Surgical Site infections: Distribution Studies of Sample, Outcome and Antimicrobial Susceptibility Testing

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Abstract

Background: The present study was conducted in the clinical lab of Microbiology department, College of Medical Sciences. The samples that were sent for culture and sensitivity from patients developing symptoms of SSIs were included in this study. A total of 206 pus samples were processed and result was interpreted based on the guidelines of different tests. Isolation and identification of the organism was done by gram stains and culture growth. Antibiotic susceptibility test was performed by Kirby Bauer disc diffusion method and result was interpreted as per Clinical and Laboratory Standards Institute (CLSI) guidelines.

Result: Out of 206 clinically suspected cases 106 (51.4%) were culture negative and 100 (48.6%) were culture positive. The growths were found to be higher in male patients than in female patients. The most commonly isolated pathogens were Staphylococcus aureus (21.8%) and Escherichia coli (21.8%), followed by Pseudomonas aeruginosa (17.4%). Amikacin (86.4%) was the most sensitive drug. 14 isolates of Methicillin resistant S. aureus (MRSA) were found.

Conclusion: The study helped in the assessment of the current anti-microbial resistance patterns of bacterial isolates in post-operative wound infections and helps in formulation of the strategy to reduce the infection rate in College of Medical Sciences-Teaching Hospital, Bharatpur.

Keywords: Surgical site infection; Culture; Isolates; Antibiotics; Resistant

Abbreviations

AST: Antibiotic Susceptibility Test; ATCC: American Type Culture Collection; BA: Blood agar; CDC: Centers for Disease Control; CLSI: Clinical Laboratory Standards Institute; CONS: Coagulase Negative Staphylococci; CTVS: Cardiothoracic Vascular Ward; GNB: Gram Negative Bacilli; GPC: Gram Positive Cocci; ICU: Intensive Care Unit; MA: Mac-Conkey Agar; MHA: Mueller Hinton Agar; MR: Methyl Red; MRSA: Methicillin-Resistant Staphylococcus aureus; MSSA: Methicillin Sensitive S. aureus; NNIS: National Nosocomial Infection Surveillance System; SENIC: Study of the Efficacy of Nosocomial Infection Control; SSI: Surgical Site Infection; VISA: Vancomycin Intermediate S. aureus; VP: Voges-Proskauer; VRE: Vancomycin Resistant Enterococci; VRSA: Vancomycin Resistant S. aureus; WHO: World Health Organization; ZOI: Zone Of Inhibition

Background

Surgical site infection (SSI) can occur anytime from 0 to 30 days after a procedure in which no implant is used and up to 1 year if foreign material (e.g. prosthetic heart valve, hip prosthesis) is implanted. About 80% to 90% of all postoperative infections occur within 30 days after the operative procedure. Most of the wound infection manifest within a week of surgery. Streptococcus pyogenes infections appear within a day or two; while Staphylococcal infections typically take four or five days and gram negative bacillary infections take six or seven days to appear. S. aureus is the most commonly isolated human bacterial pathogen and is ok an important cause of skin and soft-tissue infections (SSTIs), endovascular infections, pneumonia, septic arthritis, endocarditis, osteomyelitis, foreign-body infections, and sepsis. S. aureus can become pathogenic when conditions such as pH, temperature and nutrient availability are altered and become favourable for overgrowth [1].

The majority of SSI is caused by the native flora of the patient’s skin, mucous membranes, or hollow viscera. When skin is incised, underlying tissue is exposed to overlying endogenous flora. It is also caused by the organisms present in the hospital environment that are introduced to the patient by medical procedures. The most commonly isolated bacterial pathogens are S. aureus, Enterobacteriaceae, Coagulase Negative Staphylococci (CoNS), Enterococci and Pseudomonas aeruginosa. Although the pathogens isolated depend on the surgical procedure involved, recent reports have documented an increasing proportion of gram positive organisms and decrease in number of gram negative organisms associated with SSIs. Furthermore, there is an increase in incidence of SSIs attributed to antimicrobial resistant pathogenic bacteria like methillin resistant S. aureus (MRSA) and Vancomycin Resistant S. aureus (VRSA) [2].

