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MICROBIAL OBSERVATION AND ITS ANTIBIOTICS SENSITIVITY PATTERN IN POST-OPERATIVE WOUND INFECTION AT MICROBIOLOGY DEPARTMENT OF DMC, LAHERIASARAI, BIHAR

Microbiology

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ABSTRACT

Infections due to antibiotic resistant bacteria have increased alarmingly in both developed and developing countries. Unrestrained and rapidly spreading bacterial growth has turned the management of wound infections into a serious challenge. This study aimed to determine the prevalence of different bacterial pathogens and their antibiotic susceptibility in various types of wound infections. A cross-sectional study was conducted to collect 101 wound swabs. All isolated bacteria were identified based on colony characteristics, gram stain and standard biochemical tests, and antibiotic susceptibility testing (AST) with the disc diffusion method. The diversity of isolated bacteria and their susceptibility patterns signify a need to implement a proper infection control strategy, which can be achieved by carrying out antibiotic sensitivity tests of the isolates.

KEYWORDS

Wound Infection, Bacterial Pathogen, Antibiotic Susceptibility Pattern

INTRODUCTION

Infection is encountered by all surgeons, as they invariable impair the first line of host defences between environmental microbes and the host's internal millieu. When the bacteria breach these barrier, postoperative wound infections result. Up to the end of the 19th century, infection was the greatest risk associated with any surgical procedure .

The area of chemotherapy is now over 50 years old. Those years has been marked by continuous development and introduction of new and potent antimicrobial agents and by the relentless development and spread of significant antibiotics resistance among pathogenic bacteria. Postoperative wound infection still remains a nightmare to all surgeons, even in this era of much advanced medical science.

The ubiquity of infectious agents in man's environment, their propensity for invading the body, their potential for producing significant pathophysiological effects on various body functions, their remarkable adaptability to newer forms of treatment and wound healing, all these factors affect surgical practice and financial implications both to the person and institution.

For most period of surgical history, death from infection was the expected result, although it was not until the end of nineteenth century that the bacterial cause of surgical infection was appreciated. Death from infection was so common after compound fracture or fracture due to a gunshot wound where, amputation was the standard treatment. Before antiseptic practice were instituted, mortality rates in amputation at the times of war,1745 to 1865 were between 25 % to 90 %. Mortality rates for amputation in civilian practice during the same period ranged between 5 to 50%.

Surgical site infections has been a major problem for surgeons and surgery without sepsis has remained and unfulfilled goal, since the time lister introduced antisepsis in 1867. The bacterial flora as agents of wound infection received two devastating blows. Firstly with the introduction of aseptic surgery with emphasis on prevention of bacterial contamination of surgical wounds, by strict disinfections,. efficient sterilisation of instruments, wearing of sterile protective clothings, improve operation theatre ventilation and secondly the discovery of antibiotics.

After the general use of modern antibiotic therapy for 60 years ,clinical experience and bacteriological studies have shown that the overall incidence of infections in the surgical patient has not decreased and many related problems are still present .This is because we have a wide variety of surgical procedures to treat different conditions, which way are not available 10 to 20 years ago .Advanced medical science has given the knowledge to practice the various preventive measures to control the routinely encountered types of infections, but other types of infections have taken there place.

Widespread and indiscriminate use of antibacterial agents in hospitals has led to the progressive development of resistance to penicillin and many of the other antibiotics agents ,by a large variety of important bacteria concentrated in the hospital environment. These virulent organisms have shown the potential to become pathogenic in patients weakened by disease, injury, metabolic conditions ,surgery and other debilitating factors.

Microbiology has made significant contribution to the advancement and safety of surgery through the development of germ theory of surgical infections, aseptic techniques, passive and active immunization and antimicrobial therapy. Each of these, has had revolutionary effects on the practice of surgery. A variety of stressful forces imposed upon microorganisms during the past 60 years however, have produced reactions in the microbial world ,which in turn, have influenced the pattern and nature of infections seen in the practice of surgery.

