DOI: 10.7860/JCDR/2015/15342.6698

Original Article

Microbiology Section

Bacteriological Profile of Surgical Site Infections and Their Antibiogram: A Study From Resource Constrained Rural Setting of Uttarakhand State, India

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ABSTRACT

Introduction: Surgical site infections (SSI) constitute a major public health problem worldwide and are the second most frequently reported nosocomial infections. They are responsible for increasing the treatment cost, length of hospital stay and significant morbidity and mortality.

Aim: To determine the incidence of SSIs and the prevalence of aerobic bacterial pathogens involved with their antibiogram.

Materials and Methods: Samples were collected using sterile cotton swabs from 137 patients clinically diagnosed of having SSIs and were processed as per standard microbiological techniques. Antimicrobial susceptibility testing was done using modified Kirby-Bauer disc diffusion method. This cross sectional study was conducted for a period of six months (January 2013 to June 2013) in the Department of Microbiology at a rural tertiary care hospital of Uttarakhand state, India.

Results: Out of 768 patients, 137 (17.8%) were found to have SSIs and samples were collected from them. Out of total 137 samples, 132 (96.4%) yielded bacterial growth and 139 bacterial isolates were obtained. *Staphylococcus aureus* (50.4%) was the commonest organism followed by *Escherichia coli* (23.02%), *Pseudomonas aeruginosa* (7.9%) and *Citrobacter* species (7.9%). Antimicrobial profile of gram positive isolates revealed maximum sensitivity to vancomycin, teicoplanin and linezolid, whereas among gram negative isolates meropenem, piperacillin-tazobactam, and amikacin were found to be most sensitive.

Conclusion: The rate of SSI observed in this study was comparable to other similar studies, however we observed a higher degree of antimicrobial resistance. Adherence to strict infection control measures, maintenance of proper hand hygiene and optimal preoperative, intraoperative and postoperative patient care will surely reduce the incidence of SSIs.

Keywords: Antimicrobial resistance, Infection control, MRSA, Nosocomial infection, Post operative wound

INTRODUCTION

Surgical site infections (SSI), one of the most common causes of nosocomial infections are a common complication associated with surgery, with a reported incidence rates of 2-20% [1]. They are responsible for increasing the treatment cost, length of hospital stay and significant morbidity and mortality. Despite the technical advances in infection control and surgical practices, SSI still continue to be a major problem, even in hospitals with most modern facilities [2]. These infections are usually caused by exogenous and/or endogenous micro organisms that enter the operative wound either during the surgery (primary infection) or after the surgery (secondary infection). Primary infections are usually more serious, appearing within five to seven days of surgery [3]. Majority of SSIs are uncomplicated involving only skin and subcutaneous tissue but sometimes can progress to necrotizing infections. The usual presentation of infected surgical wound can be characterized by pain, tenderness, warmth, erythema, swelling and pus formation [4,5] . A number of patient related factors (old age, nutritional status, pre existing infection, co-morbid illness) and procedure related factors (poor surgical technique, prolonged duration of surgery, pre operative part preparation, inadequate sterilization of surgical instruments) can influence the risk of SSIs significantly [2]. In addition to these risk factors, the virulence and the invasiveness of the organism involved, physiological state of the wound tissue and the immunological integrity of the host are also the important factors that determine whether infection occurs or not [6].

Bacteriological studies have shown that SSIs are universal and the etiological agents involved may vary with geographical location, between various procedures, between surgeons, from hospital to hospital or even in different wards of the same hospital [2]. In the recent years there has been a growing prevalence of gram negative organisms as a cause of serious infections in many hospitals. In addition irrational use of broad spectrum antibiotics and resulting anti microbial resistance (AMR) has further deteriorated the condition in this regard. The problem gets more complicated in developing countries due to poor infection control practices, overcrowded hospitals and inappropriate use of antimicrobials.

This study aimed to determine the incidence of SSIs and the prevalence of aerobic bacterial pathogens involved with their antibiogram.

MATERIALS AND METHODS

This cross-sectional study was conducted for a period of six months (January 2013 to June 2013) in a rural tertiary care hospital of Uttarakhand state, India. Prior to the sample collection, approval from Institutional Ethical Committee was obtained. The study population included 137 patients suffering from SSIs in the various surgical wards (orthopedic, general surgery, ophthalmology, obstetrics and gynecology, otorhinolaryngology) of our hospital. Patients of both sex, age > 14 years, who had surgical wound pus discharge, with serous or seropurulent discharge and with signs of sepsis present concurrently (warmth, erythema, induration, tenderness, pain, raised local temperature) were included. Patients who had suture abscesses, wounds with cellulitis and no drainage were excluded from the study. A detailed history regarding age, sex, type of illness, diagnosis, type and duration of surgery performed, antibiotic therapy and the associated co-morbid diseases was obtained from the patients.