Risk factors other than microbiology can be due to systemic factors affecting the patient’s healing response, local wound characteristics, or
operative characteristics. Its risk depends on bleeding, the amount of devitalized tissue created, the need for drains within the wound, obesity and diabetes mellitus [3]. Wounds become contaminated by varying number and type of microbial pathogen; bacteria, fungi, virus, parasite, mycoplasma and rickettsia. Organisms generally encountered in wound infections and abscesses are S. aureus, Streptococcus pyogenes, Enterococcus spp., Proteus spp., Morganella spp., Providencia spp., Escherichia coli, Bacteroides spp., Clostridium spp., Peptostreptococcus spp. Other uncommon microorganisms causing culture negative SSI include Mycobacterium chelonae, Mycobacterium fortuitum, Ureaplasma urealyticum and Mycoplasma hominis [4].

MRSA is an increasingly common cause of postoperative surgical site infections Vancomycin Resistant Enterococci (VRE) and gram negative Bacilli with unusual patterns of resistance have also been frequently isolated. Since bacteria resistant to multiple antibiotics are commonly found, there is possibility of extensive outbreak which may be difficult to control. Therefore, it is imperative to establish an early diagnosis and formulate an effective antibiotic policy [5].

This study will assess the prevalence of surgical wound infections and current anti-microbial resistance patterns of bacterial isolates in post-operative wound infections in among the patients admitted to College of Medical Sciences (COMS), Teaching Hospital. It assists the clinicians in appropriate selection of antibiotics for prophylaxis and treatment.

Method

Study area

A hospital based prospective study was carried out in the College of Medical Sciences-Teaching Hospital, Bharatpur. It is a tertiary care teaching hospital affiliated with the Kathmandu University. The study was carried out for a period of six months from May 2014 to October 2014.

Study population and sample size

This included all patients with post-operative wound infections in different surgical wards and ICUs. The study covered 206 cases of post-surgical wound infection.

Type of sample

Post-surgical wound swabs and pus aspirates

Specimen collection

The sample collected for the study were pus aspirates and wound swabs from the surgical wounds. The specimens were collected aseptically. The area around the surgical wound was cleaned with 70% ethyl alcohol and the exudates was collected from the depth of the wound using two sterile cotton swabs, one for Gram stain and another for culture. Utmost care was taken not to touch the surrounding tissues to prevent contamination of the swab from endogenous resident flora.

The sample that were collected were sent to the laboratory immediately for processing, to avoid desiccation and to prevent the growth of some species at room temperature that may obliterate the true pathogens.

Rejection criteria

- Old wound swabs and pus aspirates
- Burn wounds and skin wounds
- Unlabelled and improperly labelled samples

Sample processing

The samples were processed as soon as it reached the laboratory following the standard laboratory procedures. Of the two surgical samples, one was used for gram staining and other for isolation of bacteria by culture.

Macroscopic examination

The pus samples were examined for its appearance, color, consistency and presence of granules.

Microscopic examination

An evenly spread smear of the specimen was prepared on a clean grease free glass slide. The smear was allowed to air dry, heat fixed and stained by Gram stain method. The smear was then examined for the presence of bacteria and cellular elements using microscope.

Culture

The second swab was inoculated onto plates of 5% Sheep Blood agar (BA) and MacConkey agar (MA) by rolling the swab over the agar and streaking from the primary inoculums, using a sterile bacteriological loop. These plates were incubated at 37°C for 24-48 hours.

Characterization and Identification

All types of colonies on the primary plates were examined macroscopically for haemolysis in BA, changes in physical appearance of differential media, and the colony characteristics were recorded. The colony present on these plates was gram stained, identified by motility testing, biochemical testing and antibiotic susceptibility testing.

Gram’s staining

Colonies on MacConkey’s Agar and blood agar plates were stained by gram’s staining method and the morphology, gram reaction and arrangement of the microorganisms were noted.

