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Original Article

BACTERIOLOGICAL PROFILE AND ANTIMICROBIAL SUSCEPTIBILITY PATTERN OF SURGICAL SITE INFECTIONS IN A TEACHING HOSPITAL

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ABSTRACT

Objective: Surgical site infections (SSI) are defined as infections occurring within 30 or 90 d after surgical operation or within 1 y, if an implant is left in place after procedure and affecting either incision or deep tissues at the operation site. SSI is one of the quality indicators of the healthcare system. Due to advent of newer antibiotics and emergence of multidrug-resistant bacteria, SSI requires reappraisal of the microbiological flora and *in vitro* antibiotic sensitivity pattern. The aim of the present study is to determine the incidence etiology of SSI, and determine their Antibiogram.

Methods: Cross-sectional study conducted for a period of three months from January 2024 to March 2024. A total of fifty swabs/pus specimens from various types of surgical sites suspected to be infected on clinical grounds were collected from the post-operative wards of surgical departments and processed in Microbiology laboratory. Isolation and identification of bacterial agents were done as per standard protocols. Antimicrobial susceptibility testing was performed by Kirby-Bauer's disc diffusion method.

Results: Out of 50 samples, 32 were culture-positive. 87.50% showed monomicrobial growth and 12.50% showed polymicrobial growth. Analysis of bacterial profile shows Staphylococcus aureus (27.77%) was the predominant isolate, followed by Pseudomonas aeruginosa (19.44%) and Escherichia coli (19.44%). Antibiogram of gram-positive isolates showed sensitivity to Teicoplanin, Vancomycin, and linezolid, whereas gram-negative isolates were sensitive to Meropenem, Piperacillin-tazobactam and levofloxacin.

Conclusion: Status of SSI is the main quality indicators of hospital infection control and prevention measures. Continuous surveillance on etiology and antibiogram of SSI is necessary to monitor antimicrobial resistance and guide in empirical treatment.

Keywords: Surgical site infection, Antibiogram, Antimicrobial resistance, Hospital infection control

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INTRODUCTION

Surgical site infections are one of the most common causes of nosocomial infections and common complication associated with surgery [1]. Centre for Disease Control and prevention (CDC), Atlanta, defines Surgical Site Infection (SSI) as an infection occurring within 30 or 90 d after a surgical operation or within 1 y if an implant is left in place after procedure and affecting either incision or deep tissues at the operation site. These infections may be superficial infections or deep incisional infections involving organ or body space [2]. CDC, classified wounds as Class I/Clean, Class II/Clean contaminated, Class III/Contaminated, Class IV/Dirty-infected [2-4].

Surgical site infections are responsible for an increase in the treatment cost, length of hospital stay and significant morbidity and mortality. Despite the technical advances in infection control and surgical practices, SSIs still continue to be a major problem, even in hospitals with most modern facilities [5].

Bacteriological studies show that in recent years, there has been a growing prevalence of Gram-negative organisms as a cause of serious infections in many hospitals. Although properly administered antibiotics can reduce postoperative Surgical Site Infections (SSI) due to bacterial contamination, widespread use of prophylactic broad-spectrum antibiotics can lead to the emergence of multi-drug resistant bacteria. Since initial antibiotic therapy is empirical, it is important to know the prevailing antibiotic susceptibility patterns of individual institutions by routine surveillance [6].

SSI is one of the quality indicators of the health care system of any hospital. With the increase in the incidence of nosocomial infections and multi-drug resistance, meticulous and periodic surveillance of various hospital-acquired infections became mandatory [7]. The present study was done to know the status of the SSIs in the hospital as it is one of the main quality indicators to take Hospital infection control and prevention measures. Aim of the study the incidence and aetiology of surgical site infections and determine the antibiogram of aerobic bacterial isolates.

