TITLE :

STUDY OF BACTERIAL PATHOGENS AND THERE ANTIBIOTIC SUSCEPTIBILITY PATTERN OF ISOLATES ASSOCIATED WITH POST OPERATIVE WOUND INFECTIONS IN A TERTIARY CARE HOSPITAL, OSMANIA GENERAL HOSPITAL, HYDERABAD

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Abstract :

Introduction : Post-operative wound infection is an infection that develops within 30 days after an operation (OR) within one year if an implant was placed and infection appears to be related to the surgery. These infections number approximately 500,000 per year, among an estimated 27 million surgical procedures and account for approximately one quarter of the estimated 2 million nosocomial infections each year. Post operative wound infection remains the major source of illness and one of the cause of death in surgical patient. Aims and objectives : To isolate and identify the bacterial pathogens from the infected surgical site, to determine the Antibiotic susceptibility pattern of the isolated pathogens and to study the clinical profile and to identify the risk factors in the causation of surgical site infection. Material and methods : This is a retrospective study ,conducted in the department of Microbiology, Osmania general hospital, Hyderabad , between June 2015 to May 2016.

A total of 230 clinically suspected cases of post operative wound infection in various surgical units like general surgery, orthopaedic , cardiothoracic, urology , nephrology , neurology and other superspeciality units. SPECIMEN COLLECTION: Pus collected with a sterile absorbable cotton swab **,**aspirated pus. Two Swabs were collected, one at the time of change of dressings and second Pus aspirates were collected aseptically in the operation theatre.

Results : Among the total number of specimens – 230 . Pus swabs - 190 (aerobic culture only) and aspirates -40 (aerobic and anaerobic culture). Out of a total 230 specimens, 197 (85.6%) were direct smear positive and 194 (84.3%) specimens were positive for bacteria by culture. Only 33 (14.4%) specimens were sterile by culture and also did not show any organisms on direct examination. Thus 36 specimens (15.7%) were negative by culture. Conclusion : 230 clinically suspected specimens of SSI were processed for isolation of bacterial pathogens (aerobes and anaerobes) and determining their antibiotic sensitivity pattern. The clinical profile and risk factors in association of SSI were also studied. Direct Gram's smear examination of the sample revealed the presence of bacteria in 211 (91.7%) samples along with pus cells. Of this 194(84.3%) were positive by culture . Only 36 (15.6%) were sterile. Of these 194 positive cultures, monomicrobial etiology was found in 145 (63%) samples and polymicrobial etiology in 49(21.3%) samples.

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Introduction :

Post-operative wound infection is an infection that develops within 30 days after an operation (OR) within one year if an implant was placed and infection appears to be related to the surgery.[1]These infections number approximately 500,000 per year, among an estimated 27 million surgical procedures and account for approximately one quarter of the estimated 2 million nosocomial infections each year. Post operative wound infection remains the major source of illness and one of the cause of death in surgical patient.[2]

Terms for infections associated with surgical procedures was changed from surgical wound infection to surgical site infection in 1992 by The Centres For Disease Control And Prevention (CDC).[1]SSIs are the third most commonly reported nosocomial infection and they account for approximately 14-16% of all nosocomial infections.[3]Approximately 77% of the deaths of surgical patients can be traced back to surgical wound infection and the majority (93%) were serious infections involving organs or spaces accessed during the operation. SSI patients are 60% more likely to spend time in ICU, are five times more likely to be readmitted and have twice the incidence of mortality.[4]

SSIs have been responsible for the increasing cost, morbidity and mortality related to surgical operations and continues to be a major problem even in hospitals with most modern facilities and standard protocols or pre-operative preparation and antibiotic prophylaxis.[5]SSI prolongs hospital stay and adds between 10-20% of hospital costs.[6] Despite the routine use of standard surgical scrub/ preparation and prophylactic antibiotics in many surgical patients, upto 10-20% of certain high risk patient populations are at risk for SSI.[7] Inappropriate use of broad spectrum of antibiotics or prolonged use of prophylactic antibiotics , disposes patients at even greater risk of SSI because of development of antibiotic resistant pathogens **(Dahms et al., 1998).** The incidence of infection varies from surgeon to surgeon, from hospital to hospital, from one surgical procedure to another and most importantly- from patient to patient.[8]

