

A STUDY OF BACTERIAL PATHOGENS CAUSING SURGICAL SITE INFECTION AND THEIR ANTIMICROBIAL SUSCEPTIBILITY IN TERTIARY CARE HOSPITAL

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ABSTRACT

Objectives: Surgical site infections (SSIs) remain a common and global problem that contributes to significant morbidity and mortality, prolongs hospital stay, and consequently increases health-care costs. Bacterial resistances pose a challenge and complicated the SSI treatment. This study aimed to evaluate the bacterial profile and antimicrobial susceptibility patterns of isolates among patients diagnosed with surgical site infection.

Methods: Patients who underwent either elective or emergency surgical procedures were enrolled in this study. For those who developed surgical site infections, specimens from the surgical site were collected and processed at the microbiology laboratory at the tertiary health-care center, Ahmedabad.

Results: Out of the 5003 patients, 34 developed SSI. In our study, 37 bacteria were isolated from 34 samples subjected to culture. Thirty-two samples showed monomicrobial growth and two showed polymicrobial growth. Among them, 14 (38%) were Gram-positive cocci, out of which 12 (85.7%) were *Staphylococcus aureus* and 2 (14.3%) were *Enterococci* spp. and 23 (62%) were Gram-negative bacilli, there were 21 (91.3%) enterobacteriaceae, out of which 5 (23.8%) were *Escherichia coli*, 1 (4.8%) were *Proteus mirabilis*, 15 (71.4%) *Klebsiella* spp. and 02 (8.7%) non-fermenters of which *Pseudomonas aeruginosa* and *Acinetobacter baumannii*.

Conclusions: Gram-negative bacteria were the most dominant isolates from surgical sites in the study area. Among them, *Klebsiella* spp. were the most common bacteria isolated from surgical site specimens. In our study, there is high antibiotic resistance observed which raises serious concerns and mandates strict antibiotic policy as well as antimicrobial stewardship.

Keywords: Surgical site infections, Antimicrobial resistance, Nosocomial infection.

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INTRODUCTION

Surgical site infections remain a common and global problem that may lead to significant morbidity and mortality, prolongs hospital stays, and consequently increases health-care costs. SSIs are defined as infections that develop at the surgical site within 30 days of surgery (or within 90 days for some surgeries such as cardiac, breast, and joint surgeries including implants) [1].

The World Health Organization (WHO) defined surgical site infections (SSIs) as the most surveyed and frequent type of Healthcare-Associated Infections (HAIs) in low- and middle-income countries which affect one-third of patients who have undergone various surgeries. Approximately one in ten people who have surgery in low- to middle-income countries acquire SSIs. In Africa, up to 20% of cesarean section procedures lead to a wound infection. SSIs are the most common HAI in Europe and the United States of America (USA). In Europe, SSIs affect more than 500,000 people/year, costing €19 million; in the USA, SSIs contribute to patients spending more than 400,000 extra days in the hospital, costing US\$ 10 billion a year. In the USA, 39–51% of SSIs pathogens are resistant to standard prophylactic antibiotics [2-4].

In India, although there is no national benchmark data, several studies reported SSIs rates ranging from 4 to 11 per 100 surgeries. The true data are expected to be much higher, because post discharge follow-up after surgery is very difficult and it is the big challenge in SSIs surveillance [5,6].

Many factors may promote SSIs such as the length of hospital stay, obesity, diabetes mellitus, and smoking. Most postoperative wound infections are endogenous. Exogenous infections are mainly acquired from the nose or skin flora of the operating team personnel and transmitted through the hands of the surgeon or improper operation

theater sterilization techniques, etc. Some significant factors that can influence the incidence of subsequent infection are surgical techniques, skin preparation, duration, method of wound closure, and timing of antibiotic prophylaxis after certain types of surgery. Therefore, the prevention of these infections is complex and requires the integration of a range of preventive measures before, during, and after surgery.

The antimicrobial-resistant pattern among SSI-associated pathogens varies globally, depending on the region, local epidemiological reports, and various methods for testing antimicrobial sensitivity. Bacterial resistances pose a challenge and complicated the SSI treatment. There were limited reports on the prevalence and incidence of resistant bacteria causing SSIs, especially in developing countries [7]. Therefore, this study aimed at evaluating the bacterial profile and antimicrobial susceptibility patterns of isolates among patients with surgical site infections.

METHODS

Study design

This study was carried out at the department of microbiology laboratory at the tertiary health-care center, Ahmedabad. The study was done from Aug 2019 to April 2021 during which 5003 cases were studied including patients of all ages and patients who underwent clean, clean-contaminated surgeries, contaminated, and dirty surgeries. Neonates, OPD patients, and Cases taken for second surgery at the same site for any reason, patients on antibiotics already for any other infections, presence of infection somewhere else in the body, and focal sepsis were not included in the study.

