

Bacteriological Profile of Post-Operative Wound Infections and Their Antimicrobial Resistance Pattern in a Tertiary Care Hospital

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ABSTRACT

Introduction: Hospital acquired infection (HAI) are the common source of infection in hospital set up especially surgical site infection (SSI) which is common after surgery. Various factors responsible for SSI are host factors like patient's immunity and socio-economic status, infecting micro-organisms, different surgical procedures, and hospital environment. It may lead to morbidity and mortality and increase the cost of hospital stay.

Aim: The present study was conducted to know the bacteriological profile of post-operative wound infections and their antimicrobial resistance profile in a tertiary care hospital set up.

Material and Methods: The present study was conducted with a total of 521 pus samples collected in two sterile swabs. One for direct gram staining and other for culture. Pus sample was processed on Blood agar and MacConkey agar and incubated overnight under aerobic conditions and further identification was done using biochemical reactions. Antibiotic sensitivity testing was performed using Kirby-Bauer disc diffusion method.

Results: The wound infection rate was 16.3% (85/521) in the present study. Staphylococcus aureus (35.5%) was the most predominant micro-organism isolated followed by Escherichia coli (18.8%), Pseudomonas aeruginosa (17.6%), Coagulase Negative Staphylococcus spp. (14.1%), Klebsiella spp. (7%), Acinetobacter spp. (3.5%), and Proteus vulgaris (2.3%) and Candida spp. (1.2%).

Discussion: In the present study, we have seen that vancomycin and linezolid were the sensitive drugs for Staphylococcus spp. whereas carbapenems and beta-lactamase inhibitors were promising in gram negative bacteria.

Conclusion: The bacteriological profile of surgical site infection varies from location to location depending upon infection control practices and environmental factors. The measures should be taken to reduce the SSI and generate antibiotic policy for preventing multidrug resistance micro-organisms. Thereby, reducing morbidity and mortality of post-operative wound patients.

Key words: Staphylococcus aureus, Hospital acquired infection, Post-operative wound, Surgical site infection.

HOW TO CITE THIS ARTICLE: Anjali Agarwal, Jyoti Srivastava, Seema Bose,Bacteriological Profile of Post-Operative Wound Infections and Their Antimicrobial Resistance Pattern in a Tertiary Care Hospital, J Res Med Dent Sci, 2021, 9(6): 228-233

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INTRODUCTION

Surgical site infection (SSI) accounts for one of the common hospital acquired infections (HAI) occurring both in developed and developing countries [1-4]. Surgical site infection is a post-operative wound after surgery but not the trauma wound. SSI is defined as infection occuring after invasive surgery usually within 30 days of surgery without implant [5]. As per CDC, surgical wounds can be classified as Class I-Clean; Class II-Clean contaminated; Class III-Contaminated; Class IV-Dirty [1,5]. There are three types of surgical site infection are defined by CDC-Incisional and Organ/Space. Incisional can be subdivided into Superficial (affecting skin and subcutaneous tissues) and deep (affecting soft tissues) [6].

In India, the prevalence rate of SSI ranges from 2% to 40% which depends on various factors involved affecting the harmony between agent, host and environment [1,2,7-10].

Variable factors are responsible for occurrence of surgical site infection like pre-op care, duration and type of surgery, post-op care, patient immunity, microbial factors, hospital environment and infection control practices followed [1,2,7,8]. Microbial factor also plays a crucial role in post-operative wound infection as multi-drug resistant strains are increasing nowadays which accounts for severity of infection and delayed wound healing [2].

Surgical site infection leads to morbidity and mortality if not identified and treated in time, prolongs the hospital stay further adding to socio-economic burden to the patient care and treatment [2,8,11]. Most of the surgical site infection as preventable if proper aseptic measures are adopted and infection control practices carried out in hospitals play an important role in preventing and reducing SSI [4,12].

The aim of the present study is to determine the bacteriological profile of surgical site infections and their antimicrobial resistance pattern occurring in our tertiary care hospital setup. It can help suggest clinicians to start empirical treatment before elective surgery to reduce and prevent post-operative wound infection. It further reduces the burden of disease and promote health care.

