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Research Article

Bacteriological study of surgical site infections in a tertiary care hospital at Miraj, Maharashtra state, India

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ABSTRACT

Background: Surgical site infections (SSI) are one of the common post-operative complications. Apart from bacterial contamination of wound, various patient and environment related factors play role in development and outcome of SSI. The present study is undertaken to study the frequency of SSI with reference to factors contributing to it and the antimicrobial susceptibility pattern of the causative organisms.
Methods: This single-observer, cross-sectional, complete-enumeration prospective study was carried out over a period of one year. 196 pus samples from cases of surgical site infections were processed for gram staining, culture, biochemical identification tests and antimicrobial susceptibility testing. Methicillin-Resistant *Staphylococcus aureus* (MRSA) strains were detected by using oxacillin and cefoxitin disk diffusion and minimum inhibitory concentration (MIC) of oxacillin was tested by broth dilution technique.
Results: The overall frequency of SSI was 6.17%. Most common isolates were *Staphylococcus aureus*, coagulase negative *Staphylococci* (CONS), *E. coli* and *Pseudomonas aeruginosa*. The frequency of MRSA was 8.6%. The maximum frequency was among patients operated on emergency basis in surgical department.
Conclusions: The most important determinants for SSI were emergency surgery and presence of co-morbid conditions. The frequency of occurrence was age-dependent, with maximum rate of SSI in males and females in the third and sixth decades of life, respectively.

Keywords: Surgical site infections, Post-operative wound infections, Clean and clean-contaminated wound, MRSA

INTRODUCTION

After urinary tract infection, surgical site infections (SSI) are the most common nosocomial infections in hospitalized patients.[1] As many as 1% of the patients undergoing clean and 11% of patients undergoing clean-contaminated surgery experience SSI.[2] There is substantial burden with increased morbidity and exceeding healthcare costs.[3]

Despite efforts to control infection and better understanding of sepsis, wound infection is still a clinical problem and some infections in clean wounds still remain unexplained.[4] In many SSIs, the responsible pathogens originate from patient's endogenous flora. The causative pathogen depends on the type of surgery; the most commonly isolated organisms are *Staphylococcus aureus*, coagulase negative *Staphylococci*, *Enterococcus spp* and *E. coli*.[5]

The present study was undertaken to determine the frequency of surgical site infections with reference to various directly or indirectly related pre-disposing factors. The study also aimed at ascertaining the surgical site infection rate, type of bacteria commonly involved, their antibiotic sensitivity pattern; to determine the

frequency of methicillin-resistant *Staphylococcus aureus* (MRSA) and to calculate the oxacillin minimum inhibitory concentration for MRSA.

METHODS

This single-observer, cross-sectional, complete - enumeration, prospective study was conducted over a one-year period in the tertiary care hospital attached to Government Medical College, Miraj, District Sangli, Maharashtra State in Western India. After obtaining approval from Institutional Ethics Committee, all patients of either sex, admitted in departments of Surgery, Orthopaedics, Gynaecology and Obstetrics, who had undergone surgical procedures during the study period, were included. The surgical procedures were classified as planned (elective) surgeries, emergency surgeries and clean, clean-contaminated surgeries and patients were divided accordingly.

The surgical sites were inspected at frequent intervals on days 3/5, 7 and 10, and further whenever required, for clinical evidence of infection. Samples from patients with clinical evidence of infection (purulent discharge) were processed for bacteriological examination. Details of patients' age, sex, clinical diagnosis, date of admission, personal history, and date of surgical procedure, type of procedure (clean/ clean-contaminated) and prophylactic antibiotics were recorded in a pre-tested proforma.

Sample collection

All samples were collected early in the morning before dressing of the wounds. After cleaning the surrounding skin with 70% alcohol, the pus was collected from deep inside the wound in sterile bulbs using syringes or using sterile swab sticks in duplicate.[6]

Sample processing

The first swab was used direct smear and gram staining, the second swab was inoculated on blood agar & MacConkey's agar. The plates were incubated overnight at 37º C and the morphology of colonies was observed the following morning. Identification of organisms was done by secondary smears examination and biochemical tests. [6]

Antimicrobial susceptibility testing

Broth cultures of isolated organisms were prepared and matched with McFarland 0.5 turbidity standard. Then these were tested for various antibiotics on Muller Hinton Agar plates by Kirby-Bauer Disk Diffusion Technique. The plates were incubated at 37^0 C for 18-24 hours. [6] The antimicrobials were selected for susceptibility testing according to CLSI guidelines. The MIC of oxacillin was tested for the strains resistant to oxacillin and/or cefoxitin.