METHODS AND MATERIALS

This cross-sectional study was conducted in Department of Microbiology, Andhra Medical College, Visakhapatnam for a period of three months from January 2024 to March 2024. A total number of fifty swabs/pus specimens from various types of surgical sites suspected to be infected on clinical grounds were collected from the post-operative wards of departments of Surgery, Orthopaedics and Gynaecology and Obstetrics at King George Hospital, Visakhapatnam. The study was approved by Institutional Ethics Committee.

Statistical analysis

Data collected was entered into Microsoft Excel-2010 version. Descriptive variables will be expressed in numbers and percentages. Continuous variables will be expressed as means±standard deviation. Statistical test-Chi square test will used for analyzing qualitative variable and student't' test for quantitative variable. For all statistical purposes, P value<0.05 was considered statistically significant.

Methodology

Under strict aseptic conditions, serous/purulent discharge adjacent to sutures was collected using two sterile cotton swabs. Pus samples were collected using sterile disposable syringes. Samples were immediately transported to laboratory for microbiological isolates.

Processing of samples

Processing of samples were carried out in the Department of Microbiology, Andhra Medical College, Visakhapatnam. All the samples were subjected to Gram stain to identify the presence of pus cells, morphology of bacilli and bacteriological culture to isolate the organisms. Samples were inoculated on Nutrient agar, Blood agar, MacConkey's agar and incubated at 37 °C for 24 h. After incubation, colonial and cultural characteristics of isolates were observed, biochemical tests done for identification and documented as per CLSI guidelines [8]. The antimicrobial susceptibility testing was done by the Kirby Bauer disc diffusion method [9]. MRSA was detected using cefoxitin 30µg disc. ESBL production and MBL production in Gram-negative bacteria was detected by using Potentiated Disc Diffusion test (PDT) [9, 10]. The resistance patterns were further determined by E-test by interpreting Minimum inhibitory concentration (MIC) values (mcg/ml) [9, 11].

RESULTS

Out of 50 samples processed 32 (64%) were culture positive and 18 (36%) were culture sterile. 27 samples were collected from General Surgery ward, 14 from orthopaedics ward and 9 samples from gynaecology and Obstetrics ward. The study showed male predominance 35 (70%). Majority of cases (72%) belong to low socio-economic status. The highest percentage of culture positivity (46.87%) was seen in the age group of 31-45 y. Among all the risk factors, diabetes mellitus (60%) was the commonest risk factor, followed by anaemia (52%) and alcohol consumption (36%). Out of total samples collected, 40% were from patients who underwent elective surgeries and 60% were from emergency surgeries. The study showed the incidence of surgical site infections was high in surgeries conducted for more than 2 h (75%). Only 25% of SSIs were reported from surgeries conducted for less than 2 h.

Table 1 shows that among the 50 samples, 22 were collected from clean contaminated surgeries of which 15 (46.87%) were culture positive and 17 samples were from contaminated surgeries, of which 10 (31.25%) were culture positive. 8 samples were from clean surgeries of which 5 (15.6%) were culture positive and 2 (6.25%) out of 3 samples from dirty surgeries showed culture positivity. 3.12% of surgical site infections were reported in patients who stayed in hospital for less than 5 d and discharged soon; whereas about 31.25% of SSIs were reported in patients staying in post-operative wards for more than 25 d.

Table 1: Distribution of samples and culture positivity based on type of surgeries (n = 50)

Type of surgery	No. of cases	No. of samples showing culture positivity	% of culture positivity
Clean	8	5	15.6%
Clean contaminated	22	15	46.87%
Contaminated	17	10	31.25 %
Dirty	3	2	6.25%
Total	50	32	100 %

In the present study, out of 32 culture-positive samples, monomicrobial growth was seen in (87.50%) and polymicrobial growth in 12.50% samples. 28 (77.77%) of isolates were obtained in pure culture and 8 (22.22%) of isolates were obtained in mixed culture. The total isolates obtained in both pure and mixed cultures were 17 (47.22%), gram-positive isolates and 19 (52.77%), gram-negative isolates, respectively.