Sample collection

After 48 h of surgery, dressings on the surgical wounds were removed. Evidence of wound infection was considered if the patient had local inflammatory changes such as edema, redness, warmth, or discharge

from a wound site. These were looked into each case and the changes were documented. If there was any discharge, samples were collected before dressing the wounds. The patients were made comfortable before the collection of samples and consent was taken. If only inflammatory changes were present without any discharge, the wounds were monitored until the discharge of the patient. If no inflammatory signs were noticed within 48 h, cases were followed up with the help of respective surgeons.

The surgeons in charge of the case were requested to inform/call the postgraduate scholar doing this work, whenever he/she suspected signs of SSIs in the form of fever and local signs of inflammation. In addition, these patients were educated and followed up through mobile phones for the development of SSIs for 30 days by our infection control nurses.

Preparation of wound site – the suspected as well as infected areas were cleaned with sterile normal saline followed by 70% alcohol, and then, the specimen was collected using a sterile swab. Two swabs were taken from the depth of the wound or lesion and aspirates were collected in a sterile disposable syringe and transported to the laboratory within 2 h. The samples' color, consistency, and odor were observed and recorded. The samples were properly labeled with all details of the patient and sent to the microbiology laboratory. Samples with filled laboratory request forms were received in the lab. Laboratory ID was generated using the software.

Macroscopic and microscopic examination (KOH preparation, Wet mount, Gram stain, ZN stain) was done. All the samples were inoculated on Nutrient agar, MacConkey agar, and chocolate agar plates by adopting standard microbiological techniques. The inoculated plates were incubated aerobically and also in a candle jar at 37°C in an incubator and results were read after 24 h and 48 h of incubation.

All inoculated plates were observed the next day and colony characteristics were noted. A repeat subculture was carried out on the next day for samples showing no growth on plates on the 1st day and was processed further [8]. All the isolates were identified by colony morphology, microscopic appearance, biochemical tests, and phenotypic tests for drug resistance.

Antimicrobial susceptibility testing

From each confirmed culture isolate, a suspension of a pure colony was done in sterile normal saline, which was incubated at 37°C for at least 15 min. For uniformity of a suspension on Mueller–Hinton agar, a sterile cotton tip applicator stick was used. For the antibiotic susceptibility test (AST), the Kirby–Bauer disk diffusion technique was used. For the AST, different antibiotic disks were used. These were ciprofloxacin (5 µg), Ampicillin (10 µg), Ampicillin-Sulbactam (10/10 µg), piperacillin-tazobactam (100/10 µg), cefoxitin (30 µg), cefepime (30 µg), Teicoplanin (30 µg), linezolid (30 µg), clindamycin (2 µg), gentamycin (10 µg), high-level gentamycin (120 µg), trimethoprim-sulfamethoxazole (1.25/23.75 µg), Aztreonam (30 µg), Amikacin (30 µg), Ciprofloxacin (5 µg), polymyxin B, tetracycline (30 µg), ceftriaxone (30 µg), cefotaxime (30 µg), imipenem (10 µg), ceftazidime (30 µg), vancomycin (30 µg), and cefuroxime (30 µg). The zone of inhibition was measured by a metric ruler. The AST result was interpreted as susceptible, intermediate, and resistant according to Clinical and Laboratory Standards Institute (CLSI) Performance standards for antimicrobial susceptibility testing.

Simultaneously, isolates were screened for detection of the resistance mechanisms such as Inducible methylase production (clindamycin resistant), MRSA (methicillin-resistant staphylococcus aureus), and MBL (metallo Beta-lactamase), ESBL (extended-spectrum Beta-lactamase), and AmpC beta-lactamase. For quality control, *S. aureus* (ATCC-25923) and *Escherichia coli* (ATCC-25922) were used.

RESULTS AND DISCUSSION

A total of 5003 samples were received for microbiological analysis from patients who underwent different types of surgeries from August 2019 to April 2021, out of which 34 (0.67%) were culture positive and

considered cases of SSIs; thus, overall prevalence rate of SSIs was 0.67%. Kumar *et al.* and Fahad *et al.* reported SSIs as 2.5%, which is on the higher side than our present study rate [9]. Among the 2240 males who underwent surgery, SSIs were seen in 09 (0.4%) of them and among the 2763 females, it was noticed in 25 (0.9%). A study by Hernandez *et al.* (2005) conducted in a Peruvian Hospital reported more among males 65.6%. Moses also reported male preponderance (64.3%) and this, however, differs from the study by Shanmugam *et al.* who reported almost equal among females (52%) and males (48%) [9,10].

