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NOSOCOMIAL WOUND INFECTION AMONGST POST OPERATIVE PATIENTS AND THEIR ANTIBIOGRAMS AT TERTIARY CARE HOSPITAL IN INDIA.

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ABSTRACT

Nosocomial infection constitutes a major public health problem worldwide. Increasing antibiotic resistance of pathogens associated with nosocomial infections also becomes a major therapeutic challenge for physicians. Thus, the aim of this study was to identify post operative bacterial infections in the patients developing surgical site infections at a tertiary University hospital in North India during July 2013 to Dec 2013.

Methods: One hundred and ninety six swabs/pus specimens from various types of surgical sites suspected to be infected on clinical grounds were processed, by standard methods and antibiotic susceptibility testing of all the isolates was done by using Kirby Baur disc diffusion technique.

Results: Of the one hundred and fifty-eight organisms isolated, the most common was *Staphylococcus aureus* (27.8 %), followed by *Escherchia coli* (24.05 %), *Klebsiella pneumoniae* (13.29 %), Pseudomonas aeruginosa (6.32%), *Klebsiella oxytoca* (5%), *Enterococcus* (5.6%) and other miscellaneous gram negative rods (9.4%) and *Streptococcus pyogenes* (1.30%). About 50% of the Staphylococcus aureus isolates were found to be methicillin resistant. In case of *Escherichia coli*, more than one-third of the isolates were found to be ESBL producers. The resistance to third generation cephalosporins and the quinolone ciprofloxacin was also quite high. Other isolates also showed a very high level of antibiotic resistance.

Conclusion: In addition to the economic burden for antibiotic treatment, such infections for multi-resistant organisms are a serious threat to our surgical patients. To prevent these happenings, there is ar urgent need to adopt basic principles of asepsis and sterilization and to make judicious use of prophylactic and therapeutic antibiotics and determine current antimicrobial resistance to commonly prescribed drugs.

Keywords: Wound infection; microorganisms; anti-microbial sensitivity

INFECTIONS NOSOCOMIALES DE PLAIE PARMI LES PATIENTS POST-OPERATOIRES ET LEURS ANTIBIOGRAMMES A L'HOPITAL DU SOIN TERTIAIRE EN INDE

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Résumé

Contexte: les infections nosocomiales constituent un problème majeur de santé publique dans le monde. L'augmentation de la résistance aux antibiotiques de germes associés aux infections nosocomiales devient aussi un défi thérapeutique majeur pour les médecins. Ainsi, le but de cette étude était d'identifier les infections bactériennes post-opératoires chez les patients développant les infections au niveau du site chirurgical de centre hospitalier universitaire tertiaire au Nord de l'Inde de Juillet 2013 à Décembre 2013.

Méthodes: Cent quatre-vingt-seize (196) échantillons de prélèvements de pus de plusieurs types de sites chirurgicaux suspectés d'être infectés pour des raisons cliniques ont été traités par les méthodes classiques, et le test de sensibilité aux antibiotiques de toutes les souches a été fait selon la méthode de diffusion de disque de Kirby Baur.

Résultats: Sur cent cinquante-huit (158) germes isolés, le plus dominant était *Staphylococcus aureus* **(27,8 %), suivi de** *Escherichia coli* **(24,05%),** *Klebsiellapneumoniae***(13,29%),** *Pseudomonas aeruginosa* **(6,32%),** *Klebsiellaoxytoca* **(5%),** *Enterococcus* **(5,6%) et autre germes divers àGram négatif (9,4%) et***Streptococcus pyogenes* **(1,30%). Environ, 50% de souches de** *Staphylococcus aureus* **étaient résistantes à la méthillicine. Dans le cas de** *Escherichia coli***, plus d'un tiers (1/3) de souches étaient productrices de béta-lactamases (***BLSE***). La résistance aux céphalosporines de la troisième génération et à la ciprofloxacine était également assez élevée. Les autres souches ont aussi montré de résistance de haut niveau aux antibiotiques.**

Conclusion: En plus de charge économique de traitements aux antibiotiques, ces infections de souches multi-résistantes sont de menaces sérieuses pour nos patients opérés. Pour prévenir ces évènements, il y a un besoin urgent d'adopter les principes de base d'asepsies et destérilisation et de faire un usage judicieux des antibiotiques utilisés dans les traitements prophylactique et thérapeutique et déterminer la résistance antimicrobienne courante aux médicaments couramment prescrits.