In the present study, amongst all the bacterial isolates, staphylococcus aureus (27.77%) was the predominant isolate followed by Pseudomonas aeruginosa (19.44%) and Escherichia coli (19.44%), coagulase-negative staphylococci (13.88%), Klebsiella species (8.33%), Streptococcus species (2.77%), Proteus mirabilis (2.77%), Acinetobacter species (2.77%) and Enterococcus species (2.77%). Polymicrobial growth samples are a mixture of Escherichia coli and Staphylococcus aureus, was isolated in 2 (50%) samples, Pseudomonas aeruginosa and Coagulase Negative Staphylococci were isolated in 1 (25%), and Klebsiella pneumoniae and staphylococcus aureus was isolated in 1 (25%) sample as shown in table 2.

Table 2: Distribution of total (pure and mixed) bacterial isolates (n=36)

Organism	Total		Pure		Mixed	
	No.	%	No.	%	No.	%
Staphylococcus aureus	10	27.77%	7	19.44%	3	8.33%
Pseudomonas aeruginosa	7	19.44%	6	16.66%	1	2.77%
Escherichia coli	7	19.44%	5	13.88%	2	5.55%
Coagulase negative Staphylococci	5	13.88%	4	11.11%	1	2.77%
Klebsiella pneumoniae	3	8.33%	2	5.55%	1	2.77%
Streptococcus pyogenes	1	2.77%	1	2.77%	-	-
Enterococcus faecalis	1	2.77%	1	2.77%	-	-
Acinetobacter baumannii	1	2.77%	1	2.77%	-	-
Proteus mirabilis	1	2.77%	1	2.77%	-	-
Total	36	100%	28	77.77%	8	22.22%

Among the Gram-positive cocci, Staphylococcus aureus was 100% sensitive to Teicoplanin followed by linezolid (90%), Vancomycin (90%), levofloxacin (80%), Azithromycin (70%), Cefoxitin (60%), Amoxicillin and Clavulanate (40%), Ceftriaxone (40%) and Cefotaxime (30%). Coagulase Negative Staphylococci were 100% sensitive to linezolid, Teicoplanin followed by Vancomycin (80%), Azithromycin and levofloxacin (80%), Ceftriaxone and Cefoxitin (60%), Ceftazidime (60%), Amoxicillin+Clavulanate and Cefotaxime (40%). Streptococcus pyogenes and Enterococcus faecalis showed 100% sensitive to Vancomycin and linezolid.

Among the Gram-negative isolates varied antimicrobial susceptibility pattern has been noted. Pseudomonas aeruginosa showed 100% sensitivity to Piperacillin+Tazobactam, followed by Ceftazidime+Clavulanic acid (85.71%), levofloxacin (71.40%), Amikacin and Tobramycin (57.14%), Cefotaxime and Azithromycin (28.75%). All the Pseudomonas aeruginosa isolates showed 100% resistance to Amoxyclav. Escherichia coli showed 100% sensitivity to Piperacillin+Tazobactam, 85.71% sensitivity to Ceftazidime+Clavulanic acid, Amikacin and Meropenem. Among the Klebsiella pneumoniae isolates 100% showed sensitivity to Piperacillin+Tazobactam and Ceftazidime+Clavulanic acid. 100% of Klebsiella pneumoniae isolates were resistant to Ceftriaxone. Proteus mirabilis isolates were 100% sensitive to Piperacillin+Tazobactam, Ceftazidime+Clavulanic acid, Ceftriaxone, Amikacin, levofloxacin and Meropenem. Whereas100% resistance has been noted for Amoxyclav, Ceftazidime, Tobramycin, Azithromycin and Ciprofloxacin. Acinetobacter baumannii showed 100% sensitivity to Piperacillin+Tazobactam and Meropenem. Whereas 100% resistance has been reported for Amoxyclav, Ceftazidime, Ceftazidime+clavulanic acid, Ceftriaxone, Amikacin, Tobramycin, Azithromycin and levofloxacin.