The age of the study subjects ranged from 21 years to 87 years. Twenty (28.4%) of them belonged to >45 years of age followed by 10 (25%) and 4 (21.5%) in the age group of 16–34 years and 35–44 years, respectively. On the contrary, it was more among those above 45 and may be attributable to the nature of the wound. In general, the occurrence of SSIs was more as age advances since these cases were suffering from diabetes mellitus and/or other comorbid conditions which contribute to decreased physiological defense mechanisms and poor immune function. It is supported by many studies, for example, Owens *et al.* and Bharatnir *et al.* reported that a greater number of SSIs occurred among 36–50 years (1.3 times higher risk of acquiring SSIs than the ones who were in the age group of 10–35 years). Similarly, a high rate of infection was noted in the later age groups by Mundhada *et al.* [11,12].

The present study included 3441 elective surgeries and 1562 emergency surgeries, in which the SSIs rate was 14 (0.4%) and 20 (1.2%), respectively. Emergency surgeries showed a higher rate of SSI as compared to elective surgeries. The increased rate of SSIs in emergency surgeries may be due to a very narrow period without proper patient preparation and surgical preparedness as well as contaminated wounds as in cases of road traffic accidents. The same has been cited in most of the studies done earlier on SSIs. Tabiri *et al.* also reported that emergency cases had a higher number of SSIs (23.8%) as compared to elective cases (7.4%) [13].

Among 5003 patients, 1928 underwent clean surgeries, of these 14 developed SSI (0.7%). The occurrence of SSIs among clean-contaminated (n=2405), contaminated (n=516), and dirty wounds (n=154) were (14) 0.5%, (4) 0.8%, and (2) 1.3%, respectively. These variations may be attributable to increased microbial load in the operative field which is of higher risk to SSIs. Similar to this study, Shrestha *et al.* reported SSIs in 2.9%, 15.3%, and 18.7% of clean-contaminated, contaminated, and dirty wounds, respectively, and none in clean wounds [14].

As per CDC, SSIs have been categorized into superficial, deep, and organ/space SSIs. In the present study, it was observed that 30 (88%) had superficial SSI and the rest (n=04) deep ones. There was no organ/space SSIs observed during the study period. Superficial SSI was found to be higher. Walraven *et al.* reported the same with the majority of these (n=8188, 57.5% of all SSIs) having a superficial component [15].

All 34 SSIs occurred in patients with one or more risk factors such as diabetes mellitus, smoking, and alcohol. Among them, 10 (29.4%) had only single risk factor (diabetes mellitus), 8 (23.5%), 7 (20.6%), 6 (17.6%), and 3 (8.8%) had combination of 2, 3, 4, and 5 risk factors, respectively.

Among the 5003 surgeries, abdominal surgeries constituted (n=1520; 30.4%) the highest rate of SSI (18, 52.9%) occurred in the category of hysterectomy followed by hernia repair. One hundred and fourteen patients underwent hysterectomy, and 5 developed SSIs (4.38%).

Abdominal surgeries show the highest rate of SSIs (n=18, 52.9%), followed by pelvic surgeries (n=5, 14.71%), bone and joint (n=4, 11.76%), breast and axilla (n=3, 8.82%), skin and plastic surgeries (n=3, 8.82%), and neurosurgery (n=1, 2.94%).

When compared with other studies, Allegranzi *et al.* and Azoury *et al.* also reported that abdominal surgeries are commonly done and have

high rates of surgical site infections, which may be due to spillage of the bowel content during gastrointestinal tract surgery [3,16]. Maksimovic et al. reported that orthopedic surgeries were more commonly associated with SSI [17].

Distribution of various bacteria in SSIs

In our study, 37 bacteria were isolated from 34 samples subjected to culture. Thirty-two samples showed monomicrobial growth and two showed polymicrobial growth (*E. coli*+*Enterococcus* spp., *S. aureus*+*Enterococcus* spp.+*Klebsiella* spp.). Hence, a total of 37 isolates were obtained. Among them, 14 (38%) were Gram-positive cocci and 23 (62%) were Gram-negative bacilli, there were 21 (91.3%) enterobacteriaceae and 2 (8.7%) non-fermenters.