Mots clés: infection de plaie post-opératoire; microorganismes; sensibilité aux antimicrobiens

INTRODUCTION

Despite an improved understanding of the patho-physiology, methods of prevention and prophylaxis and technological advances that have been made in surgery and wound management, surgical wound infections remain the most common cause of post operative morbidity and mortality1. A surgical wound may get infected by the exogenous bacterial flora which may be present in the environmental air of the operation theatre or by the endogenous flora2. Surgical wound infection remains one of the most important post-operative complications, accounting for 10 to 20% of the hospital costs. Although total elimination is not possible, a reduction in the infection rate to a minimal level could have significant benefits in terms of both the patient comfort and the medical resources which are used3. The rate of infection of the surgical wounds is influenced by the duration of the pre-operative hospitalization, administration of the prophylactic antibiotics, the duration of the surgery and by the fact as to whether the surgery was emergency or elective. Patient factors and environmental factors, both local and general, like age and nutritional status and preexisting illnesses also determine the final outcome. Postoperative wound infection can occur from first day onwards to many years after an operation but commonly occurs between the fifth and tenth days after surgery4. It may originate during the operation i.e. as a primary wound infection or may occur after the operation from sources in the ward or as a result of some complications i.e. secondary wound infection5.6 and can be characterized by various combinations of the signs of infection (e.g. pain, tenderness, warmth, erythema, swelling, drainage)4. Most post-operative wound infections are hospital acquired and vary from one hospital to the other and even within a given hospitals and they are associated with increased morbidity and mortality5.

The site of infection may be limited to the suture line or may become extensive in the operative site and the infecting microorganisms are variable, depending on the type and location of surgery, and antimicrobials. Surgical site infections (SSIs) which account17% of all health care-associated infections are the second most common HAIs next to urinary tract infections. They occur after approximately 3% of all operations and result in greater lengths of stay and additional costs6.

The emergence of poly antimicrobial resistant strains of hospital pathogens has also presented a challenge in the provision of good quality inpatient care7. The battle between bacteria and their susceptibility to drugs is yet problematic among public, researchers, clinicians and drug companies who are looking for effective drugs8. Therefore, the aim of this study was to isolate bacterial pathogens from hospital acquired surgical site infection and determine their current antimicrobial sensitivity patterns among patients who had clean and clean contaminated operations at MM Institute of Medical Sciences and Research Mullana, Ambala.

MATERIALS AND METHODS

Study Centre

The present study was conducted at a tertiary care university hospital and study centre in North India, between July 2013 and Dec 2013.The hospital has more than 5000 surgical patients in general surgery, orthopaedics and gynaecology wards per year and on average ten major operations are performed per day. In addition, the hospital accepts referred patients from different parts of the region.

*Clean Operations***:** a type of wound in which no inflammation is encountered and the respiratory, alimentary or genitourinary tracts are not entered and there is no break in aseptic operating procedure.

Clean-contaminated operations: a type of wound in which the respiratory, alimentary or genitourinary tracts are entered but without significant spillage (without visible contamination).

Contaminated operations: a type of wound where acute inflammation (without pus) is encountered, or where there is visible contamination of the wound. Examples include gross spillage from a hollow viscous during the operation or compound/open injuries operated within four hours.

Dirty Operations: a wound in the presence of pus, where there is a previously perforated hollow viscous or compound/open injury more than four hours old.

Postoperative nosocomial infection: a surgical site or blood stream infection occurring after 48 hours of operation until the time of discharge from hospital with clinical signs and symptoms and laboratory confirmation.

The Centre for Disease Control, (CDC), USA, classifies the surgical site infections into: (a) Superficial incisional SSI which involves only skin and subcutaneous tissue of incision, (b) Deep incisional SSI which involves deep soft tissues (e.g. fascia and muscle layer) of the incision, (c) Organ \Space SSI includes infection apparently related to the operative procedure and infection involves any part of the body, excluding skin incision, fascia, muscle layer that is operated or manipulated during operative procedure.