Post operative wound infections occur when the inoculum of contaminating micro-organisms is not contained by host defences, proliferates and produces established infection.[9]The chances of developing an establishing infection after surgery are determined by pathogenicity of the invading micro-organisms and by the size of bacterial inoculum.[10]The virulence and invasive capability of the organisms have been reported to influence the risk of infection, but the physiological state of the tissue in the wound and immunological integrity of the host seem to be of equal importance in determining whether infection occurs or not.[11]Despite improved understanding of the pathophysiology and improved methods of prevention and prophylaxis, SSI remains the most common cause of post-operative morbidity and mortality.[12]

MATERIALS AND METHODS

STUDY CENTRE: Osmania General Hospital, AfzalgunJ, Hyderabad.

STUDY PERIOD : June 2015 to may 2016.

SELECTION OF CASES

A total of 230 clinically suspected cases of post operative wound infection in various surgical units like general surgery, orthopaedic , cardiothoracic, urology , nephrology , neurology and other superspeciality units.

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INCLUSION CRITERIA

Wound infection was diagnosed if any one of the criteria was fulfilled

1. Serous / non-purulent discharge from wound.
2. Serous / non-purulent discharge from wound with signs of inflammation like
   - oedema
   - redness
   - warmth
   - raised local temperature
   - fever >38^{o}c
   - tenderness
   - induration
3. Pus discharge from wound.
4. Wound deliberately opened up by the surgeon due to localized collection (serous/ purulent).

EXCLUSION CRITERIA

1. Stitch abcesses(minimal inflammation and discharge confined to the points of suture penetration)
2. Infection of an episiotomy or neonatal circumcision site.
3. Infected burn wounds

SPECIMEN COLLECTION

SPECIMEN: Pus collected with a sterile absorbable cotton swab.
Aspirated pus

TIME OF COLLECTION:

1. Swabs were collected at the time of change of dressings.
2. Pus aspirates were collected aseptically in the operation theatre.

CULTURE FOR AEROBES AND FACULTATIVE ANAEROBES

METHOD OF COLLECTION

To reduce the chances of contamination by normal skin flora during collection the following method was followed for collection of specimens.

1. The infected site is first cleaned with normal saline on removal of dressing.
2. If any eschar is present, it is removed with the help of sterile blade.
3. The infected site is again washed with normal saline and allowed to dry.
4. Then with the application of slight pressure beside the infected site, a sterile absorbable cotton swab is rolled over it and placed in a sterile bottle.
5. Pus swabs are collected in duplicate.
6. The specimen was immediately brought to the Microbiology lab for further processing of the sample.

METHOD OF PROCESSING

Macroscopic Examination

- Colour
- Odour
- Consistency

Direct smear examination

One of the swab collected was used for preparation of direct smear and stained by Jensen's modification of Gram's stain .After allowing it to air dry, the smears were screened

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under oil immersion objective to note the morphological features of the bacteria and the presence of inflammatory cells.

Culture

1. Second swab was used for culture by inoculating it on routine media like
    a. Blood Agar
    b. MacConkey Agar
    c. Chocolate Agar
2. The inoculated Blood agar and MacConkey agar plates were incubated at 37°c overnight. The inoculated Chocolate agar plates were incubated in an atmosphere of 5-10% CO_2 in a candle jar.
3. After overnight incubation the plates were examined for bacterial growth. Further identification and confirmation of organisms was done by the standard identification tests. **(Mackie and McCartney, 14th ed, pg 131-146)**

4. After 48 hours incubation Chocolate agar plates were similarly examined and the colonies further processed.

Antibiotic susceptibility testing

Antibiotic sensitivity assay was performed on all isolates using Kirby- Bauer Disc Diffusion method on Mueller-Hinton Agar , using the following Hi-media Antibiotic discs.

RESULTS

The present study was undertaken in various surgical units, Osmania General Hospital, Hyderabad to isolate bacterial pathogens from surgical site infections and to determine their antibiotic sensitivity pattern.