Table 3: Antibiotic susceptibility pattern of gram-positive cocci (n=17)

Organism	LZ	TEI	VA	CX	AZM	CTX	CTR	AMC	CAZ	LE
Staphylococcus aureus (n=10)	9 (90%)	10 (100%)	9 (90%)	6 (60%)	7 (70%)	3 (30%)	4 (40%)	4 (40%)	3 (30%)	8 (80%)
Coagulase Negative Staphylococcus (n=5)	5 (100%)	5 (100%)	4 (80%)	3 (60%)	4 (80%)	2 (40%)	3 (60%)	2 (40%)	3 (60%)	4 (80%)
Streptococcus pyogenes (n=1)	1 (100%)	-	1 (100%)	-	1 (100%)	-	-	_	1 (100%)	1 (100%)
Enterococcus faecalis (n=1)	1 (100%)	-	1 (100%)	-	1 (100%)	-	-	-	1 (100%)	1 (100%)

Note: lZ-linezolid, TEI-Teicoplanin, VA-Vancomycin, CX-Cefoxitin, AZM-Azithromycin, CTX-Cefotaxime, CTR-Ceftriaxone, AMC-Amoxyclav,

Table 4: Antibiotic susceptibility pattern of gram-negative bacilli (n=19)

Isolate	AMC	PIT	CAZ	CAC	CTX	MRP	AK	TOB	AZM	LE
*Pseudomonas aeruginosa (*n=7)	0	7 (100%)	5 (71.4%)	6 (85.71%)	2 (28.57%)	6 (85.71%)	4 (57.14%)	4 (57.14%)	2 (28.57%)	5 (71.4%)
Escherichia coli (n =7)	1 (14.28%)	7 (100%)	5 (71.4%)	6 (85.71%)	5 (71.4%)	6 (85.71%)	6 (85.71%)	5 (71.4%)	2 (28.57%)	3 (42.85%)
Klebsiella pneumoniae (n=3)	1 (33.33%)	3 (100%)	2 (66.66%)	3 (100%)	0	2 (66.66%)	2 (66.66%)	2 (66.66%)	1 (33.33%)	1 (33.3%)
Proteus mirabilis (n=1)	0	1 (100%)	0	1 (100%)	1 (100%)	1 (100%)	1 (100%)	0	0	1 (100%)
Acinetobacter baumannii (n=1)	0	1 (100%)	0	0	0	1 (100%)	0	0	0	0

Note: AMC-Amoxicillin+Clavulanate, PIT-Piperacillin+Tazobactam, CAZ-Ceftazidime, CAC-Ceftazidime+Clavulanate, MRP-Meropenem, CTX-Cefotaxime, CIP-Ciprofloxacin, TOB-Tobramycin, AK-Amikacin, AZM–Azithromycin, lE-levofloxacin

Out of total 10 Staphylococcus aureus isolates, 4(40%) were Methicillin Resistant Staphylococcus aureus (MRSA) and 6(60%) were Methicillin Sensitive Staphylococcus aureus (MSSA).

In the present study, out of total 19 Gram-negative isolates, Extended Spectrum Beta-Lactamase (ESBL) production was seen in 4 (21.05%) isolates, and Metallo Beta-Lactamase (MBL) production was seen in 3 (15.78%) isolates.

DISCUSSION

Surgical site infections are those infections, occurring in the surgical incisions and structures adjacent to the wounds exposed during the surgery [12]. Despite the advances made in asepsis, antimicrobial drugs, sterilisation and operative techniques, SSIs are responsible for the increasing cost, morbidity and mortality related to surgical operations. Surgical site infections are caused by exogenous and endogenous microorganisms that enter the operative wound during the procedure [13]. Predisposing underlying conditions for surgical site infections include immunosuppression, irradiation, steroid administration, diabetes mellitus and malnutrition. The risk of infection after surgery depends upon the factors including the type and length of surgical procedure, age, underlying conditions and previous history of the patient, skill of the surgeon, diligence with which infection control procedure are applied and the type and timing of pre-operative antibiotic prophylaxis [14].