Motility testing

Hanging drop method was used for demonstration of motility. A drop of the broth culture was taken on the centre of a cover slip and a cavity slide placed over it with the cavity covering the centre of cover slip and inverted. The preparation was then observed under low (10X) and high power lens (40X) of the microscope.

Biochemical testing

Catalase and coagulase testing were done for confirmation of Gram positive bacterial isolates. For Gram negative bacterial isolates, different tests were done-Indole, MR, VP, Citrate utilization test, OF and Urease.
Antibiotic susceptibility testing

Antibiotic sensitivity testing was performed using the standard disc diffusion method recommended by the Clinical and Laboratory Standard Institute [6] for the following antibiotics: Amikacin, Amoxycillin-clavulanic acid, Gentamicin, Ampicillin-sulbactam, Cefoperazone-sulbactam, Meropenem, Levofloxacin, Ciprofloxacin, Cefazidime, Cefotaxime, Ceftriaxone, Cefixime, Cephalexin, Ofloxacin, Cotrimoxazole, Azithromycin, Erythromycin, Penicillin, Ampicillin, Oxacillin and Vancomycin. S. aureus resistant to oxacillin will be identified as MRSA and those susceptible as methicillin sensitive S. aureus (MSSA).

Quality control for tests

The quality control was performed in every required step. The samples were collected using sterile swab aseptically in order to avoid contamination. The sterility of each batch of the test medium was confirmed by incubating uninoculated plates and tubes overnight at 37°C and were not used if those plates and tubes showed the evidence of bacterial growth and other visual reactions after incubation. The positive and negative control were incubated along with test for comparing the results. Control strains of E. coli (ATCC 25922), S. aureus (ATCC 25923) and P. aeruginosa (ATCC 27853) were used to check the quality of the medium from each batch. Similarly, indicator media with correct pH antibiotics discs having correct amount as indicated was used.

Statistical Analysis

All the study data were entered into the computer database using standard format, checked for errors and verified. Data maintained in the computer sheets were organized and analyzed by SPSS 16.0 software for windows. Data will be presented in appropriate Table, Figures by calculating of percentage, rate, etc.

Results and Discussion

Wound infections have been a problem in the field of surgery for a long time. Antimicrobial resistance can increase complications and costs associated with procedures and treatment. An infected wound complicates the postoperative course and results in prolonged stay in the hospital and delayed recovery [7]. In the present study, an attempt has been made to know the various pathogens associated with surgical site infections and their antibiotic.

The present study of postoperative wound infections was carried out on patients who had undergone surgery in the College of Medical Sciences-Teaching Hospital, Bharatpur, Chitwan. The study included the pus aspirates and wound swabs collected from post-surgical wound infections. A total of 206 wound samples were collected and processed. They were gram stained and cultured in appropriate culture media, identification was made and antimicrobial susceptibility testing was carried out for appropriate selection of antibiotic.

Cultural Characteristics of Sample

In this study, out of 206 specimens from clinically suspected cases, 106 (51.4%) were culture negative and 100 (48.6%) were culture positive as shown in Figure 1.
Sex-wise distribution of samples

In the present study, out of 206 samples, 134 specimens (65.04%) were from male patients and 72 (34.96%) were from female patients. The number of samples from male patients was higher than from female patients. Among the 100 culture positive cases, 71 (71%) were from specimens of male patients and 29 (29%) were from specimens of female patients. Growth was found to be higher in samples from male patients. In all age groups, male patients were affected more frequently than female patients. Therefore, wound infection is higher in male patients than in female patients.

Working age group and males were found to be more affected. This may be because males and matured are more active outdoors. Similarly, younger and older age groups are comparatively less active and less prone to accidents and wounds.