Total number of specimens - 230.
Pus swabs - 190 (aerobic culture only)
Pus aspirates - 40 (aerobic and anaerobic culture)

TABLE :1 AGE WISE DISTRIBUTION OF CASES

S.No	AGE GROUPS (X)	CASES (230)	NUMBER OF CASES POSITIVE (194)	RATE OF ISOLATION (X/230)	PERCENT OF ISOLATION (X/194)	AGE WISE ISOLATION App.
1.	≤20 yrs	9	5	2.2%	2.6%	55%
2.	21- 40 yrs	58	45	19.5%	23.2%	78%
3.	41- 60 yrs	84	77	33.4%	39.7%	92%
4.	≥ 60 yrs	79	67	29.1%	34.5%	85%

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The maximum rate of isolation of the pathogens was from 41-60 years (33.4%), followed by > 60years (29.1%), 21-40 years (19.5%) and < 20years (2.2%). The maximum rate of isolation when adjusted age- wise was also the same.

TABLE :2 EFFECT OF TYPE OF SURGERY ON SSI

S. No	TYPE OF SURGERY (X)	CASES (230)	NUMBER OF CASES POSITIVE (194)	RATE OF ISOLATION (X/230) app.	PERCENT OF ISOLATION (X/194) app.	SURGERY WISE ISOLATION app.
1.	Emergency	151	138	60%	71.1%	91.3%
2.	Elective	79	56	24.3%	28.9%	70.8%

The maximum rate of isolation of pathogens was from emergency surgery (60%) than elective surgery(24.3%).

TABLE :3 EFFECT OF DURATION OF SURGERY ON SSI

S.No	DURATION OF SURGERY (hrs) X	NUMBER OF CASES (230)	NUMBER OF CASES POSITIVE (194)	RATE OF ISOLATION (X/230) App.	PERCENT OF ISOLATION (X/194) App.	DURATION OF SURGERY WISE ISOLATION app.
1.	< 1	43	27	11.7%	13.8%	62.7%
2.	1 -2	76	65	28.3%	33.5%	86.6%
3.	> 2	111	102	44.3%	52.7%	91.9%

The maximum rate of isolation of pathogens was seen in surgeries lasting >2 hours (44.3%) followed by surgeries lasting 1-2 hours (28.3%) and <1hour (11.7%).

TABLE :4 SSI RATE DEPENDING ON SURGICAL WOUND CLASSIFICATION

SURGICAL CLASS	NUMBER OF CASES	NUMBER OF CASES POSITIVE (X)	RATE OF ISOLATION (X/230)	PERCENT AMONG THE ISOLATES (X/194)	SURGICAL WOUND CLASSIFICATION WISE App.
I	35	14	6%	7.3%	40%

II	43	37	16%	19%	86%
III	70	65	28.2%	33.5%	92%
IV	82	78	33.9%	40.2%	95%

The surgical site infection rate was maximum in class IV wounds (33.9%), followed by class III wounds (28.2%), class II(16%)

TABLE : 5 RESULT OF DIRECT SMEAR AND CULTURE

SPECIMEN	NUMBER	%
Smear +ve & culture +ve	194	84.3%
Smear +ve & culture –ve	3	1.3%
Smear –ve & culture –ve	33	14.4%
TOTAL	230	100%

Table 5 compares the results of direct smears and culture of the processed specimens. Out of a total 230 specimens, 197 (85.6%) were direct smear positive and 194 (84.3%) specimens were positive for bacteria by culture. Only 33 (14.4%) specimens were sterile by culture and also did not show any organisms on direct examination. Thus 36 specimens (15.7%) were negative by culture.

TABLE :6 OVERALL RESULTS OF THE CASES STUDIED

DETAILS OF ISOLATION	NUMBER	RATE OF ISOLATION
Positive Cultures	194	84.3%
Monomicrobial	145	63%

Polymicrobial	49	21.3%
Negative Cultures	36	15.7%

The positive culture findings are summarized in this table. Out of 194 positive cultures, polymicrobial etiology was found only in 49cases(21.3%) in contrast to monomicrobial isolates in 145 cases (63%).

TABLE :7 DETAILS OF AEROBIC ORGANISMS ISOLATED

Total Number of specimens - 230

ORGANISMS	No. OF SPECIMENS SHOWING GROWTH (X)	TOTAL No. OF ORGANISMS ISOLATED	RATE OF ISOLATION (X/230)	PERCENT AMONG THE ISOLATES (X/237)
Monomicrobial	138	138	60%	58.2%
Polymicrobial	49	99	21.3 %	41.8%
TOTAL	187	237		

The positive culture findings of aerobic organisms are summarized in this table. Out of 230 specimens, positive cultures for aerobic organisms were obtained in 187(81.3%) while 36(15.6%) were sterile. A total of 237 aerobic organisms were isolated. Polymicrobial etiology was found only in 49 cases (21.3%) in contrast to monomicrobial isolates in 138 cases (60%).