Postoperative wound infections are the second most common cause of nosocomial infection .It has been estimated that postoperative infection develop in at least 2 % of hospitalized patients undergoing operative procedures, although this is a likely under estimate because of incomplete post discharge data ,other data indicates that postoperative wound infections develop following 3 to 20 percent of certain procedures. There seems to be a perception among some surgeons that postoperative wound infections is a relatively trivial infection. However Kleeven et al in 2002 in their study on estimating healthcare associated infections and deaths in United States of America hospital, there were 2,90,000 infections in hospitalized patients and surgical site infection was estimated to be directly responsible for 8205 deaths of surgical patients that year thus, mortality rate was 3 % among patients who developed surgical site infections. A large number of patients develop long term disabilities as a result of poor wound healing and overt tissue destruction following these infections. Finally, the economic cost of surgical site infection to the patients and health care delivery system are high.

Alexander fleming discovery of penicillin in 1928 give the world first powerful weapon against bacteria. As the time passed, with the influx of voluminous number of antibiotics, the clinician often times finds himself overwhelmed by the variety of options. Determination of the etiological agent is vital in the final choice of antibiotics. A working knowledge of the most likely causative organism and the prevailing antibiotic sensitivity / resistant will be of great help.

Each hospital has its own unique bacterial flora to which patients are at risk for acquiring infection during hospitalization. In such situations; microorganisms exhibit unique pattern of antimicrobial activity during a certain period of time. Only when such an epidemiological data are available can the surgeon employ a logical approach towards surgical site infection control. Also resistance to antimicrobial has become a serious problem necessitating the in-depth study of surgical site infection to prevent the future complications in operated cases.

AIMS AND OBJECTIVE

In the developing countries, post-operative wound infection still remains a commonest complication in surgical patients. Surgical wound infection increases the morbidity, hospital stay and thereby the cost of medical treatment. At times it can also be the cause of mortality.

In order to prevent or to reduce the incidence of post-operative wound infection maximum emphasis was given to the practice of :

- strict aseptic measures.
- use of meticulous surgical techniques
- regular surveillance of factors influencing post-operative wound infection
- prophylactic administration of antibiotics

Inappropriate and indiscriminate use of antibiotics may increase the cost, through unnecessary use of these drugs without a proper sensitivity test result, thus resulting in development of antibiotic resistance among the bacteria. Therefore a study of this kind helps in recognising the range of microorganisms causing wound infection.

This would act as a guide towards routine surveillance. The CDC, surgical infection society and other regulatory agencies advocate the use of surveillance, which reduces the incidence, thereby improving medical care and decreasing the cost of hospital care.

The present study of surveillance was taken up in the department of microbiology at Darbhanga Medical College, Laheriasarai, Bihar with the following objective:

1) To isolate and identify the organism causing surgical post-operative wound infection.
2) To know the predominant organism causing surgical post-operative wound infection.
3) To determine the current antibiotic sensitivity pattern of the isolates.
4) To detect Extended Spectrum Beta Lactamase (ESBL) producing Escherichia coli and Klebsiella pneumoniae.

METHOD AND MATERIAL

The present study of Bacteriology of post operative surgical wound infections was carried out in the Department of Microbiology, Darbhanga Medical College, Laheriasarai, Bihar.

The material for the present study was obtained mostly from patients in the Gastro-Intestinal Surgery Department, who had undergone operations and who had developed Signs & Symptoms of post-operative wound infections.

Surgical sites were considered to be infected according to the set of clinical criteria recommended by the Surgical wound infection task force.

The wounds were classified using the wound contamination class system, Proposed by U.S. National Research Council (1964)).

INCLUSION CRITERIA

- Cases of Clean and Clean contaminated surgeries conducted in the G.I surgery Department.
- Age >14 years.
- Patients of either sex

EXCLUSION CRITERIA

- Refusal to participate in the study.
- Patients already receiving antibiotics for >1 week.
- Patients operated in emergency.
- Patients undergoing re-operation.
- Patients failing to come for follow-up of upto 30 days since the day of operation

COLLECTION OF MATERIAL

The wounds were examined for suggestive Signs/Symptoms of infection in the post operative period, during wound dressing or when the dressings were soaked, until the patient was discharged from the hospital and also in the Out-patient department after discharge.