Anguzu and Olila [9], in a study carried out in Jinja Regional Referral Hospital, Uganda found that out of 94 patients studied, 56 (59.6%) were males and 38 (40.4%) were females which was in accordance with the present study. Similar findings were recorded in the study conducted by Khan et al. [13] in Ayub Medical College, Abbottabad, where out of 104 patients, 64.42% were males and 35.58% were females. In the similar manner, Adegoke et al. [14], carried out a study in Nigeria where higher percentage of male (76.5%) patients was found than females.

Age-wise distribution of samples

In the present study, samples were collected from age group ranging from 1 to 90 years and categorised into three main age groups, 1-30, 31-60 and 61-90 years representing younger, matured and older age groups. Among 206 cases, highest number of infections (102) was recorded in the age group 31-60 years and the highest incidence was in the same age group. The lowest incidence was seen in older age group 61-90 followed by younger age group 1-30. The prevalence of wound infection was not significantly affected by age (p>0.05) (Table 1). Similar findings were obtained in the study of Khosravi et al. [15], in Pakistan, the median age was 35 (± 15.8) years. Similarly, Ranjan et al. [16], in a referral hospital in Haryana, India found the modal age group as 21-40 years with the frequency of 146.

Pattern of culture isolates

In this study, among 100 culture positive samples, a total of 110 bacterial isolates were obtained comprising 27 (24.5%) gram positives and 83 (75.5%) gram negatives (Figure 3). Similar result was obtained by Goswami et al. [8], in a study carried out in a tertiary care hospital in Gujarat, India, where out of 183 organisms, 31.15% pathogens were gram positive and 68.85% were gram negative. In a study by Manyahi [2], gram negative organisms were more prevalent than gram positive bacteria accounting for 77.5% and 22.5% of isolates respectively.

In the present study, the most commonly isolated pathogens were S. aureus 24 (21.8%) and E. coli 24 (21.8%) (Figure 4), followed by P. aeruginosa 17 (15.4%), K. pneumoniae 15 (13.6%), Acinetobacter anitratus 12 (10.9%), Enterobacter spp. 11 (10%), Coagulase negative Staphylococcus (CoNS) 3 (2.7%), Proteus mirabilis 2 (1.8%), Proteus vulgaris 1 (0.9%) and Klebsiella oxytoca 1 (0.9%).
S. aureus were confirmed by tube coagulase test (Figure 5) and E. coli were confirmed by biochemical tests (Figure 6).

The predominance of S. aureus in surgical site infection is consistent with reports from other studies, both in India and abroad. S. aureus has been the most common organism isolated from SSIs over decades and across continents. S. aureus was isolated in 63% of cases in a study by Shriyan et al. [18] which is very high. In a study carried out by Manyahi [2], S. aureus was the most prevalent organism accounting for 9 out of 44 (20%) isolates.

**Antimicrobial susceptibility testing**

Antimicrobial susceptibility testing is important for the correct prescription of antibiotics for the treatment of patients. Antibiotic sensitivity testing is an in vitro method for estimating the activity of drugs which will assist clinician in selecting an antimicrobial agent effective in inhibiting the growth of an infecting microorganism in vivo. Total numbers of bacterial isolates were 110.

| S. No | Antibiotics                  | Sensitive | Intermediate | Resistant | Total |
|-------|-----------------------------|-----------|--------------|-----------|-------|
|       | No. | %   | No. | %   | No. | %   | No. |
| 1     | Amikacin                    | 95        | 86.4 | 1   | 0.9 | 14  | 12.7 | 110     |
| 2     | Ciprofloxacin               | 67        | 60.9 | 1   | 0.9 | 42  | 38.2 | 110     |
| 3     | Ceftriaxone                 | 51        | 46.4 | 2   | 1.8 | 57  | 51.8 | 110     |
| 4     | Meropenem                   | 76        | 69.1 | 5   | 4.5 | 29  | 26.4 | 110     |
| 5     | Ampicillin-sulbactam        | 76        | 69.1 | 2   | 1.8 | 32  | 29.1 | 110     |
| 6     | Cefoperazone-sulbactam      | 89        | 81   | -   | -   | 21  | 19   | 110     |
| 7     | Ceftazidime                 | 30        | 36.1 | 3   | 3.6 | 50  | 60.2 | 83      |
| 8     | Ofloxacin                   | 36        | 43.4 | 4   | 4.8 | 43  | 51.8 | 83      |