DISCUSSION

Surgical site infection is the commonest post operative complication and causes significant post-operative morbidity and mortality, prolongs hospital stay and adds between 10-20% to the hospital costs. Although the total elimination of wound infection is not possible, a reduction in the infection rate to a minimal level could have significant benefits in terms of both patient comfort and medical resources used.[13]

Despite advances in infection control practices, SSIs remain a substantial cause of morbidity and mortality among hospitalized patients. This may be partially explained by the emergence of antimicrobial-resistant pathogens and the increased numbers of surgical patients with risk factors. Thus, to reduce the risk of SSI, a systematic but realistic approach must be applied with the awareness that this risk is influenced by characteristics of the patient, operation, personnel, and hospital.[14]

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The present study was undertaken to describe the spectrum of bacterial isolates in causation of SSI, their antibiotic sensitivity patterns and association of considerable risk factors in the causation of SSI.

Out of 230 clinically suspected cases of SSI, the highest incidence of SSI was observed in 41-60 years (33.4%) , followed by > 60years(29.1%) age group. This finding correlated with the work published by others. De Sa LA, Sathe LJ, Bapat RD(1984) reported an increased incidence of SSI in 51-70 years age group.[33] Suchitra and Lakshmidevi (2009) also reported an increased incidence in patients >45years age group.[15]

The maximum rate of isolation of the pathogens was seen in emergency(60%) surgeries compared to elective surgeries (24.3%).This finding correlated with the work of Renvall et al(1980) [16] and Gil Egea et al(1987) [17] who reported an higher incidence in patients requiring emergency operations.

SSI was also directly related to duration of surgeries. The maximum rate of isolation of the pathogens was seen in surgeries lasting for > 2 hours (44.3%), followed by 1-2hours (28.3%).This finding was in accordance with the study of Miftari et al (1990)[18] who reported an increased incidence of infection in surgeries lasting >1 hour(50%) when compared to surgeries lasting <1 hour (10%).Lilani et al(2005)also observed the same finding , reporting an SSI rate of 1.47% and 38.46% in surgeries lasting <1 hour and >2 hours respectively.

SSI were classified into four classes according to the degree of contamination of the surgical site by CDC. According to this classification maximum rate of isolation of the pathogens were from class IV (33.9%), followed by class III(28.2%), class II (16%) and class I(6%).This finding correlated with studies carried out by Renvall et al (1980), Abu Hanifah et al (1990), Twum- Danso et al (1992), Narotam et al (1994), Lilani et al (2005) and Sangrasi et al.[20,21,22,19]

The association of risk factors in the causation of SSI was also studied. A higher incidence of SSI was seen in surgeries with pre-operative stay >7days (30.4%),followed by the time of local preparation of >12 hours (24.3%), the presence of drains (20%) ,smoking (19.5%), alcohol (17.9%), presence of septic focus(9.1%) and diabetis mellitus (7%).This finding correlated with the work carried out by others. Kowli et al (1985)[23] reported an infection rate of 17.4% and 71.4% with a pre-operative stay of 0-7 days and >21 days respectively. Suchitra and Lakshmidevi (2009)[15] also reported an increased incidence of SSI in patients with pre-operative stay of >7days and in diabetic patients. Lilani et al (20005) [19] reported an SSI rate of 22.41% in cases where drain was used than in non-drained wounds (3.03%). Nagachinta et al (1987), Vinton et al(1991), Bryan et al (1992), Holley et al (1995) reported cigarette smoking as an independent risk factor in causation of SSI. De Sa LA, Sathe LJ, Bapat RD (1984) reported an infection of 36.6% in patients with septic focus.

Out of 230 specimens, 197 (85.6%) were positive for bacteria by direct Gram stain of the sample and 194 (84.3%) were positive for culture. Only 33(14.4%) samples were both direct smear and culture negative. Thus 36 (15.7%) samples are negative by culture. The cause for culture negative but direct smear positive samples was explained by Giacometti et al who said that Atypical Mycobacteria, Mycoplasma hominis, Ureaplasma urealyticum, small colony variant Staphylococcus aureus, Nocardia, Actinomycetes, Legionella, Coxiella burnetti, Herpes simplex virus etc as some of the causes for culture negative SSI.[24]

Out of 194 positive cultures, 145 were positive for monomicrobial growth (63%) and 49 for polymicrobial growth(21.3%). This finding correlated with the study of Giacometti et al (2000)[24] who reported 76% of monomicrobial growth and 25% of polymicrobial growth.