Using sterile cotton swabs, two pus swabs/ wound swabs were collected aseptically from each patient suspected of having SSI. Gram stained preparations were made from one swab for provisional diagnosis. The other swab was inoculated on 5% sheep blood agar (BA) and Mac Conkey agar (MA) plates and incubated at 37°C for 48 hours before being reported as sterile. Growth on culture plates was identified by its colony characters and the battery of standard biochemical tests [7,8]. Antimicrobial sensitivity testing (AST) was carried out by modified Kirby Bauer disc diffusion method on Muller Hinton agar and results were interpreted in accordance with Clinical Laboratory Standards Institute guidelines [9]. Methicillin resistance was detected by taking cefoxitin (30µg) as a surrogate marker and was confirmed by using PBP2a latex agglutination test (Oxoid Ltd, Hampshire, UK). *Staphylococcus aureus* - ATCC 25923, *Escherichia coli* - ATCC 25922 and *Pseudomonas aeruginosa* - ATCC 27853 were used as control strains for AST. All dehydrated media, reagents and antibiotic discs were procured from Hi Media Laboratories Pvt. Ltd., Mumbai, India.

RESULTS

From January 2013 to June 2013 a total of 768 patients underwent the major surgeries in various departments of our hospital, out of which 137 patients (17.8%) were clinically diagnosed of having SSIs. Out of the total 137 samples processed, 132 (96.4%) yielded aerobic bacterial growth and a total of 139 bacterial isolates were obtained. Monomicrobial growth was seen in 125 samples while seven samples showed polymicrobial growth. The mean age of the patients was 43.8 years (range 14 to 85 years) and the peak incidence of SSI was observed in age group > 50 years (51.8%). [Table/Fig-1] shows the age wise distribution of various morphotypes in SSIs. Males (74.6%) were more commonly affected than females (25.5%) and the sex ratio male: female was 2.9:1.

Among the 139 bacterial isolates, *S. aureus* (50.4%) and *E. coli* (23.02%) were the commonest organisms. [Table/Fig-2] depicts the characterization of various bacterial isolates obtained from patients with SSIs. Antimicrobial susceptibility testing was carried out for all 139 isolates and the results are depicted in [Table/Fig-3]. *Staphylococcus aureus* strains showed a high degree of resistance for ampicillin (85.7%). Methicillin resistance was seen in 15.7% of all the *S.aureus* isolates. Gram negative isolates showed even higher rate of resistance and commonly prescribed agents like gentamicin, cotrimoxazole and ciprofloxacin were found resistant for most of the gram negative isolates. Meropenem showed good activity against most of the gram negative isolates, except for *P. aeruginosa* strains which showed high resistance for meropenem (72.7%) also.

Age (years)	Morphotypes			Total
	Monomicrobial	Polymicrobial	Sterile	
14 – 20	5	1	3	9
21 – 30	8	0	0	8
31 – 40	19	0	2	21
41 – 50	25	3	0	28
>50	68	3	0	71
Total	125	7	5	137

[Table/Fig-1]: Age wise distribution of various morphotypes in surgical site infections

Organism	No. of isolates (%) (n=139)
Staphylococcus aureus MSSA MRSA	70 (50.4) 59 (84.3) 11 (15.7)
Escherichia coli	32 (23)
Pseudomonas aeruginosa	11 (7.9)
Citrobacter species *Citrobacter freundii* *Citrobacter koseri*	11 (7.9) 7 (63.6) 4 (36.4)
Acinetobacter species *Acinetobacter baumannii* *Acinetobacter lowfii*	7 (5.0) 5 (71.4) 2 (28.6)
Klebsiella species *Klebsiella pneumoniae* *Klebsiella oxytoca*	4 (2.9) 1 (25) 3 (75)
Proteus species *Proteus mirabilis* *Proteus vulgaris*	4 (2.9) 2 (50) 2 (50)

[Table/Fig-2]: Characterization of various bacterial isolates obtained from patients with surgical site infections
MSSA : Methicillin sensitive *Staphylococcus aureus*;
MRSA: Methicillin resistant *Staphylococcus aureus*

DISCUSSION

Despite the advances in surgical techniques and better understanding of the pathogenesis of wound infection, management of SSIs remains a significant concern for surgeons and physicians in a health care facility. Patients with SSIs face additional exposure to microbial populations circulating in a hospital set up which is always charged with microbial pathogens. The unrestrained and rapidly spreading resistance to the available array of antimicrobials further contributes to the existing problem. Most of the SSIs are hospital acquired and vary from hospital to hospital. The rate of SSIs has been reported to be 2.5% to 41.9% [10]. In the present study the overall rate of SSI was 17.8% which was in concordance with the study conducted by Satyanarayana et al., who reported the overall rate of SSI as 13.7% in their study [11]. Various other studies from India have shown the rate of SSI to vary from 6.1% to 38.7% [10,12-14] . However in comparison to the Indian hospitals the rate of infection reported from other countries is quite low, for instance in USA it is 2.8% and in European countries it is reported to be 2-5% [11]. The lack of attention towards the infection control measures, inappropriate hand hygiene practices and over crowded hospitals can be the major contributory factors for high infection rate in Indian hospitals.