The common antibiotic discs used for all types of bacterial isolates were Amikacin, Ciprofloxacin, Ceftriaxone, Meropenem, Ampicillin-sulbactam and Cefoperazone-sulbactam. Among these, Amikacin (86.4%) was the most effective drug. Other effective drugs were Cefoperazone-sulbactam (81%), Meropenem (69.1%) and Ciprofloxacin (60.9%). While the least effective drug was Ceftriaxone (46.4%). Erythromycin, Penicillin, Oxacillin, Amoxycillin-clavulanic acid, Ampicillin, Levofloxacin and Vancomycin were used for gram positive isolates. Tobramycin and Piperacillin were used for P. aeruginosa only. Similar findings were given by Kranthi et al. [19] where Amikacin is most effective drug against gram positive and gram negative bacteria which is 100% true and in accordance to our result (Table 2).
Shriyan et al. [18], found that S. aureus was generally sensitive to Amoxicillin-clavulanic acid followed by Vancomycin (100%), Teicoplanin (100%) and linezolid (100%). 37.7% of the S. aureus isolates were resistant to Erythromycin and Ampicillin-clavulanic acid and 1.8% of the them were MRSA which is very less as compared to 58.3% in the present study.

**Table 2: Antibiotic susceptibility pattern of total isolates.**

|   | Azithromycin | Gentamicin | Penicillin | Amoxycillin-clavulanic acid | Erythromycin | Oxacillin | Vancomycin | Cotrimoxazole | Levofloxacin | Ampicillin | Tobramycin | Piperacillin |
|---|--------------|------------|------------|-----------------------------|--------------|-----------|------------|--------------|--------------|------------|------------|-------------|
|   | 61           | 73.5       | 2          | 2.4                         | 20           | 24.1      | 83         | 19           | 70.4         | 8          | 27         | 15          |
|   | 44           | 53         | -          | -                           | 39           | 47        | 83         | 11           | 40.7         | -          | 16         | 59.3        |
|   | -            | -          | -          | -                           | 27           | 100       | 27         | 13           | 70.4         | -          | 8          | 29.6        |
|   | 11           | 11         | -          | -                           | 16           | 59.3      | 27         | 14           | 44.4         | -          | 15         | 55.6        |
|   | 27           | 100        | -          | -                           | -            | -         | 27         | 15           | 100          | -          | -          | -           |
|   | 18           | 66.7       | -          | -                           | 9            | 33.3      | 27         | 16           | 67.7         | -          | -          | -           |
|   | 19           | 70.4       | -          | -                           | 8            | 29.6      | 27         | 17           | 70.4         | -          | 8          | 29.6        |
|   | 14           | 51.9       | -          | -                           | 13           | 48.1      | 27         | 18           | 82.4         | -          | 3          | 17.6        |
|   | 15           | 88.2       | -          | -                           | 2            | 11.8      | 17         | 20           | 88.2         | -          | -          | -           |

In a study carried out by Raza et al. [20], in a tertiary care hospital, Kathmandu, Nepal, all *S. aureus* isolates were sensitive to Amikacin, Ceftriaxone, Meropenem, Ampicillin-sulbactam, Cefoperazone-sulbactam and Vancomycin followed by Ciprofloxacin (95.8%), Erythromycin (70.8%), Levofloxacin (75%) Cotrimoxazole (66.7%) and Ampicillin (54.2%). Highest resistance, with 62.5% strains being resistant was seen with Amoxicillin-clavulanic acid. The Gram positive bacterial isolates were found to be 100% resistant towards Pencillin. Methicillin resistant *S. aureus* (MRSA) was found to be 14 (58.3%) which was resistant to Oxacillin, and was found to be sensitive to Vancomycin (Figure 7).