In the present study, out of the total number of samples processed, 64% were culture positive and 36% were culture sterile. These findings correlated with A. Ramesh, *et al.*, (2012) [15] who reported 66% of culture positivity, Jeena Amatya, *et al.*, (2015) [16] reported 60.6%. Majority of the samples in the present study were obtained from General Surgery department followed by orthopaedics department. These findings were comparable to Rudratej Patil, *et al.*, (2015) [17] and Hemalatha *et al.*, (2007) [18]. The higher frequency of surgical site infections observed in the department of General Surgery could be because of higher number of emergency procedures conducted in the department. In the present study, out of a total of 35 male patients, 65.62% showed culture positivity and out of 15 female patients, 34.37% showed culture positivity. These findings correlated with Arti Jain, *et al.*, (2015) [19] who reported a culture positivity of 66% in males and 34% in females. In the present study, the incidence of surgical site infections is highest in the working age group. Similar preponderance of surgical site infections in the advancing age group (>50 y) was observed by Narsinga Rao Bandaru, *et al.*, (2012) [20]. In the present study, diabetes mellitus is the predominant risk factor in a total of 30 patients; diabetes along with hypertension in 15 patients. The present study supports the conclusion of Malone, *et al.*, (2002) [21] and Nutanbala, *et al.*, (2005) [22] that diabetes mellitus is the significant pre-operative risk factor for surgical site infections. In the present study, 60% of surgical site infections were reported from emergency surgeries and 40% from elective surgeries. These findings correlated with Sivasankari Selvaraj, *et al.*, (2016) [23] and Ravinder Reddy, *et al.*, (2012) [24]. In the present study, 75% of surgical site infections occurred in surgeries of greater than two hours duration and only 25% were reported from surgeries that were performed in less than two hours duration. These findings correlated with Aniruddha, *et al.*, (2017) [25], SP lilani, *et al.*, (2015) [26] and Moro *et al.*, (2005) [27]. In the present study, 46.87% of surgical site infections have been reported from clean, contaminated surgeries. It correlated with the findings of A. Ramesh *et al.*, (2012) [15] who reported 40% and Sivasankari Selvaraj, *et al.*, (2016) [23] who reported 53%. In the present study, increased incidence of surgical site infections has been identified in patients staying in hospital for up to 7 d preoperatively with 56.25%. These findings correlated with Naveen, *et al.*, (2014) [28]. In the present study, the percentage of monomicrobial growth was 87.50% which correlated with M. Saleem *et al.*, (2015) [22] who reported 90.75% of monomicrobial growth; Vikrant Negi, *et al.*, (2015) [1] with 94.7% and Rudratej Patil, *et al.*, (2015) [5] with 86%. The percentage of polymicrobial growth in the present study was 12.50% which correlated with Rudratej Patil, *et al.*, (2015) [5] with 14%.

In the present study, amongst the culture-positive samples, the incidence of Gram-positive cocci in pure culture is 36.11%. This finding correlated with M. Saleem, *et al.*, (2015) [29] who reported an incidence of 37.3% of Gram-positive cocci and Jyothi Sonawane, *et al.*, (2010) [11] who reported 36.48%. In the present study, amongst the culture-positive samples, the incidence of Gram-negative bacilli in pure culture is 41.66%. This finding correlated with Kyathi Jain, *et al.*, (2014) [28] who reported an incidence of 32.86% of Gram-negative bacilli. Mixed isolates in the present study were 22.22% of culture-positive samples. Therefore, amongst all the bacterial isolates combined, Staphylococcus aureus was the predominant isolate in the present study with an incidence of 27.77%. This finding correlated with Jyothi Sonawane *et al.*, (2010) [30] who reported an incidence of 29.26% of Staphylococcal isolates; Aniruddha *et al.*, (2017) [25] who reported 29%. In the present study, Pseudomonas aeruginosa was the second most predominant isolate with 19.44% of culture positivity. This finding correlated with Aniruddha, *et al.*, (2017) [25] who reported an incidence of 19%; M. Saleem *et al.*, (2015) [29] who reported 18.67%.