MATERIAL AND METHODS

The present study is a prospective cross-sectional study carried out in the Bacteriology laboratory in the department of Microbiology, Hind Institute of Medical Sciences, Barabanki. The duration of the study ranges from 1st June to 31st December 2019.

A total of 521 samples were collected for the study from post-operative patients admitted in all the surgical wards of various departments in our hospital. The collected samples were transported to the laboratory, and if there was a delay, the samples were stored at 4oC till further processing.

Inclusion criteria

- Elective surgery.
- Patients with age ≥ 18 yrs.

Exclusion criteria

- Emergency surgery(trauma) and Burn patients.
- Patients with age <18yrs.

Ethical clearence

Patient's consent was taken on an Informed Consent form. The study was ethically approved by the Institutional Ethical Committee (IEC).

Sample processing

Pus samples were collected in two sterile swabs. One swab for gram staining (Direct microscopy) and one for culture. If frank discharge, it was collected in a wide mouth sterile container. In the laboratory, first swab after direct gram staining was visualized under oil field immersion(1000x) magnification to identify epithelial cells, pus cells and micro-organisms. The second swab was cultured on Blood agar and MacConkey agar and incubated overnight at 37oC under aerobic conditions. Samples with growth on Blood agar and MacConkey were further processed and identification was done by colony morphology and biochemical reactions as per standard protocol [13].

Antibiotic sensitivity test was performed using Kirby Bauer disc diffusion method on Muller Hinton agar (Himedia) and organism was reported as susceptible or resistant to the antibiotics as per CLSI guidelines 2018 [14]. The antibiotics used (potency in μ g/disc; Himedia) for Gram negative bacteria were ampicillin (10), gentamicin (10), amikacin (30), ceftazidime (30), ceftriaxone (30), cefepime (30), amoxicillin-clavulanate (10/10), piperacillin-tazobactam (100/10), ciprofloxacin (5), levofloxacin (5), imipenem (10), meropenem (10), ertapenem (10).

For Gram positive bacteria, penicillin(10Units), cefoxitin (30), erythromycin(15), clindamycin(2), vancomycin(30), linezolid (30).

Statistical analysis

All the data was entered in an Excel sheet and the data was presented in the form of tables and charts. The results were statistically analysed using Advanced excel software. p value<0.05 was considered statistically significant.

OBSERVATIONS AND RESULTS

Out of 521 non-duplicate pus samples collected from wards of surgical departments, 85 are culture positive. Depending upon the demographic factors like age, sex etc. the samples are described as in Table 1. It has been found that males (62.3%) have more culture positive wound infection compared to females (37.6%). According to the age, it is seen that wound infection is common between 45-60yrs (50.6%) and >60 yrs(29.4%) of age compared to 18-45 years of age (20%).

In our study, in culture we have seen that gram positive organism predominates gram negative microorganism. The bacteriological profile of micro-organims is as follows Staphylococcus aureus (35.3%) is the most micro-organism isolated followed common by Escherichia coli (18.8%), Pseudomonas aeruginosa (14.1%), Klebsiella spp. (7.05%), (17.6%), CONS Acinetobacter spp.(3.5%) and Proteus vulgaris(2.3%).One sample showed Candida spp. (1.2%) (Figure 1).



Figure 1: Distribution of micro-organisms in postoperative wound infection.

The antibiotic sensitivity profile for gram positive bacteria in their ascending order for Staphylococcus aureus is Penicillin (33%), Erythromycin (50%), Ciprofloxacin (73.3%), Cefoxitin (73.3%), Clindamycin (80%), Gentamicin (87%), Ampicillin & Levofloxacin (100%) also Vancomycin and Linezolid (100%). For CONS, Penicillin (25%), Erythromycin (50%), Clindamycin (58.3%), Cefoxitin (66.7%), Genatmicin & Ciprofloxacin (83.3%), Levofloxacin (91.6%), Ampicilin, Vancomycin & Linezolid (100%) as described in Table 2.