The predominance of male patients was seen in this study with male: female ratio of 2.9:1and this finding was in contrast to the other studies where a much higher number of female patients have been reported [4,5]. The patients with age >50 years had a higher incidence of SSI (51.8%) in comparison to an incidence of 12.4% among the patients who were ≤30 years of age. Advancing age is an important factor for the development of SSIs, as in old age patients there is low healing rate, low immunity, increased catabolic processes and presence of co-morbid illness like diabetes, hypertension, etc [13]. Regarding the duration of the operation a prolonged time was found to be a significant risk factor for SSI and it was observed that as the order and the duration of surgery increased, the rate of infection also increased.

Staphylococcus aureus, gram positive cocci, is a major human pathogen and a predominant cause of SSIs worldwide with a prevalence rate ranging from 4.6% to 54.4% [14]. In the present study predominance of *S. aureus* (50.4%) was seen and this finding was consistent with reports from other studies [5,10,12,15,16]. Infection with *S. aureus* is most likely associated with endogenous source as it is a member of the skin and nasal flora and also with contamination from environment, surgical instruments or from hands of health care workers [16,17]. *S. aureus* was the single predominant gram positive bacterial isolate obtained in this study. Special interest in *S. aureus* SSI is mainly due to its predominant role in hospital associated infection and emergence of methicillin resistant *S.aureus* (MRSA) strains. In our study methicillin resistance was seen in 15.7% of *S.aureus* isolates. This finding was in tandem with the study conducted by Aggarwal et al., [18], who reported methicillin resistance in 10% of the isolates, however it is in contrast with the study conducted by Kownhar et al., [15] who reported

Antibiotics	Organisms *						
	S. aureus (n=70)	*E. coli* (n=32)	*P. aeruginosa* (n=11)	*Citobacter* spp.(n=11)	*Acinetobacter* spp. (n=7)	*Klebsiella* spp.(n=4)	*Proteus spp.* (n=4)
AMP	85.7	81.3	100	100	85.7	100	100
AMC	34.3	81.3	81.8	100	71.4	100	75
AMK	8.6	15.6	45.5	18.1	14.3	0	25
AZT	NT	81.3	72.7	100	71.4	100	75
CFP	NT	81.3	NT	100	71.4	100	75
CFT	NT	NT	72.7	NT	NT	NT	NT
CTR	22.9	43.8	81.8	36.4	28.6	50	50
CFX	15.7	NT	NT	NT	NT	NT	NT
CIP	32.9	40.6	90.9	27.3	28.6	25	50
GEN	17.1	46.9	81.8	45.5	57.1	25	50
LNZ	0	NT	NT	NT	NT	NT	NT
MRP	NT	12.5	72.7	9.1	0	0	0
PIP	NT	NT	45.5	NT	NT	NT	NT
PTZ	NT	12.5	18.1	9.1	14.3	25	0
TEC	0	NT	NT	NT	NT	NT	NT
VAN	0	NT	NT	NT	NT	NT	NT
Chi square test	x^2=248.046 df= 9 p =0.001	x^2 =102.867 df= 9 p =0.001	x^2 =29.355 df= 10 p =0.001	x^2 =66.000 df= 9 p =0.001	x^2 =20.696 df= 9 p =0.014	x^2 =25.185 df= 9 p =0.003	x^2 =16.000 df= 9 p =0.067

[Table/Fig-3]: Antibiotic sensitivity pattern of aerobic bacterial isolates in surgical site infections
*Sensitivity pattern shown in the table is the percentage of isolates resistant to the antibiotic. Intermediately sensitive isolates were considered as resistant.
AMP: ampicillin; AMC: amoxicillin-clavulanate; AMK: amikacin; AZT: aztreonam; CFP: cefotaxime; CFT: ceftazidime; CTR: cotrimoxazole; CFX: cefoxitin; CIP: ciprofloxacin; GEN: gentamicin; LNZ: linezolid; MRP: meropenem; PIP: piperacillin; PTZ: piperacillin-tazobactam; TEC: teicoplanin; VAN: vancomycin; NT: not tested.

higher incidence of 21.7%. Still higher incidences of 45% and 58.2% of MRSA have been documented by Eagye et al., [19] and Kaye et al., [20] respectively. We found that all the *S. aureus* strains (irrespective of methicillin resistance) were sensitive to vancomycin, teicoplanin and linezolid. This finding can be of relevant clinical use for the formulation of antibiotic policy of our hospital.

