Antimicrobial susceptibility pattern of organisms causing surgical site infection in a tertiary care hospital, Valsad, South Gujarat

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Abstract
Introduction: A surgical site infections (SSI) are a 3rd most common nosocomial infection and is responsible for morbidity, mortality and increased healthcare costs.

Aims and Objectives: The main aim of this study is to identify the isolates causing surgical site infections and its anti-microbial susceptibility pattern.

Materials and Methods: A total 50 cases of surgical site infections were taken for the study. The suspected samples were processed by using standard microbiological methods. The modified Kirby-Bauer’s disc diffusion method was used for antibiotic susceptibility pattern. The isolates of Enterobacteriaceae family were initially screened for ESBL production and were further confirmed by Double Disk Synergy Test as per Clinical and Laboratory Standards Institute (CLSI) guidelines. Reference strains of E. coli (ATCC 25922), P. aeruginosa (ATCC-27853), S. aureus (ATCC 25923) and Klebsiella 700603 were used as controls.

Result: Out of 50 samples, 19(38%) were culture positive and total 23 organisms were isolated. Pseudomonas aeruginosa 9 (39.13%) was the most common organism isolated, followed by Klebsiella spp. 5 (21.73%), Staphylococcus aureus 3 (13.04%). Most of the Gram-negative isolates were sensitive to imipenem and meropenem followed by piperacillin-tazobactam. Gram-positive organisms were found to be more sensitive to levofloxacin, linezolid and vancomycin. ESBL production was seen among 44.44% isolates of Enterobacteriaceae family.

Conclusion: The rate of infection reflects on patient’s care and standard of treatment in any hospital. Thus, better stewardship of SSI is required with accurate antibiotic policies. Periodic monitoring of etiology and antimicrobial susceptibility in the community and hospital settings is recommended.

Keywords: Surgical site infection, Bacteriological profile, Antibiotic susceptibility test, ESBL (Extended Spectrum Beta-Lactamase).

Introduction
Surgical Site Infection (SSI) is defined as infection related to an operative procedure that occurs at/near surgical incision within 30 days of the procedure and involves only skin or subcutaneous tissue of the incision.1-3 Globally, SSI rates have been reported from 2.5% to 41.9%.1,2 Recently published papers reported the incidence of SSIs as 3.38% in Karamsad,1 18.14% in Telangana,4 and 11.6% in W. Rajasthan.5

Surgical Site Infection (SSI) is the 3rd most frequently reported nosocomial infections4 and account for 14% to 16% of all nosocomial infections among hospitalized patients.2,6 The incidence of surgical site infection (SSI) varies from hospital to hospital and also varies in different studies that have been reported from time to time.7

Despite recent advances in aseptic techniques including operation theatre and surgical techniques, sterilization methods and standard protocols of preoperative preparation and antibiotic prophylaxis, SSI continues to be a major cause of hospital-acquired infections and a major source of morbidity and mortality amongst hospitalized patients in developing countries.2,4

The risk of developing SSIs is multifactorial. It includes emergency procedures, pre-morbid illness, extremes of age, gender, altered immune response, malnutrition, metabolic diseases and wound classification.2

SSIs increase the rate of rehospitalization, the use of health care, diagnostic, and therapeutic resources, and hospital costs.2,5 Patients who develop SSIs are 5 times more likely to be readmitted to the hospital, 60% more tending to require to stay in an intensive care unit (ICU), and twice as likely to die compared with patients without SSIs.6

Nosocomial infection due to multi-drug resistant organisms like Methicillin-Resistant Staphylococcus aureus (MRSA), Metallo-beta-lactamase producing Pseudomonas aeruginosa, vancomycin-resistant Enterococcus (VRE), Extended-spectrum beta-lactamase (ESBL) producing Klebsiella which has added a new dimension to the problem in the management of SSI. It has made the choice of empirical therapy more difficult and costlier. The condition is serious in developing countries owing to irrational prescriptions of antimicrobial agents.9 The treatment depends on determining its susceptibility to antibiotics.7

World health organization (WHO) and other studies indicated that periodic surveillance and giving feedback to surgeons on SSIs rate and associated factors can decrease up to 50% of SSIs.5 The literature suggests that 60% of SSIs are preventable.6

The frequent studies of anti-microbial susceptibility pattern of organisms causing SSI enables hospital departments to keep an eye on illegitimate use of anti-microbial and set rules on use of the antibiotics.

