihar17,18,19. Although MRSA from clinical specimens showed higher susceptibility to individual antibiotics when compared with others, we obtained high percentage of multidrug resistant MRSA from these specimens. Poddar CK, et al. From Bihar had reported 24% of the MRSA isolated from clinical specimens to be multidrug resistant.20 Pappu RK, Poddar CK, et al. From Northern Bihar had reported a higher percentage of multidrug resistant MRSA.18 Rajak KC, Poddar CK, et al. From Bihar reported even a higher percentage of multidrug MRSA but from high risk patients admitted in burns and orthopaedic units.19 In our study we also looked forward for treatment options 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 100% susceptible to linezolid and vancomycin, followed by moderate susceptibility (70, 14%) to gentamicin, cefuroxime and least susceptibility to doxycycline, ciprofloxacin (22.81% and 20.95% respectively). This finding is in concordance to other studies that also found that all the Methicillin resistant S. aureus isolates were uniformly susceptible to linezolid and vancomycin.20,21,22

CONCLUSIONS
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 only antibiotic found in our study to give uniform sensitivity (100%). When antimicrobials including vancomycin are considered for treatment, choice inevitably requires the need for in vitro susceptibility testing of every isolate of MRSA in the clinical laboratories. Our study is a preamble to enable epidemiologists to understand the nature of MRSA isolates in Kosi region Bihar, India.

REFERENCES