Gram negative isolates comprised of 49.6% of all the aerobic bacterial isolates. *E. coli* (46.4%) was the commonest gram negative bacteria isolated followed by *P. aeruginosa* (15.9%) and *Citrobacter spp* (15.9%). Similar observations have been reported by various other authors also [4,5,14]. Few studies have reported *P.aeruginosa* as the most frequent isolate in SSI [6,21] which remains a third most isolated strain in this study. Presence of enteric organisms could be attributed to the patient's normal endogenous microbial fecal flora [10] *E. coli* invasion of the wound is a clear case of poor hospital hygiene.

Antibiotic susceptibility results revealed that a high degree of resistance was seen for majority of the bacterial isolates. For gram positive bacteria vancomycin, teicoplanin, linezolid and amikacin were found to be the most effective antibiotics. The degree of resistance was even higher among the gram negative bacteria and the commonly used drugs were found to be more resistant with an average resistance range from 50% to 100%. Meropenem, piperacillin-tazobactam, and amikacin were found to be the most effective antimicrobial agents whereas ampicillin, amoxicillin-clavulanate and cefotaxime were among the most resistant drugs. *P.aeruginosa* strains isolated in the present study were found highly resistant in comparison to the previous studies [6,5]. The development and spread of resistant bacterial strains has emerged as a global problem. The appearance of multi drug resistant (MDR) strains over the past decades has been regarded as an inevitable genetic response to the strong selective pressure imposed by antimicrobial chemotherapy which plays a crucial role in evolution of antibiotic resistant bacteria.

All cases in our study received prophylactic antimicrobials prior to the surgery. Current recommendations for antimicrobial prophylaxis to prevent SSI advise that an antimicrobial agent be administered within 60 minutes prior to surgery and discontinued soon afterward [22]. However, more than 50% of our patients received preoperative antimicrobials more than six hours before surgery and almost all patients were treated with antimicrobials after surgery. Many of them were even treated until the day of discharge in an attempt to prevent infection while they were hospitalized. The most widely used combination was a third generation cephalosporin and an aminoglycoside. However, the antimicrobial susceptibility results showed that the isolated bacterial strains were mostly resistant to these agents. Invariably the maximum resistance was observed for ampicillin by nearly all the isolates and this was found to be statistically significant for all except *Proteus* species [Table/Fig-3]. The frequent empirical prescription of these antimicrobials as a treatment and prophylaxis in our hospital might have contributed for observed high degree of resistance. This situation raises a serious concern and calls for immediate revision of antibiotic policy and antibiotic prescribing guidelines.

The rate of SSI observed in this study was comparable to other similar studies carried out in developing countries including India; however the bacterial isolates detected from our patients showed a high degree of resistance for commonly prescribed antimicrobials in our setup. Although the studies with bigger sample size are sought to make it statistically more relevant.

Inspite of the modern surgical and sterilization techniques and the use of prophylactic antimicrobials, SSIs still continue to pose an important clinical challenge. They impose a substantial burden of disease both on patients as well as healthcare services in terms of pharmacotherapeutic and pharmacoeconomic losses. Although SSIs cannot be completely eliminated, but reduction of the rate of infection to minimal can have significant benefits by reducing the wastage of healthcare resources, patient morbidity and mortality. This can be achieved by optimal preoperative, intraoperative and postoperative patient care. Also there is good evidence that attention to multiple patient related and procedure related risk factors can decrease the risk of SSIs significantly. This would be supplemented with proper infection control measures and a sound antibiotic policy which will surely result in better patient care, safety and healthcare outcomes.

LIMITATION

The limitation of our study was that, anaerobic bacterial profile and fungal cultures were not done on the wound swabs obtained from SSIs. Further prospective studies can be undertaken in this regard.

CONCLUSION

To establish the most suitable empirical treatment for each patient, it is very important to know the microbial epidemiology of each institution. The information obtained from this study allows a better understanding of the microbial etiology of SSIs in our hospital which may have epidemiological and therapeutic implications. Using the results of this study, an initiative for establishing improved hospital antimicrobial policy and antimicrobial prescribing guidelines should be undertaken. Also the inappropriate and prolonged use of antibiotics should be avoided as this can lead to the development of resistant micro organisms which are even more difficult to get rid of.

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FINANCIAL OR OTHER COMPETING INTERESTS: None.

Date of Submission: **Jun 23, 2015**
Date of Peer Review: **Jul 07, 2015**
Date of Acceptance: **Aug 06, 2015**
Date of Publishing: **Oct 01, 2015**