Patients

The study group included all the clean and clean contaminated surgeries which were conducted in this hospital during that period. Procedures in which healthy skin was not incised such as opening of an abscess, burn injuries and donor sites of split skin grafts and contaminated and dirty surgeries were excluded from the study. The samples were collected from those patients who showed an evidence of surgical wound infections like a serous, sanguineous or purulent discharge, soaked dressing or gaping wounds. Purulent materials were collected on sterile commercial cotton swabs aseptically and gently to avoid contamination of the specimens with normal microbial flora of the skin.

Specimens were collected before redressing and administration of antibiotic therapy. Specimens were labeled, kept in a vial and transferred immediately to the laboratory for bacteriological examination. Then, one of the wound swabs was inoculated on to Blood agar and MacConkey agar plate9. The inoculated agar plates were incubated aerobically at 37 °C overnight. The other wound swab was used for Gram staining smears to make presumptive diagnosis10.

The smear was screened for pus cells, the gram reaction, morphology, arrangement and number of types of the organisms and to select significant organism based on the quantitative measurements made on direct microscopy i.e. finding of bacteria on a given microscopic smear were taken as presence of 106 or more bacteria per swab which is reliably predicts a microbial load of >105 CFU/g of tissue11.

Antimicrobial susceptibility testing was performed using Kirby Bauer agar disc diffusion technique for the isolated pathogen12. A loop full of bacteria was taken from a pure culture colony and was transferred to a tube containing 5ml of phosphate buffer saline and mixed gently until it formed a homogenous suspension and the turbidity of the suspension was adjusted to the turbidity of McFarland 0.5 standard in a tube. The standardized inoculums of each isolate were inoculated on to Mueller-Hinton antibiotic sensitivity medium. Finally, all the isolates were tested for the antibiotic discs as per Clinical and Laboratory Standards Institute (CLSI) guidelines. Gram positive isolates were tested for drugs such as penicillin, oxacillin, linezolid, vancomycin, erythromycin, clindamycin, chloramphenicol, gentamycin, ciprofloxacin, tetracycline and cotrimoxazole. Gram negative isolates were tested for drugs such as gentamycin, ampicillin, chloramphenicol, amikacin, piperacillin-tazobactum, cefamandole, cefixime, ciprofloxacin, meropenem, imipenem, Cotrimoxazole, streptomycin, tobramycin, netilmycin ceftazidime and cefotaxime.

The plates were incubated aerobically at 370C for 18-24 hours and the interpretation of the results of the antimicrobial susceptibility was made based on the CLSI criteria as sensitive, intermediate and resistant by measuring diameter of inhibition the zone. All intermediate readings were taken as resistant during data entry. The standard reference strains, *Staphylococcus aureus* (ATCC25923), *Escherichia coli* (ATCC25922 and *P. aeruginosa* (ATCC 27853) were used to assure testing performance of the potency of drug discs well as quality of culture media. Methicillin resistance was detected by disc diffusion technique using 1µg oxacillin discs.13 Test for ESBL production was done by double disc approximation test.14

RESULTS

A total of 1568 patients were undergone major operations and admitted in Surgical (n=860) and Gynecology (n=458) wards and Orthopedics (250) of which 314 (20.02%) had clean and 1254 (79.98%) clean

contaminated operations during the study period (Table1).

TABLE.1 INCIDENCE OF SSI ACCORDING TO RISK CLASS

Risk Class	Surgeries Performed No.	SSI	SSI %
Clean	**314**	**14**	**4.4%**
Clean Contaminated	**1254**	**134**	**10.6%**
Total	**1568**	**148**	**9.43%**

The sex profile of these patients showed that 678 (43.23%) were males and 890 (56.76%) were females making male to female ratio of 1:31.1. The mean age of patients was 32.2 years and1254 (80%) of them were older than 15 years. The overall culture confirmed nosocomial infection rate on these patients was 9.43 % (Table1). The infection rate was higher in males than females. The infection rate was relatively high (27.3%) in the age group of >51 years old followed by 21-30 years of age group (12.6%).