It is therefore essential to identify microbes in post-operative surgical wounds and study anti-microbial susceptibility pattern of the microbes causing SSIs.
Aims and Objectives
1. To identify the isolates causing surgical site infections.
2. To study anti-microbial susceptibility pattern of isolated organisms.

Materials and Methods
Study Area and Study Population
A cross sectional study of 2 months (02 June 2017-02 August 2017) was conducted in the Microbiology department of a tertiary care hospital, Valsad, South Gujarat as a part of STS-ICMR 2017.

This study was commenced after approval by the Institutional Human Ethics Committee of GMERS Medical College and Hospital Valsad. Confidentiality was observed.

The sample size obtained was 50.

Sample Selection Criteria
The following information of patient was taken according to the proforma prepared which included name, age, sex, case history.

Inclusion Criteria Included
1. Specimens (pus swabs, wound swabs) of post-operative patients of all clinical departments, developing infections within 30 days after operation.
2. Patients of age above 18 years.
3. Patients able to provide consent
4. Patients able to participate in an interview (He/she has no speech or hearing impairment).

Procedure
1. The pus swabs and wound swabs from all cases of SSI were routinely collected aseptically and sent to the Microbiology department without delay for analysis.
2. The samples in the laboratory were processed for direct microscopy, aerobic/anaerobic culture and sensitivity as per the standard protocols.
3. The swabs were used to make smear and Gram's staining was done to ascertain the morphological form of bacteria present.
4. The samples were inoculated on the required agar plates like Nutrient agar (NA), MacConkey Agar (MA), Blood Agar (BA) and Sabouraud's dextrose agar (SDA) in two sets.
5. One set was incubated aerobically at 37°C for 18-24 hours and another anaerobically.
6. After incubation, different microbes were identified from positive cultures by their morphological and biochemical characteristics.
7. The modified Kirby-Bauer’s disc diffusion method was used for antibiotic sensitivity pattern.
8. It was done on Mueller Hinton agar using various antibiotics as per Clinical and Laboratory Standards Institute (CLSI) guidelines.
9. The isolates of Escherichia coli, Klebsiella and Proteus showing resistance to ceftazidime and cefotaxime were further tested for ESBL production by Double Disk Synergy Test.
10. More than 5 mm increased in zone diameter for ceftazidime-clavulanic acid vs. zone diameter of ceftazidime disk incubated for 18 hours on Mueller-Hinton agar was interpreted as positive for ESBL production.
11. Reference strains of E. coli (ATCC 25922), P. aeruginosa (ATCC-27853), S. aureus (ATCC 25923) and Klebsiella 700603 were tested as controls.

Analysis
The observations were recorded and analyzed in MS Excel 2010.

Result
Out of 50 samples received for culture and sensitivity in the microbiology laboratory, 19 (38%) samples showed growth and 31 (62%) samples showed no growth. [Fig. 1]

Among the 19 culture positive cases majority i.e. 78.94% (15/19) were males and 21.05% (4/19) were females.

The cases were more in the age group of 48-58 years i.e.6 (31.57%) followed by 4 (21.05%) cases in the age group of 38-48 years as shown in. [Fig. 2]
Surgical site infection rate varies in different surgical procedures. The infection rate was more in amputation followed by fractures operation. [Fig. 3] Most common surgical diagnosis was Diabetic foot.

Among 19 culture positive samples, 15 (78.95%) yielded pure bacterial isolates and 4 (21.05%) yielded mixed infections. So overall 23 organisms were isolated from 19 culture positive samples.