When infection was clinically suspected, the area around the surgical wound was cleaned with 70% ethyl alchohol. The exudate was collected from the depth of the wound using two sterile cotton swabs and for anaerobic culture, the material was aspirated in a sterile syringe or when it was not possible, the discharge was collected using a third swab.

Two extra swabs were collected and immediately, one of them was put in Nutrient broth and the other into a pre-reduced RCMB at the bedside.

The wounds and the samples collected were inspected for characteristics that indicate anaerobic infection, which include foul odour, brick red fluorescence due to protoporphyrin production, black necrotic tissue or black discharge, sulfur granules, blood and purulence.

Relevant clinical history of the patient was taken.

TRANSPORT

All the specimens collected were transported immediately to the laboratory for further processing. The Nutrient broth and RCMB were incubated at 37° C.

METHODS

The samples collected were processed as follows:

a) Direct microscopic examination of gram stained smear.
b) Inoculation of the samples onto different culture media for aerobic and anaerobic organisms.
c) Preliminary identification
d) Bio-chemical tests
e) Antibiotic sensitivity

CULTURE FOR ANAEROBIC ORGANISMS

The aspirate in the syringe or the 3rd swab was inoculated into a plate of freshly poured 5% Blood agar, a plate of blood agar with Haemin 5 µg/ml and Menadione 10 µg/ml. and a blood agar plate with Gentamicin 10 µg/ml.

The plates were placed in the anaerobic Jar of Dynamicro GR anaerobic system.

Silicon grease was applied on the rim of the jar and lid generously. The contents of the Dyanox Anaerobic charge (P2) were placed at the bottom of the jar.

40 ml of 1:4 Sulphuric acid was poured on the charge quickly, the plates were placed the lid closed and clamped tightly. The external cup was filled with saturated sodium carbonate solution a vigorous bubbling was seen immediately.

A plate of blood agar streaked with a known pseudomonas strain was kept inside the jar as a biological indicator.

The anaerobic plates are incubated at 37'C for 48-72 hrs. Subcultures were done similarly from the RCMB after 4 days when there was no growth on the primary plates.

Colony Morphology was recorded in detail; this included colony size, shape, colour, internal appearance profile, opacity, general appearance (eg. mucoid, glistening, breadcrumb like, dull) and other distinctive characteristics.

Transmitted light was used to look for beta hemolysis, a double zone of beta-hemolysis and greening of the agar which usually is more apparent after exposure to air.

The presence of pigments, colours varying from light tan to black or pink to red was observed.

The plates were held at an angle to a light source to detect corroding or pitting of the Agar.

Woods lamp was used to detect fluorescing colonies with colours including brick red, pink and yellow green. Gram stained smears of the colonies were examined. Catalase test 1 ml of 15% Hydrogen peroxide was taken in a test tube, the suspected colonies were harvested on a glass rod and dipped into the Hydrogen peroxide solution.

The evolution of bubbles denoted a positive test.

SPOT INDOLE TEST

The growth from the blood agar plate was rubbed into filter paper saturated with Para dimethyl amino cinnamaldehyde reagent.

A blue colour denotes a positive test.

AEROTOLERANCE TEST

Each colony type on the anaerobic blood agar plates was subcultured onto two plates of blood agar and one was incubated aerobically and the other anaerobically.

If there was growth on the aerobic plate, the organisms were considered aerobes and correlated with the aerobic culture, if there was no growth in the aerobic plate and growth was seen only in the anaerobic plate, the organisms were considered anaerobes.