In the present study, Gram-positive cocci were mostly sensitive to Teicoplanin, linezolid and Vancomycin which correlated with Vikrant Negi *et al.* [1]. In the present study, Gram-negative bacilli were sensitive to Piperacillin+Tazobactam, Ceftazidime+Clavulanic acid and Meropenem, which correlated with M. Saleem *et al.* [29]. In the present study, among a total of 10 Staphylococcal isolates, Methicillin resistance was observed in 4 (40%) isolates, which correlates with study of Kyathi Jain *et al.*, (2014) [32] who reported 48.78% and Rudratej Patil *et al.*, (2015) [17] who reported 53.9% of MRSA. The chance of post-operative wounds being infected by MRSA is dependent on the duration of surgery, type of surgery and the nasal carriage rate among the attending personnel. Prompt diagnosis of MRSA infection is, therefore, important for patients, health caregivers and for epidemiological purposes. Among the total Gram-negative isolates in the present study, Extended Spectrum Beta-Lactamase (ESBL) production was seen in 21.05% and MBL production was seen in 15.78%, which correlates with Reba Kanunga *et al.*, (2015) [32] and Priya Datta *et al.*, (2015) [33] who reported incidence of 20.5% and 12.6% respectively.

CONCLUSION

The present study concludes that surgical site infections are high among post-operative cases of emergency surgeries. Risk factors like diabetes and hypertension are enhancing morbidity. The present study showed that Staphylococcus aureus was the most common aerobic bacterial isolate causing SSI followed by Pseudomonas aeruginosa. Antibiotic susceptibility test showed that Most of Gram-positive isolates were sensitive to Teicoplanin, linezolid, Vancomycin, Azithromycin and Amikacin. And most of Gram-negative isolates are sensitive to Imipenem, Amikacin, Piperacillin+tazobactam, Ceftazidime+clavulanic acid and levofloxacin. Emergence of beta-lactamase producers like Pseudomonas*,* Escherichia coli, Klebsiella species and others further worsened the condition. Hence, timely reporting of presence of ESBL and MBL-producing Gram-negative bacteria in surgical site infections is very essential for reducing their incidence. To achieve the goal of prevention of multidrug resistant organisms we have to focus on the preventive measures, including fundamental principles of asepsis. Apart from these measures, surgical expertise and theatre discipline are also essential components against surgical site infections.

Hospital infection control committee of the hospitals need to strengthen the surveillance activities of capturing surgical site infections as it is one of the quality indicators to take corrective and preventive actions to improve the infection control programme. They should make recommendations at all levels for prevention of surgical site infections. The patients undergoing surgery and the hospital staff should be screened for colonisation with MRSA to prevent surgical site infections and spread of hospital-acquired infections [36]. Strict adherence to the standardised infection control policies and antibiotic policy will decrease the incidence of surgical site infections due to hospital acquired multidrug resistant microorganisms. Simple measures like Hand hygiene recommendations are important to prevent cross-infection through the colonised hands [35]. The present study emphasizes on, working knowledge of the prevalent organism, virulence and resistance profile and help the infection control practitioner and surgeon to treat the infection effectively at the earliest and also decreases economic burden due to surgical site infections.

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AUTHORS CONTRIBUTIONS

All the authors have contributed equally

CONFLICT OF INTERESTS

Declared none

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