Detection of methicillin resistant *staphylococcus aureus* (MRSA) was done by cefoxitin disk diffusion testing; Oxacillin disk diffusion testing and minimum inhibitory concentration of oxacillin by Broth dilution method using Muller-Hinton broth. MIC ≥4µg/ml indicated methicillin resistance.[7,8]

RESULTS

A total of 3,160 patients admitted in the tertiary care hospital had undergone surgical procedures during the study period. Of these surgeries, 1,328 (904 elective, 424 emergency) were conducted by the Surgery Department, 1025 (391 elective, 634 emergency) by Obstetrics and Gynaecology Department, and the remaining 807 (463 elective, 344 emergency) by Orthopedics Department. Out of 3,160 surgeries, 1814 (57.3%) were "clean", 688 (21.8%) were "clean contaminated" and 658 (20.82%) were "contaminated".

The infection rates in clean, clean-contaminated and contaminated wounds were 2.86%, 7.84% and 13.52%, respectively. The occurrence of SSI in elective and emergency surgeries was 4.32% (n=76) and 8.48% (n=119), respectively. The frequency of SSI was 8.35% (n=117, 68 males and 43 females) in the Department of Surgery, 4.68% (n=48 females) in the Department of Obstetrics and Gynaecology, 4.46% (n=36, 24 males and 12 females) in the Department of Orthopaedics. Though the rate of SSI was higher in females (male-female ratio 1: 1.11), the gender difference was not statistically significant. The overall rate of SSI (both sexes combined) was in the age group of 21-30 years. However, the highest rates among males and females were seen in age group of 21-30 years and 51-60 years, respectively.

Table 1: Isolated organisms from patients.

Organism	Isolates (n = 208)
Staphylococcus aureus	46 (22.1%)
Coagulase negative staphylococci (CoNS)	34 (16.3%)
E. coli	29 (13.9%)
Pseudomonas aeruginosa	22 (10.5%)
Klebsiella pneumonia	16 (7.6%)
Enterococcus spp	13 (6.2%)
Citrobacter freundii	12 (5.7%)
Enterobacter spp	9 (4.3%)
Proteus mirabilis	8 (3.8%)
Proteus vulgaris	6 (2.8%)
Acinetobacter spp	6 (2.8%)
Citrobacter diversus	4 (1.9%)
Streptococcus spp	3 (1.4%)

No organism was grown from 11 (5.64%) samples.

Out of 3,160 cases, 195 (6.17%) patients had SSI. Of the 195 samples collected in duplicate, cultures of 11 (5.64%) samples did not show any growth of organisms.

Out of 184 wound infections, 24 were caused by two micro-organisms. In remaining 160 samples, only one organism was isolated. The frequency of mixed infections was higher in patients who had undergone gastrointestinal surgeries. The commensal flora of the gastro-intestinal tract could be the associated factor. On cultivation, 208 strains were obtained (Table 1).

Table 2: Antibiotic susceptibility of gram positive cocci.

Antibiotic	*Staphylococcus aureus*	CoNS	*Enterococcus spp.*	*Streptococcus* spp.
Penicillin	6 (13%)	9 (26.4%)	6 (46.1%)	2 (66.6%)
Amoxycillin	10 (21.7%)	14 (41.1%)	7 (53.8%)	2 (66.6%)
Oxacillin	28 (60.8%)	24 (70.5%)	10 (76.9%)	3 (100%)
Vancomycin	46 (100%)	34 (100%)	13 (100%)	3 (100%)
Erythromycin	20 (43.4%)	20 (61.2%)	-	3 (100%)
Cotrimoxazole	21 (45.6%)	19 (55.8%)	-	-
Amikacin	26 (56.5%)	26 (76.4%)	-	3 (100%)
Ciprofloxacin	17 (36.9%)	14 (41.1%)	6 (46.1%)	3 (100%)

CoNS = Coagulase Negative *Staphylococci*

Table 3: Antibiotic susceptibility of gram negative bacilli *(Enterobacteriaceae).*