Among the 14 Gram-positive cocci, 12(85.7%) were *S. aureus* and 2 (14.3%) were *Enterococci* spp. Out of 21 Enterobacteriaceae, 5 (23.8%) were *E. coli*, 1(4.8%) were *Proteus mirabilis*, and 15 (71.4%) *Klebsiella* spp., which included 13 *Klebsiella pneumoniae* and two *Klebsiella oxytoca*, the remaining 2 were non-fermenters 1 (50%) *Pseudomonas aeruginosa* and 1 (50%) *Acinetobacter baumannii* and no candida species were isolated (Fig. 1).

The most common organisms isolated in abdominal surgeries were *Klebsiella* spp. followed by *S. aureus* and *E. coli*. In pelvic surgeries, *E. coli* was commonly encountered, whereas it was *S. aureus* and *Klebsiella* species in orthopedic surgeries. A study by Abubaker et al. shows *Klebsiella pneumoniae* as the most predominant organism (50%) followed by *S. aureus* (27.8%), these findings are similar to our study [18].

Antimicrobial susceptibility pattern in Gram-positive cocci

There were 12 *S. aureus* and 2 *Enterococcus* species isolated during the study period. Vancomycin and Linezolid were highly active drugs against *S. aureus* showing 100% sensitivity. *S. aureus* was isolated from a total of 12 samples, out of these, 5 (41.67%) were methicillin-resistant *S. aureus* (MRSA), and the remaining 7 (58.33%) were methicillin-sensitive *S. aureus* (MSSA). A study done by Ranjan et al. showed that 27.96% of *S. aureus* were methicillin-resistant strains, which is also in line with our results [19].

Teicoplanin, Vancomycin, and Linezolid were highly active drugs against Gram-positive organisms with 100% sensitivity. We did not find any vancomycin-resistant enterococcus (VRE).

Antimicrobial susceptibility pattern in Gram-negative bacilli

Out of 37 isolates, there were 1 (2.7%) *A. baumannii* and 1 (2.7%) *P. aeruginosa* isolated. Tigecycline and a combination of sulbactam were 100% sensitive for *A. baumannii*. *P. aeruginosa* shows 100% sensitivity to all drugs tested. Twenty-three – Gram-negative organisms were isolated. Among these isolates, 19 (82.61%) were ESBL positive, 10 (43.48%) were ESBL and AmpC producers, and 10 (43.48%) were carbapenemase producers (Fig. 2).

Distribution of ESBL-producing Gram-negative bacilli in SSIs

Out of 23 – Gram-negative organisms, 19 were ESBL producers (82.61%). Among them, 5 (26.31%) were *E. coli*, 13(68.42%) were

Klebsiella spp., and 1 (5.26%) was *A. baumannii*. The prevalence of ESBL producers was high in a study by Golia et al. who noticed 80% of *E. coli* and 100% of *Klebsiella* species isolated from SSIs, to be ESBL producers [20].

Distribution of ESBL and AmpC producing Gram-negative bacilli in SSIs (Fig. 3)

In the present study, out of 23 – Gram-negative organisms, 10 (43.48%)

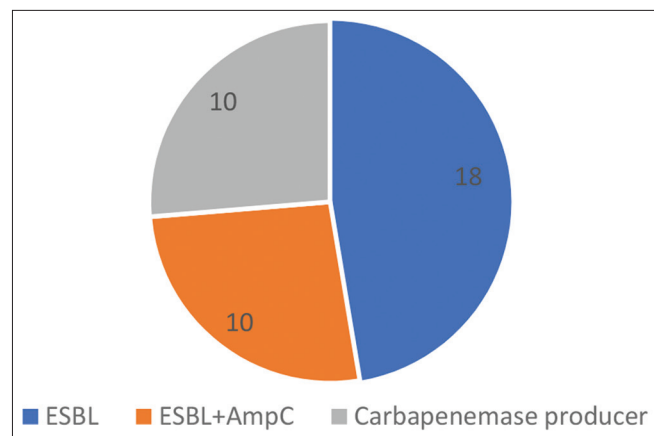


Fig. 2: Distribution of ESBL, ESBL and AmpC, and carbapenemase producer organisms

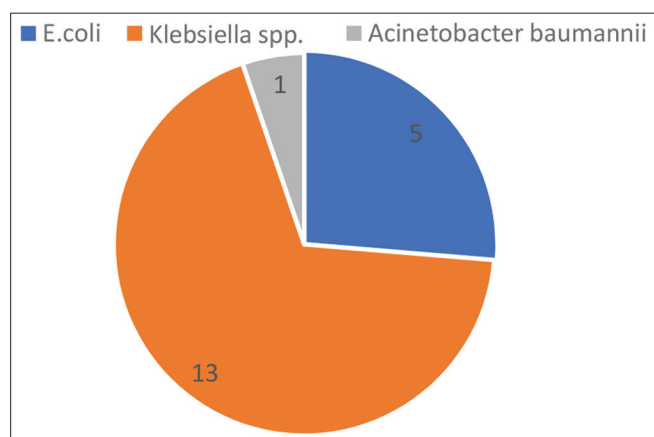


Fig. 3: Distribution of ESBL and AmpC producing Gram-negative bacilli in surgical site infections