The antibiotic sensitivity profile for gram negative bacteria in their descending order is as follows-Meropenem, Imipenem & Ertapenem (80-100%) showed high sensitivity to all the gram negative bacteria (both fermenters and non-fermenters) as described in detail in Table 3. They also showed moderate to high sensitivity to Beta-lactamase inhibitors like Amoxicillinclavulanate(80-100%), Cefoperazone-sulbactam Piperacillin-tazobactam (67-100%), (67-100%)followed by aminoglycosides like Gentamicin and Amikacin ((50-80%). Among fluoroquinolones, Levofloxacin (80-100%) is more sensitive than Ciprofloxacin (25-67%). High resistance was seen in third & fourth generation cephalosporins like Ceftriaxone, Ceftazidime and Cefepime (17-67%).The sensitivity to Ampicilin varies from 24% to 100%.

Table 1: Demographic features in post-operative wounds.

Variables	Culture positive n=85	Culture negative n=436	Total samples	p value*		
		Based on sex				
Male	53(62.3%)	309(70.8%)	362	0.118		
Female	32(37.6%)	127(29.1%)	159			
Based on age (in years)						
>18-45	17(20%)	139(31.8%)	156	0.089		
>45-60	43(50.6%)	184(42.2%)	227			
>60	25(29.4%)	113(25.9%)	138			
*Chi-square test is performed & p value<0.05 is considered as statistically significant.						

Table 2: Antibiotic sensitivity profile of gram-positive bacterial isolates.

Drugs	S. aureus (n=30)	CONS (n=12)
Penicillin	33.30%	25%
Ampicillin	100%	100%
Cefoxitin	73.30%	66.70%
Erythromycin	50%	50%
Clindamycin	80%	58.30%
Gentamicin	87.70%	83.30%
Ciprofloxacin	73.30%	83.30%
Levofloxacin	100%	91.60%
Vancomycin	100%	100%
Linezolid	100%	100%

Table 3: Antibiotic sensitivity profile of gram-negative bacterial isolates.

Drugs	Escherichia coli (n=16)	Klebsiella spp (n=6)	Proteus spp (n=2)	Acinetobacter spp (n=3)	Pseudomonas spp (n=15)
Ampicillin	24%	33%	100%	66.70%	100%
Ceftriaxone	25%	17%	50%	66.70%	60%
Ceftazidime	25%	17%	50%	66.70%	60%
Cefepime	69%	33%	50%	66.70%	60%
Amoxicillin-Clavulanate	81%	100%	100%	100%	100%
Cefoperazone-Sulbactam	69%	67%	100%	100%	93%

Piperacillin-Tazobactam	75%	67%	100%	66.70%	74%
Amikacin	75%	67%	50%	66.70%	80%
Gentamicin	69%	50%	50%	66.70%	80%
Ciprofloxacin	25%	33%	50%	66.70%	67%
Levofloxacin	81%	100%	100%	100%	100%
Ertapenem	81%	84%	100%	100%	100%
Meropenem	100%	84%	100%	100%	100%
Imipenem	100%	84%	100%	66.70%	100%

DISCUSSION

In the present study, the prevalence rate of SSI is 16.3% (85/521) which is similar to other studies conducted in India like 16% in western India [10], 14.5% in Rajasthan [8], 11.7% in Gujarat [2], it is higher than some studies like Shahane et al. which showed 6% prevalence[1] and 10% [16]. Also some studies had higher prevalence like 39% where the study was conducted in rural setup [9] and 21.6% in Mysore [15].

The rate of SSI varies from type of surgery, duration of surgery, emergency or elective surgery, skill and experience of surgeon, operative location, rural or urban centres and so on along with hospital infection control practices [4,9,10].

In our study, we have seen that wound infections are more common in males (62.3%) compared to females (37.6%), although it is not statistically significant (p=0.118) (Table1). Other studies also reported male predominance of wound infection [1,9,10,15]. The reason may be more tobacco consumption, smoking, blood transfusion etc. in males [9,17,18]. However, sex determination is not the pre-determinant factor for SSI [19]. Female preponderance is seen in a study in Aligarh [20].