Antibiotic	*E. coli*	*Klebsiella spp.*	*C. freundii*	*Enterobacter*	*P. mirabilis*	*P. vulgaris*	*C. diversus*
Amoxycillin	4 (13.8%)	2 (12.5%)	3 (25%)	3 (33.3%)	2 (25%)	1 (16.6%)	1 (25%)
Cephalexin	12 (41.3%)	5 (31.2%)	2 (16.3%)	6 (66.6%)	6 (75%)	3 (50%)	0 (0%)
Gentamicin	18 (62%)	10 (50%)	8 (66.6%)	5 (55.5%)	5 (62.5%)	4 (66.6%)	3 (75%)
Amikacin	21 (72.4%)	12 (75%)	9 (75%)	7 (77.7%)	6 (75%)	4 (66.6%)	4 (100%)
Cefoperazone	16 (55.1%)	11 (68.7%)	5 (41.7%)	7 (77.7%)	6 (75%)	4 (66.6%)	2 (50%)
Cefepime	21 (72.4%)	11 (68.7%)	8 (66.6%)	8 (88.8%)	7 (83.5%)	4 (66.6%)	4 (100%)
Ciprofloxacin	12 (41.3%)	6 (37.5%)	5 (41.7%)	4 (44.4%)	4 (50%)	3 (50%)	1 (25%)
Cotrimoxazole	15 (51.7%)	6 (37.5%)	7 (58.3%)	5 (55.5%)	5 (62.5%)	3 (50%)	2 (50%)

Table 4: Antibiotic sensitivity of gram negative bacilli *(Pseudomonas and Acinetobacter).*

Antibiotic	*Pseudomonas aeruginosa*	*Acinetobacter spp*
Amoxycillin	4 (18.1%)	0 (0%)
Amikacin	10 (45.4%)	5 (83.2%)
Gentamicin	9 (40.9%)	5 (83.2%)
Ciprofloxacin	7 (31.8%)	2 (33.3%)
Ceftriaxone	11 (50%)	2 (33.3%)
Ceftazidime	12 (54.5%)	3 (50%)
Imipenem	20 (90.9%)	6 (100%)
Piperacillin	18 (81.8%)	3 (50%)

DISCUSSION

SSI

The microbiology of SSI largely depends upon the kind of operation performed, the types of work load and the hospital environment.[9,10] Comparative statistics from hospitals in different parts of the world may be unreliable and even misleading. The prevalence of pathogen varies from place to place and hospital to hospital.[11] Periodic studies carried out in the same hospital over a number of years would provide more reliable information.[10]

Frequency of infection

Surgical site infections remain a major issue of patient safety despite of improvements in surgical practice and infection control techniques.[12] The overall infection rate in current study is 6.17% whereas that reported by other studies ranges from 4-33%.[4, 13-18]

Department-wise distribution of patients

The higher frequency of SSI observed in Department of Surgery could be because of higher number of emergency procedures conducted in the department. Similarly,

substantial number of surgeries had dealt with gastro-intestinal and urinary systems, which contributed to clean contaminated wound.

Age and sex-wise distribution of patients

The reason for higher rate of SSI in third decade of life in this study could be the greater number of young adults getting operated for exploratory laparotomy for antral or appendicular perforation or appendicitis.

Also, the number of females undergoing Caesarean sections and other gynaecological operations in third and sixth decade is higher. Similar observations have been made by other researchers.[4,18]

Organisms isolated

Frequency of culture negative SSI in the present study was 5.4%, which compares with 7.28% reported in a tertiary care hospital in Bangalore.[14] The cause of failure to grow any bacterium on culture could either be due to anaerobic nature of the organism or non-viability of organisms in patients on antibiotic therapy.

Staphylococcus species

The frequency of isolation of Staphylococci is depicted in Table 1. The frequency of isolates of *Staphylococcus aureus* from infected surgical wounds reported by other researchers was in the range of 19-75%.[11,14,15,19,20]

Enterococcus species

Among catalase negative organisms, 6.2% were Enterococci in the present study while that reported by other authors ranged from 0.06-7.1%.[13,14,19]

β haemolytic Streptococci

Streptococcus spp was isolated from 3 cases (Table 1). A study from Nagpur has reported its frequency as 3.78%.[11] The decreasing frequency of *Streptococcus spp* as a causative agent of SSI may be ascribed to its susceptibility to even age-old antimicrobial drugs. The emerging multidrug resistant organisms are now taking the place of *Streptococci*.