One hundred and ninety six cases were processed for bacteriological study, in 48 (24.5 %) cases there was no growth and 148 (75.5%) cases were culture positive and were considered definitive cases of SSI. Out of 148 positive cases, 138 cases showed single organism as causative factor and 10 showed two organisms. A total of 158 organisms were isolated.(Table2)

TABLE.2 DIRECT MICROSCOPY AND CULTURE POSITIVITY

Direct Microscopy	Microscopy Positive	Culture
Pus Cells+ GPC	**12**	**12**
Pus Cells + GNB	**40**	**37**
Pus Cells +GPC+GNB	**88**	**78**
Few Pus Cells + No Organisms	**20**	**11**
No Pus Cells + No Organisms	**36**	**10**

GPC – Gram positive cocci; GNB – Gram negative bacilli

TABLE.3 INCIDENCE OF MONOMICROBIAL / POLYMICROBIAL GROWTH

No. of organisms	No of cases
No Growth	**48**
One	**138**
Two	**10**
Total	**196**

On direct microscopy of samples collected from 196 clinically infected cases, 140 samples were positive for Gram staining for pus cells and organisms. In 20 samples, few pus cells and no organisms were seen and in 36 samples no pus and no organism was seen in direct microscopy. (Table3) *Staphylococcus aureus* (27.84%) and *E coli (24.05%*) were the commonest pathogens which were isolated, followed by Klebsiella pneumoniae (13.03%) and Pseudomonas aeruginosa (6.32%).(Table4).

S.aureus (n=44) was the commonest isolate of which 52% (n=23) isolates were MRSA; all sensitive to vancomyicn and linezolid followed by chloramphenicol, gentamicin, ciprofloxacin and, 48% (n=21) isolates were MSSA (Methicillin sensitive *Staphylococcus aureus*). *Enterococcus sp.*(n=9) was 100% sensitive to vancomycin and linezolid (Table5). Piperacillin-Tazobactum, Ceftazidime, Tobramycin and Gentamicin are the common antimicrobials used for surgical prophylaxis and also for empirical therapy of SSIs. Gram negative bacilli isolated in our study were highly sensitive to these antibiotics (Table-6). ESBL producers included *Klebsiella sp.* (50%) (n=10), E.coli (20%) (n=4), and *Pseudomonas sp.* (30%) (n=6). *Pseudomonas sp.* (n=14) were mostly sensitive to Piperacillin-Tazobactum combination, meropenem and imipenem and amikacn. Most of the Gram negative bacilli were resistant to cefamandole, cefixime & cotrimoxazole. (Table6)

DISCUSSION

Nosocomial infections, including surgical site infection, still form a large health problem and contribute substantially to patient morbidity, mortality, prolonged hospital stay, expensive hospitalization and prolonged therapy15,16. Emergence of poly antimicrobial resistant strains of

hospital pathogens has also presented a major challenge in the provision of good quality in patient care7. The overall infection rate was 9.43 % in our study. This was in agreement with the overall infection rate which ranged from 2.8% to 20.19% in other studies17,18,19,20.

Cruse and Foord observed that the rate of infection of clean wounds was more useful as an indicator of control of infection of surgical wounds than the overall incidence17. So a detailed analysis of Clean and clean contaminated cases was conducted in this study, which definitely are the most useful measures in microbiological surveillance and research17. Accordingly, contaminated and Dirty surgeries were excluded from the study.In our study surgical site infection was significantly associated with class of wounds. For clean contaminated operations, 10.6% presented with SSIs and 4.4% for clean operations. This high rate of infection among former wound type is probably because of profound influence of endogenous contamination during the time of operation. The present study confirms the understanding that there is a gradual rise in incidence of wound infection as age advances. The infection rate was relatively high (27.3%) in the age group of >51 years old followed by 21-30 years of age group (12.6%). The higher incidence in patients above 60 years in our study is perhaps due to decreased immune-competence and increased chances of co-morbid factors like Diabetes mellitus, Hypertension, other Chronic ailments and personal habits like Smoking and alcoholism.

In the present study, on direct microscopy of samples collected from 196 clinically infected cases 140, samples were positive for Gram staining for pus cells and organisms. In smears from 20 samples, few pus cells and no organisms were seen but 11 were culture positive. This may be probably due to low number of organisms which could not be detected by microscopy but, yielded growth on culture. *Staphylococcus aureus* (27.84%) and *E coli (24.05%*) were the commonest pathogens which were isolated. Similar findings were recorded by S.P. Srivastava et al21 and S.V.Bhatia et al22. The predominance *S. aureus* infection seen in this study is most likely associated with endogenous source as the organism is a member of the skin and nasal flora of the patients as it was explained by Isbori et al5 and Angu and Olila23. Infection with this organism may also be associated with contamination from the environment, surgical instruments or contaminated hands of the health professionals5,23. *E.coli* (24.05%) was the second most common isolated bacteria from SSI. This could be because of the profound influence of endogenous contamination from the bowel and hollow muscular organs of patients.