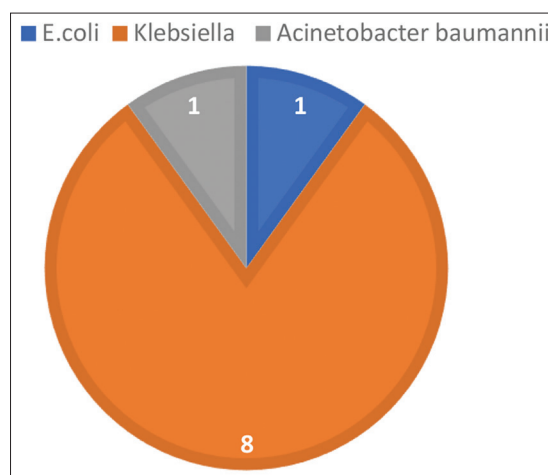


Fig. 4: Distribution of carbapenemase producers

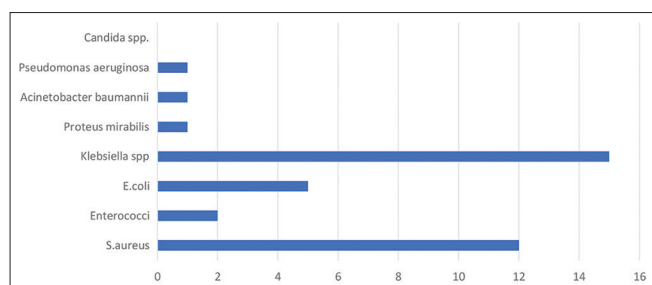


Fig. 1: Distribution of organisms in surgical site infections

were ESBL and AmpC producers, among them, 1 (10%) were *E. coli*, and 9 (90%) were *Klebsiella* spp. Compared to our findings, Sultan *et al.* and Tapan *et al.* reported a 48.5% prevalence of ESBL and AmpC coproducers [21,22].

Distribution of carbapenemase producers in SSIs (Fig. 4)

Out of 23 – Gram-negative organisms, 10 (43.48%) were carbapenemase producers, of which 1 *E. coli* and 8 *Klebsiella* spp. and 1 *A. baumannii*. Antoinette reported that 86% of *E. coli*, 52% of *A. baumannii*, and 86% of *K. pneumoniae* isolates were resistant to imipenem [23].

CONCLUSIONS

Gram-negative bacteria (62.16%) were the most dominant isolates from surgical sites in the study area. Among the Gram-negative bacilli, *Klebsiella* spp. (71.4%) were the most common bacteria causing surgical site infection. In Gram-positive bacteria, *S. aureus* (32.43%) were the most dominant isolates from surgical sites.

Gram-negative bacilli which showed resistance to third-generation cephalosporins and carbapenems in antibiotic susceptibility testing were subjected to a phenotypic confirmatory test for ESBL, AmpC, and MBL production. Phenotypic tests such as the AmpC screening test and Modified Hodge test showed 82.61%, 43.48%, and 43.48% ESBL, AmpC, and MBL production, respectively, in 23 – Gram-negative bacilli isolated.

The study revealed that the most sensitive drugs for Gram-positive isolates are linezolid, teicoplanin, and vancomycin (100%); and the most sensitive drugs for Gram-negative bacteria are colistin (95.24%) and tigecycline (95.24%) followed by aminoglycosides (71.43%), carbapenems (57.15%), and beta-lactam and beta-lactam inhibitors combination drugs (52.38%).

The increasing prevalence of drug-resistant organisms in SSIs such as MRSA, and organisms producing ESBL, AmpC, or carbapenemase raise serious concerns about antibiotic resistance and mandate strict antibiotic policy as well as antimicrobial stewardship. The present study indicates that every institution has to maintain a surveillance of SSIs and find out changing trends to curtail SSIs and infections due to multidrug-resistant strains. It also reiterates the necessity of an actively functioning infection control team and antimicrobial stewardship program.

AUTHORS' CONTRIBUTIONS

All the authors have contributed equally to the data collection, its interpretation, and preparation of the manuscript.

CONFLICTS OF INTEREST

The authors have no conflicts of interest.

AUTHORS' FUNDING

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