In our study, all isolates of *S. aureus* were sensitive to Amikacin, Ceftriaxone, Meropenem, Ampicillin-sulbactam, Cefoperazone-sulbactam and Vancomycin followed by Ciprofloxacin (95.8%), Erythromycin (70.8%), Levofloxacin (75%) Cotrimoxazole (66.7%) and Ampicillin (54.2%). Highest resistance, with 62.5% strains being resistant was seen with Amoxicillin-clavulanic acid. The Gram positive bacterial isolates were found to be 100% resistant towards Pencillin. Methicillin resistant *S. aureus* (MRSA) was found to be 14 (58.3%) which was resistant to Oxacillin, and was found to be sensitive to Vancomycin (Figure 7).

Among Gram negative bacterial isolates, the most effective drug was Amikacin (81.9%) followed by Cefoperazone-sulbactam (74.7%) and the least effective drug were Ceftriaxone (31.3%) and Ceftazidime (36.1%). In the present study, all isolates of *E. coli* were sensitive to Amikacin followed by Cefoperazone-sulbactam (87.5%), Azithromycin and Gentamicin (83.3% each). Resistance was maximum with Ceftriaxone and Ofloxacin (62.5% each).

Suchitra et al. [23], in a study carried out at Bangalore, India found 90% of their *E. coli* were sensitive to Amikacin and Gentamicin which is comparable with our study. Malik et al. [24], at a tertiary care hospital in India also reported a similar sensitivity pattern. In their study, *E. coli* isolates showed maximum susceptibility to imipenem and Cefoperazone-sulbactum (93.10%).

The scenario of isolates and their antibiotic susceptibility pattern varies from place to place, time to time and patient to patient. It depends on the patients who were taking broad-spectrum antibiotics as prophylaxis, infrequent usage of drugs, lower immune status, poor nourishment and age. Approaches to the prevention and control of SSI have evolved over many years and traditionally have been classified into those interventions before surgery, during surgery and after surgery. However, prevention must be underpinned by a knowledge and understanding of the microbial pathogenesis, and the importance of surveillance.

**Conclusion**

The present study was conducted in the Department of Microbiology, College of Medical Sciences, Teaching Hospital, Bharatpur, Chitwan. A total of 206 cases were examined for postsurgical site infections. The samples were pus aspirates and wound swab of which 100 were culture positive and 106 were culture negative. Highest number of infections was recorded in the same age group 31-60 years but the highest incidence was in age above 60. Out of 206 pus samples, highest number of samples 131 (63.6%) were collected from General Surgical ward followed by 35 (17%) from Orthopaedics ward. Based on culture findings, 90 yielded growth of single organism while 10 yielded growth of two organisms, thus accounting for 110 organisms isolated. The most commonly isolated pathogens were *S.*
aureus 24 (21.8%) and E. coli 24 (21.8%), followed by P. aeruginosa. On antimicrobial susceptibility testing, Amikacin (86.4%) was the most effective drug. The second most effective drugs were Cefoperoxazone-sulbatam (81%) for overall bacterial isolates. Further, Vancomycin was also found to be 100% sensitive in gram positive isolates. The present study has given us an idea about the incidence of Surgical Site Infection in our hospital. S. aureus and E. coli were the most common organism causing SSI, followed by Pseudomonas sp., Klebsiella sp., Acinetobacter sp. MRSA, which was resistant to Oxacillin, and was found to be sensitive to Vancomycin (100%). So, Vancomycin could be the drug of choice for MRSA. The study suggests that although surgical site 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 wastage of health care resources.

Ethics approval and consent to participate

Not applicable.

Availability of data and material

The data and materials supporting the conclusions of this article are included within the article.

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Competing interests

The authors declare that they have no competing interests.

Authors’ contributions

Rama Bastola participated in designing methodology, carrying work, analyzing results and writing manuscript. Pramila Prajuli participated in designing methodology, writing manuscript and supervised the carried-out work. All authors read and approved the final manuscript.

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