The present study has also indicated that most of *S. aureus* were resistant to penicillin and oxacillin. Most sensitive antibiotics in our study were: imipenem, meropenem, amikacin, piperacillin-tazobactum in *Klebsiella sp.*, *E.coli*, and *Pseudomonas sp.*, and vancomycin, linezolid in MRSA and MSSA. These drug combinations should be used for empirical therapy, though; the prophylaxis must be continued with lower drugs according to the available surgical prophylaxis guidelines to prevent selection pressure and spread of resistance.
Predominant role of MDR bacteria in nosocomial infections similar to our study has been proved by many previous workers19,20,22,23. Infection by Multi drug resistant bacteria enhances the need of antibiotic stewardship and also indicates the need of proper disinfection of hospital environment.

Conclusion

In conclusion, the rate of nosocomial infection obtained in this study was comparable to other similar studies carried out in other countries. However, the bacterial isolates detected from our patients were resistant for commonly available and prescribed antimicrobial drugs. Therefore, antibiotics such as Ampicillin, Amoxicillin, Penicillin, Trimethoprim-sulphamethoxazole, Chloramphenicol and Ceftriaxone are not the drug of choice for treating patients with nosocomial infections in the study area. Hospital also needs to make a concerted effort to minimize hospital acquired infections by following strict aseptic operation procedures, effective methods of sterilization and patient management.

TABLE.4 NUMBER OF ORGANISMS

Organism	Number	Percent
Staph aureus	44	27.84%
Esch coli	38	24.05%
Kleb Pneumoniae	21	13.30%
Pseudomonas sp	10	6.32%
Enterococcus sp.	9	5.70%
Kleb Oxytoca	8	5.05%
Coagulase negative staph	6	3.80%
Citrobacter sp.	6	3.80%
DiphtheroidesI	4	2.53%

Organism	Number	Percent
Enterobacter sp.	2	1.27%
Morganella morganii	2	1.27%
Acinetobacter sp	2	1.27%
Streptococcus sp	2	1.27%
Candida sp	1	0.63%
Total	158	100%

TABLE.5 GRAM +VE ORGANISM WITH ANTIBIOTIC SENSITIVITY (%)

Antibiotic	MRSA	MSSA	CONS	Enterococcus sp	Streptococcus Sp.
Pencillin	45.5	49	33.33	50	100
Oxacillin	0	100	50	0	-
Linezolid	100	100	100	100	
Vancomycin	100	100	100	100	-
Erythromycin	85.7		33.33	-	-
Clindamycin	15.3	70	-	-	
Gentamicin	70	70	13.33	100	100
Ciprofloxacin	64.3	60	33.33	100	50
Chloramphenicol	76.9	80	66.66	100	-
Tetracycline	60	50	50		-
Co-trimoxazole	15.3	20	50	-	-

TABLE.6 GRAM –VE ORGANISMS WITH ANTIBIOTIC SENSITIVITY (%)

Antibiotics	Esch. coli.	Kleb pneumonia	Kleb oxytoca	Citrobacter sp.	Enterobacter sp	Pseudomonas aeruginosa	Morganella morganii	Acinetobacter sp	Proteus sp.
Gentamicin	62.5	33.33	25	50	0	80	100	100	100
Ampicillin	5.2	2	0	0		10	0	50	0
Chloramphenicol	75	65	25	50	50	30	100	50	33.33
Amikacin	90	80	12.5	33.33	50	80	100	50	100
Tazobactam+ Pipracilin	87	88.6	0	50	0	70	100	100	33.33
Cefamandole	15	9	0	33.33	50	10	0	50	33.3
Cefixime	42.9	40	0	33.33	0	40	0	0	0
Ciprofloxacin	62.5	45	O	33.33		80	0	100	66.66
Metropenem	38	85	-	16.66	50	-	50	50	0
Imipenem	90	90	50	16.66	50	-	50	50	-
Co-trimoxazole	0	10.52	50	33.33	50	70	100	-	-
Tobramycin	100	80	25	50	100	90	50		100
Netilmycin	56		25	50	50	80	50	100	100
Ceftazidime	92.7	100	25	100	100	70	50	100	100
Cefotaxime	94.4	94.1	25	100	100	70	50	100	100

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