Among the 23 organisms isolated from culture positive pus samples, 4 (17.39%) were Gram-positive bacteria and 19 (82.61%) were Gram-negative bacteria. [Fig. 4]  

Fig. 5 shows the bacteriological profile of infected samples. Out of Gram-positive cocci isolates, *Staphylococcus aureus* 3 (13.04%) was the commonest organism followed by *Streptococcus* 1 (4.35%). Amongst Gram-negative bacteria, *Pseudomonas aeruginosa* 9 (39.13%) was the most common organism followed by *Klebsiella* 5 (21.73%), *Escherichia coli* 2 (8.7%), *Proteus*
vulgaris 2 (8.7%) and the least isolated organism was Acinetobacter species 1 (4.35%)

**Fig. 5**

Klebsiella was found to be 80% sensitive to Imipenem and Meropenem followed by 60% sensitive to Amikacin, Gentamycin, and Kanamycin. It was 40% sensitive to piperacillin/Tazobactam, cefoperazone, cefotaxime, ceftriaxone, ceftazidime; cefepime, Levofloxacin, ciprofloxacin, Ofloxacin, Cotrimoxazole and 20% sensitive to Cefazolin and Cefaclor and 0% sensitive to Ampicillin. [Table 1]

Proteus was found to be 100% sensitive to Piperacillin/Tazobactam, Imipenem, Meropenem and Cotrimoxazole and 50% sensitive to Cefoperazone; Cefotaxime; Ceftriaxone; Ceftazidime, Cefepime, amikacin, Gentamycin, Cefaclor, Levofloxacin, Ciprofloxacin, and Ofloxacin.

Ampicillin, Amoxicillin/clavulanate, cefazolin, and cefaclor were 0% sensitive to Proteus and E. coli. [Table 1]

E. coli was found to be 100% sensitive to Imipenem, Meropenem, Levofloxacin; Ciprofloxacin; Ofloxacin and 50% to piperacillin/Tazobactam, Amikacin; Gentamycin; Kanamycin and fully resistant to ampicillin, cefazolin and cefaclor. [Table 1]

Two strains of Klebsiella, one strain of Escherichia coli and one strain of Proteus were found to be Extended Spectrum Beta-Lactamase (ESBL) producer (44.44%) i.e. resistant to first, second, and third generation cephalosporins and monobactams but sensitive to imipenem and meropenem. Most of these ESBL producing isolates were isolated from the patients of amputation.

Among non-fermenters, *Pseudomonas* showed 100% sensitivity towards Polymyxin-B, followed by 78% to piperacillin/Tazobactam, Imipenem, Meropenem. It showed 33% sensitivity towards Cefepime, Aztreonam, Ciprofloxacin, Ofloxacin, Levofloxacin, Gatifloxacin followed by 22% shown by Ceftazidime and 11% sensitivity towards Piperacillin, Amikacin and Gentamycin.

*Acinetobacter* was 100% sensitive to piperacillin/Tazobactam, Ceftazidime, Cefepime, Imipenem, Meropenem, Amikacin; Gentamycin; Ciprofloxacin, Ofloxacin, Levofloxacin and Cefotaxime except for piperacillin which was resistant. [Table 2]

Gram-Positive isolates, *S. aureus* were 100% sensitive to Rifampin, Tetracycline, Levofloxacin, Ciprofloxacin, Moxifloxacin, Clindamycin, Trimethoprim, Linezolid and Vancomycin followed by 67% sensitive to Amikacin; Gentamycin; Kanamycin; Azithromycin; Clarithromycin, and erythromycin. All strains of Staphylococcus were resistant to penicillin and ampicillin but sensitive to cefoxitin and oxacillin. Thus isolated strains were *Methicillin Sensitive Staphylococcus aureus* (MSSA). [Table 3]