The present study showed that the infection rate is higher among age group between >45-60 yrs. (50.6%) compared to age group >60 yrs. of age (29.4%) and >18 yrs. to 45 yrs. (20%), it is statistically insignificant(p=0.08) (Table1). It is also seen in other studies conducted where the maximum number of patients were from 5th and 6th decade of age [2]. Also, other studies like Setty et al, Mekhla et al and Patel et al showed more incidence in >50yrs of age. This shows that incidence of SSI increases with the increasing age. The reason may be the poor immune system and comorbidities associated with old age [9,10,15].

In our study, gram positive micro-organisms and gram negative are equal in number in gram staining so no predominance can be suggested on this basis. Although the most common micro-organism isolated varies in different hospital settings depending upon the different nosocomial pathogens and commensals.

In our setup, the most common micro-organism isolated from post-operative wound in our study is *Staphylococcus aureus* (35.3%) followed by *Escherichia coli* (18.8%),

Pseudomonas aeruginosa (17.6%), CONS (14.1%), *Klebsiella* (7.05%), *Acinetobacter* (3.5%) and Proteus vulgaris (2.3%). Other studies also support our findings with *Staphylococcus aureus* as the commonest pathogen [2,8,15,21]. Studies suggest that as *Staphylococcus aureus* is a colonizer in anterior nares of healthy individual it may be responsible for SSI [11]. There are studies where *Escherichia coli* is the most common micro-organism isolated may be due to endogenous flora [1,10,21,23].

In our study, we have seen Pseudomonas aeruginosa (17.6%) also contributing to post-operative wound infection. Some studies have shown it to be the most common micro-organism among gram negative bacteria followed by *Escherichia coli*.[3] Pseudomonas is common in hospitals where there is poor hygiene maintenance and relaxed infection control practices.

In many cases, it is found that the pathogenic organism is usually the commensal flora of skin,mucous membrane and hollow viscera like gastro-intestinal tract leading to gram positive, gram negative and anaerobic microorganism predominance depending upon the site and type of surgery [11,24]. Pre-operative antibiotics plays an important role in reducing the risk of surgical site infection [19].

We have one isolate having Candida species (1.2%) It has been found in a study that yeast also forms an important risk of surgical site infection [11].

Antimicrobial resistance plays an important role in controlling hospital acquired infection (HAI). In the present study, *Staphylococcus aureus* showed complete sensitivity to vancomycin and linezolid [8]. All *Staphylococcus aureus* are 100% sensitive to ampicillin and levofloxacin contrary to Narula et al where they are completely resistant and Setty et al showed 61% resistance to ampicillin.[8,15]We have seen nearly 80% sensitivity to gentamicin & clindamycin similar to another study whereas penicillin showed poor efficacy with sensitivity only 33.3% as also discussed in a study. [1] *S. aureus* showed 50% resistance to erythromycin and 28% resistance to ciprofloxacin whereas another study showed 72% resistance to erythromycin and 47% resistance to ciprofloxacin in Setty et al. [15].

Eight isolates (26.7%) were methicillin resistance *Staphylococcus aureus* (MRSA), other studies also showed

high percentage of MRSA in cases of surgical site infection like 43.75% MRSA [8] and 44% MRSA [11,21].

In Coagulase negative *Staphylococcus spp* (CONS), we have seen 25% sensitivity to penicillin. There are 4 isolates with methicillin resistance (33%) in our study whereas another study reported 84% MRCONS [21]. It has been found that nearly 50% sensitivity is reported to erythromycin and clindamycin whereas 80 to 90% sensitivity to gentamicin, ciprofloxacin, and levofloxacin. All the CONS isolates were completely sensitive to vancomycin and linezolid.

Among gram negative bacteria, *Escherichia coli* is the predominant micro-organism with complete sensitivity to meropenem and imipenem and 80% sensitivity to ertapenem which is similar to other studies [8,16]. Our study showed moderate sensitivity to amikacin (75%) and gentamicin (69%) similar to some other studies [1,16].