All the Gram positive cocci isolated were sensitive to Vancomycin(100%), followed by Ciprofloxacin (86%), Amikacin (85.4%) and Amoxiclav(82.6%).90% of the isolates of S.aureus were resistant to Oxacillin. These findings of sensitivity pattern was comparable with the studies of Lilani et al (2005), Le et al (2006) who have reported 100% sensitivity to Vancomycin and 90% of Staphylococcus resistant to methicillin .[19]

Among the Gram negative isolates, Imipenam showed 100% sensitivity.

Klebsiella pneumoniae showed 92.4% sensitivity to Aztreonam , followed by Cefoperazone+ sulbactam(92.3%), Gentamicin(90.4%), Ceftriaxone+sulbactam (90%) .

Escherichia coli showed 94.3% sensitivity to Piperacillin , followed by Aztreonam (93.1%), Ceftriaxone+ sulbactam(91%), Cefoperazone+sulbactam((91%) and Amikacin(90%) .

Proteus species showed 91.5%% sensitivity to Ceftriaxone+ sulbactam followed by, Cefoperazone+sulbactam(90.5%) ,Aztreonam (90%) and Amikacin(89.2%).

Pseudomonas aeruginosa showed 90.8% sensitivity to Ceftriaxone+ sulbactum followed by Aztreonam(90.4%),Cefoperazone+sulbactam(89%) and Gentamicin (81.3%)

Citrobacter species showed 93% sensitivity to Ceftriaxone+ sulbactam followed by Aztreonam (91%),Cefoperazone+sulbactam(89%) and Amikacin(84.4%)

This pattern of antibiotic sensitivity was comparable with studies of Giacometti et al (2000) and Jonathan et al (2008)[24.]

SUMMARY AND CONCLUSION

In the present study, 230 clinically suspected specimens of SSI were processed for isolation of bacterial pathogens (aerobes and anaerobes) and determining their antibiotic sensitivity pattern. The clinical profile and risk factors in association of SSI were also studied.

Majority of cases belonged to the 41-60 years age group and the overall incidence was found to be higher in males as compared to females.

Direct Gram's smear examination of the sample revealed the presence of bacteria in 211 (91.7%) samples along with pus cells. Of this 194(84.3%) were positive by culture . Only 36 (15.6%) were sterile. Of these 194 positive cultures, monomicrobial etiology was found in 145 (63%) samples and polymicrobial etiology in 49(21.3%) samples.

The maximum rate of isolation of pathogens was seen in emergency surgeries (60%), surgeries lasting for > 2hours (44.3%) , surgical class IV (33.9%) and III (28.2%), patients with pre-operative stay >7 days (30.4%), patients with pre-operative shaving >12 hours(24.3%), presence of drains (20%), H/O smoking and alcoholism(19.5%, 17.5%).

A total of 244 isolates were recovered, out of which 153 (62.7%) were Gram positive and 91 (37.3%) were Gram negative organisms. Staphylococcus aureus was the predominant isolate (44.3%), followed by Coagulase negative Staphylococcus (14.8%) , Klebsiella pneumoniae (13.9%) and Pseudomonas aeruginosa (11.7%) .

Out of 40 pus aspirates, 7 anaerobic organisms (17.5%) were isolated. No aerobe, facultative anaerobe and micro-aerophilic organism were isolated from these samples. Peptostreptococcus species (10%) was the predominant anaerobe isolated , followed by Bacteroides species (5%).

All the Gram positive organisms showed 100% sensitivity to Vancomycin and Gram negative organisms showed 100% sensitivity to Imipenam. All anaerobic organisms showed 100% sensitivity to Metronidazole.

The high rate of isolation of pathogens in patients with pre-operative stay >7 days and with pre-operative shaving >12 hours is primarily due to colonization of patients with hospital acquired resistant micro-organisms. Local preparation of the operating site ideally done on the day of surgery, just prior to surgery minimizes colonization of hospital acquired pathogens. Thus it is of utmost importance that surgeons and other medical personnel involved in patient care need to minimize these risk factors in order to prevent post-operative wound infections to a minimum.

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