Enterobacteriaciae

In the current study, *E.coli* was isolated from 29 (13.9%) patients (Table 1). Our finding correlates with the frequencies (7.5-21%) reported by other studies.[4,11,14,15,19] There were 14 isolates of *Klebsiella pneumoniae* and 2 of *Klebsiella oxytoca* (Table 1).

This correlates with the findings reported by other studies.[4,11,14,15] The present study showed 7.6% of isolates were that of *Citrobacter spp* (12 *C. freundii* and 4 *C. diversus*). Other studies from Nagpur and New Delhi have reported frequencies of 2.27% and 4.1%, respectively.[11,13]

Of the isolates of *Proteus spp*, *P. mirabilis* and *P. vulgaris* comprised 8 (3.8%) and 6 (2.8%), respectively (Table 1). Other studies have reported the frequency of *Proteus spp*. isolates in range of 2.9-6.8%.[11,14,15,17] The present study showed 9 (4.3%) were *Enterobacter spp*, whereas a study from New Delhi has reported the frequency of isolation of the same organism as 2.7%.[13]

Pseudomonas aeruginosa

Twenty two (10.5%) strains of *Pseudomonas aeruginosa* were isolated in the current study (Table 1). Other studies have reported the frequency of isolates of this organism in the range of 10.7-19%.[4,11,13,14,17]

Acinetobacter species

Out of 208 organisms isolated in the present study, 6 (2.8%) were *Acinetobacter spp*., (Table 1). Other studies have reported higher frequency of *Acinetobacter sp* ranging from 10.53-29%.[4,13,17]

Antibiotic sensitivity tests

Staphylococcus spp (Table 2). A Mumbai-based study has reported 100% penicillin resistance among *S. aureus*, whereas ampicillin resistance reported by a Nagpur-based study and a Bangalore-based study was 91.8% and 77.7%, respectively.[11,14,16] These compare with the findings of our study.

MRSA: (Table 2)

A seven-year review of MRSA susceptibility has reported a constant susceptibility rate of 98% to co-trimoxazole with significant decrease in susceptibility of quinolones (63%) and also reported that susceptibility to erythromycin has decreased from 15% in the year 2000, to 4% in the year 2006.[21]

The current study obtained comparable susceptibility in relation to erythromycin, but a lower susceptibility to co-trimoxazole. All the MRSA in current study were sensitive to vancomycin, as found in many other studies.[13,14,16,21]

Coagulase negative staphylococci (CoNS) (Table 2)

Using disc diffusion method, the frequency of oxacillin-resistant CoNS was 29.5% in the current study while other studies have reported frequencies ranging from 14-40%.[22-25] The frequency of co-existing resistance to oxacillin and ciprofloxacin, which was proposed to be an alternative therapy for MRSA infection, has ranged from 23% to 100%.[22,26]

Enterobacteriaceae

E. coli with higher resistance against fluoroquinolones and 100% susceptibility to fluoroquinolones and 66.6% susceptibility to Amikacin have been reported.[13,14] Amikacin remains the antimicrobial with highest susceptibility according to our study. The high degree of resistance reported in *Klebsiella spp* by many researchers was also observed in the current study (Table 3).[13,14,17]

Pseudomonas aeruginosa

Gentamicin resistance of 45.8% and 100% have been reported, while in the present study, the resistance was 60% (Table 4).[11,16]

Acinetobacter spp

Earlier studies do not mention this drug resistant pathogen, but recent studies have mentioned presence of highly drug resistant Acinetobacter in SSI (Table 4).[13,17]

CONCLUSION

To conclude, the overall frequency of SSI was 6.17% (2.86% in clean, 7.84% in clean contaminated and 13.52% in contaminated wounds). Maximum frequency of SSI was in the third decade of life for males and sixth decade for females. The most common gram positive and gram negative isolates were *Staphylococcus aureus* (22.1%) and *E. coli* (13.9%), respectively. The frequency of MRSA was 8.6%. All *Staphylococci* and *Enterococci* were vancomycin sensitive. Among Gram-negative bacilli, the highest percentage susceptibility was to amikacin. Imipenem was most effective anti-pseudomonal drug with 100% susceptibility.

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