*Streptococci* was sensitive to Rifampin, Penicillin G, Tetracycline, Levofloxacin, Ciprofloxacin, Moxifloxacin, Trimethoprim, Linezolid and Vancomycin and resistant to Azithromycin; Clarithromycin, Erythromycin, and Clindamycin. [Table 3]
Table 1: Antibiotic sensitivity pattern of Enterobacteriaceae (n=9)

| Microbes       | Klebsiella (5) | Proteus (2) | Escherichia coli (2) |
|----------------|---------------|-------------|----------------------|
| Drugs          | Sensitivity % | Sensitivity % | Sensitivity %       |
| Ampicillin     | 0             | 0           | 0                    |
| Amoxicillin/clavulanic acid | 0          | 0           | 0                    |
| Piperacillin/Tazobactam | 40          | 100         | 50                   |
| Cefazolin      | 20            | 0           | 0                    |
| Ceftalex       | 20            | 0           | 0                    |
| Cefoperazone   | 40            | 50          | 50                   |
| Cefotaxime     | 40            | 50          | 50                   |
| Ceftriaxone    | 40            | 50          | 50                   |
| Cefazidime     | 40            | 50          | 50                   |
| Cefepime       | 40            | 50          | 50                   |
| Imipenem       | 80            | 100         | 100                  |
| Meropenem      | 80            | 100         | 100                  |
| Amikacin       | 60            | 50          | 50                   |
| Gentamycin     | 60            | 50          | 50                   |
| Cefalex        | 60            | 50          | 50                   |
| Levofloxacin   | 40            | 50          | 100                  |
| Ciprofloxacin  | 40            | 50          | 100                  |
| Ofoxacin       | 40            | 50          | 100                  |
| Cotrimoxazole  | 40            | 100         | 50                   |

Table 2: Antibiotic sensitivity pattern of Non-fermenters (n=10)

| Microbes       | Pseudomonas (9) | Acinetobacter (1) |
|----------------|-----------------|-------------------|
| Drugs          | Sensitivity %   | Sensitivity %     |
| Piperacillin   | 11              | 0                 |
| Piperacillin/Tazobactam | 78          | 100              |
| Cefazidime    | 22              | 100               |
| Cefepime      | 33              | 100               |
| Aztreonam     | 33              | -                 |
| Imipenem      | 78              | 100               |
| Meropenem     | 78              | 100               |
| Amikacin      | 11              | 100               |
| Gentamycin    | 11              | 100               |
| Ciprofloxacin | 33              | 100               |
| Ofoxacin      | 33              | 100               |
| Levofloxacin  | 33              | 100               |
| Gatifloxacin  | 33              | -                 |
| Polymyxin-B   | 100             | -                 |
| Cefotaxime    | -               | 100               |

Table 3: Antibiotic sensitivity pattern of Gram positive cocci (n=4)

| Microbes       | Staphylococcus (3) | Streptococci (1) |
|----------------|---------------------|------------------|
| Drugs          | Sensitivity %       | Sensitivity %    |
| Rifampin       | 100                 | 100              |
| Penicillin G   | 0                   | 100              |
| Ampicillin     | 0                   | -                |
| Amoxicillin/clavulanic acid | 0          | -                |
| Amikacin       | 67                  | -                |
| Gentamycin     | 67                  | -                |
| Kanamycin      | 67                  | -                |
| Azithromycin   | 67                  | 0                |
| Clarithromycin | 67                  | 0                |
| Erythromycin   | 67                  | 0                |
Discussion

The present study was carried out in 50 patients who underwent various surgeries. Out of 50 cases, 19(38%) cases yielded positive culture while 31(62%) cases showed no growth. This is similar to another study by Anirudh S. et al in which the infection rate was 32%.7 However, few others have found out a very high proportion of culture positive cases in their studies which is contrary to our findings.12

The infection rate in Indian hospitals is much higher than that in other countries; for instance, in the USA, it is 2.8% and it is 2-5% in European countries.1,9 The low infection rate in developed countries may be due to vast differences in working conditions prevailing in these countries.7 The higher rates reported by some authors may be due to the inclusion of contaminated and dirty wound types and also emergency surgeries in their studies.7