Among the beta-lactam group, cephalosporins and fluoroquinolones, Escherichia coli showed high resistance upto 75% to 80%. Similar findings have been observed in other studies also [1]. In a study in Rwanda,53.3% resistance was reported to ceftriaxone. These are the drugs which are most used in prophylaxis before the surgical procedure to be conducted [16]. These group of drugs are becoming resistant may be due to the indiscriminate use of these antibiotics and increasing prevalence of ESBL producing micro-organisms. It can be further supported by findings in our study that combination drugs like amoxicillin-clavulanate (81%), cefoperazone-sulbactam (69%), piperacillin-tazobactam (75%) showed high sensitivity to Escherichia coli compared to third and fourth generation cephalosporins. Several studies support our finding that there is increase in multidrug resistance micro-organism causing postoperative wounds [1,8,22]. There are also studies where increasing resistance to amoxicillin-clavulanate (98.8%) is found [16].

In the present study, *Klebsiella spp.* showed higher sensitivity to levofloxacin and carbapenems, however ciprofloxacin showed 33% sensitivity to the microorganism whereas in another study, ciprofloxacin and levofloxacin are 63% sensitive [2].

Proteus vulgaris showed high sensitivity to betalactamase inhibitors, carbapenems and levofloxacin and moderately sensitivity to third generation cephalosporins, aminoglycosides and ciprofloxacin like another study [2].

Pseudomonas aeruginosa showed 67% sensitivity to ciprofloxacin and 100% sensitivity to levofloxacin whereas in a study by Nutanbala et al, P. aeruginosa showed good sensitivity to ciprofloxacin (83.8%) and low to levofloxacin (43%). We have seen 40% resistance to cephalosporins and 20% resistance to aminoglycosides. Another study showed nearly 60-70% resistance to third & fourth generation cephalosporins and 50-70% resistance to amikacin and gentamicin [3]. In our study, Pseudomonas aeruginosa are completely sensitive to

carbepenems like meropenem, imipenem & ertapenem like a study [1] whereas other studies showed 50% resistance to meropenem and 25- 30% resistance for meropenem and imipenem [2,3]. Carbapenems are the reserve drugs and should be used judiciously for the multidrug resistance micro-organisms [3].

We have isolated Acinetobacter spp. (3.5%) in nontrauma surgical patients with post-operative wound. One of the studies have shown Acinetobacter spp as the most common micro-organism among gram negative bacteria having multi-drug resistance [25]. In our study also, antibiotic resistance is a bit higher in non-fermenters compared to fermenters like Enterobacteriaceae family members.

LIMITATION

Due to the short duration of study, we could not study the underlying factors like diabetes, blood transfusion, smoking etc. associated with surgical site infection and their impact on the delayed wound healing.

CONCLUSION

The prevalence of surgical site infection is multifactorial depending upon several factors like infection control practices, hospital environment, surgery performed, patient's immunity and many others. In the present study, we tried to focus on the bacteriological profile and their antibiotic resistance pattern in our hospital setup which helps generate antibiotic policy and choose the appropriate drugs for empirical therapy by clinicians in cases of post-operative wound infection. Among gram positive organisms, vancomycin and linezolid are the promising drugs in surgical site infections and for gram negative organisms, beta- lactamase inhibitor drugs and carbapenems play an important role.

Due to the multidrug resistance, only fewer drugs are left for the treatment of surgical site infections. Multidrug resistance (MDR) micro-organisms are difficult to treat which delays the wound healing. To reduce the MDR micro-organisms, irrational use of antibiotics should be prevented. The empirical treatment should be replaced by specific treatment after getting culture and sensitivity report. Periodic surveillance studies should be conducted to update the changing pattern of predominance of micro-organism and their antimicrobial drug resistance profile. It is emphasized that measures should be taken to minimise the hospital acquired infection (HAI) by modifying the infection control practices and conducting more awareness and better training programmes for health care workers.

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