In our study, the incidence of SSI was higher 78.94% (15/19) in male in comparison to 21.05% in female (4/19). This may be due to the fact that men are more prone to trauma because of their outdoor activities. A study by Dr. Ashok Kumar showed a similar trend, 25.6% in male and 17.6% in female. The study showed that males were more prone to SSI.13

Maximum number of cases belonged to age group 48-58 years (31.57%) followed by 38-48 years (21.05%) which is comparable to the other studies.1,4,13 Increasing age is correlated with a greater likelihood of certain chronic conditions, malnutrition and a fall in the body immunological efficiency, predisposing to SSI.4,7,13

Shah et al1 and Brian Mawalla9 reported a higher rate of infection in patients with diabetes mellitus which is similar to the present study.

When the association of type of surgery with infection rate was assessed it was revealed that maximum post-operative infection rate was found in amputation surgery (22%) followed by in fractures operations (12%) which is contrary to other studies in which laparotomy was most common.9

The rate of poly-microbial pathogens in the current study 21.05% was comparable to study done by Lopiso Dessalegn et al [20.1%]12 but more than 11.6% detected in the study done by Dr. Mahesh Sharma.5

Microbiological profile of wound infection shows that Pseudomonas were the most common isolated organisms which is comparable with other studies4,14 while it’s contrary with other studies5,7,12 which found that E. coli was most common isolated organism. S. aureus was the predominant causative agent among gram positive cocci which corroborated with other studies.4,5,9,14

In our study the members of Enterobacteriaceae family showed high sensitivity to Meropenem and Imipenem followed by Amikacin, gentamycin, and kanamycin. This correlates with other studies.1,4

Two strains of Klebsiella, one strain of E. coli and one strain of Proteus were found to be Extended Spectrum Beta-Lactamase (ESBL) producer (44.44%) which is comparable with other studies.7,9

Our study revealed that among non-fermenters piperacillin-tazobactam and Polymyxin-B were the most effective antibiotic which is comparable with other study.14

In Gram-Positive Bacteria, S. aureus was not sensitive to penicillin, ampicillin at all which is comparable with a study done by Aniruddha S. et al.7 But it was 100% sensitive to cefoxitin and oxacillin. Thus isolated strains were Methicillin-Sensitive Staphylococcus aureus (MSSA) which was similar to the study by Aniruddha S7 but contrary to other studies where Methicillin-Resistant Staphylococcus aureus (MRSA) were isolated.9,14 Also they were sensitive to linezolid and vancomycin which was comparable with other studies.13,14 While some other study revealed gentamycin as the most effective antibiotic.7

The usage of ineffective drugs in severe bacterial infections could be havoc as it can complicate management and increase morbidity and mortality. The majority of Gram-negative isolates were sensitive to meropenem while gram-positive being sensitive to vancomycin and clindamycin; this could be explained by the fact that these antibiotics are relatively rare in the hospital and are more expensive so they are rarely misused.9

The high isolation rate of bacteria and increased drug resistance to the commonly used antibiotics warrants the need for immediate measures ensuring effective infection prevention and rational use of antimicrobial agents leading to minimize infection rate and emergence of drug resistance.

Imipenem and Meropenem are effective for most isolates of Enterobacteriaceae and Non-fermenters and becomes the best choice when empiric treatment of surgical site infection is unavoidable. Levofloxacin, Linezolid and Vancomycin were sensitive against Gram-positive cocci. Piperacillin-tazobactam was effective against almost all isolates in our institute.
Conclusion
Surgical site infection is a significant convolution of surgeries in today’s era. It is one of the important causes of postoperative morbidity and mortality. The rate of infection is a reflection of patient care and standard of treatment in any hospital. Thus a better stewardship of SSI is required with accurate antibiotic policies can help reduce major problem of antimicrobial resistance in hospital-acquired infections and ultimately SSI rate in developing countries. Periodic monitoring of etiology and antimicrobial susceptibility both in the community and hospital settings is recommended.

Conflict of Interest: None.

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