SPECIAL POTENCY ANTIBIOTIC DISC SUSCEPTIBILITY

Colistin 10µg, vanco mycin 5µg and Kanamycin 1 mg disks were placed on the first quadrant of a well streaked blood Agar plate.
A zone size of 10mm or less was considered resistant. These disks were used as an aid in determining Grams reaction, and in separating the Bacteroides and Fusobacterium species Gram positive organisms are resistant to Colistin and sensitive to Vancomycin, whereas Gram negative organisms are resistant to Vancomycin. Fusobacterium are susceptible to both Kanamycin and Coiistin and Bacteroides are generally resistant to Kanamycin but variable in susceptibility to Colistin.

All the results were recorded.

RESULTS

101 clinically diagnosed cases of Post-operative wound infection of all age groups and both sexes, irrespective of the preoperative admini stration of antibiotics admitted in various GI-surgical wards were studied.

83 (82.18%) samples yielded growth on culture and 18 (17.82%) samples yielded no growth. Out of culture positive samples. There were 83 isolates altogether. 60 isolates were of Gram negative bacteria, accounting for 72.29% of the isolates and Gram positive bacteria accounted for 23 (27.71%) of the isolates. The most frequent isolate was Escherichia coli, which represented 16 (19.28%) of the total isolates, followed by Staphylococcus aureus and Klebsiella pneumonia 14 (16.87%). Escherichia coli was found to be the most common Gram negative organism isolated and Staphylococcus aureus was the most common Gram positive organism isolated.

Staphylococcus aureus (42.71%), was the most common organism isolated from class I wound infection. Escherichia coli (29.23%) was the predominant organism isolated from class II wound infection. The most common organism isolated from class III wound infection was Proteus mirabilis (40%) and Klebsiella pneumonia (66.66%) was found to be the most common organism causing class IV wound infection. The Gram negative organisms were most sensitive to imipenem (100%), followed by piperacillin/tazobactam (98.33%), ceftazidime (71.67%), cefepime (63.33%) , cefotaxime (61.67%), amoxyclav (45%).The least sensitive antibiotic against Gram negative organisms was ampicillin(0%). The Gram positive organisms were most sensitive to vancomycin (100%), followed by clindamycin (91.30%), linezolid (86.96%) and gentamicin and erythromycin (82.61%). The least sensitive antibiotic against Gram positive organisms was penicillin Out of 54 isolates of Staphylococcus aureus, 12 (22.22%) were found to be MRSA strains. 25% of MRSA strains were inducible clindamycin resistant. Vancomycin (100%) was found to be the most sensitive antibiotic against methicillin resistant Staphylococcus aureus followed by linezolid (66.6%) and clindamycin (58.3%).

Extended spectrum beta lactamase production was observed in 6 (42.86%) of 14 Klebsiella pneumoniae isolates and in 4 (25%) of 16 Escherichia coli isolates. Klebsiella pneumoniae was found to be more Extended spectrum beta lactamase producer when compared to Escherichia coli. Among Extended spectrum beta lactamase producing isolates imipenem and piperacillin/tazobactam were found to be the most sensitive antibiotics followed by amikacin, gentamicin and ofloxacin. Imipenem resistant strains of Pseudomonas aeruginosa was not seen in the study.

CONCLUSION

The present study which was conducted in DMCH, Laheriasarai, has given us the knowledge of the most common organisms causing Post-operative wound Infection and their antibiotic sensitivity pattern.
The most common causative agent of SSI Escherichia coli was followed by Staphylococcus aureus ,Klebsiella pneumoniae etc. The Gram positive organisms were most sensitive to vancomycin followed by clindamycin, linezolid and gentamicin. The Gram negative organisms were most sensitive to imipenem followed by piperacillin /tazobactam, ceftazidime and amikacin. Methicillin resistance is a common problem in Staphylococcus aureus which can be routinely detected by cefoxitin disc test. The MRSA strains in our study were most sensitive to vancomycin followed by linezolid and clindamycin. The presence of inducible clindamycin resistance among MRSA strains indicates the importance of identification of such strains by D test to avoid treatment failure with clindamycin.

Microorganisms responsible for post-operative wound infections like Klebsiella pneumoniae and Escherichia coli have the ability to produce Extended spectrum beta lactamase resulting in limiting of therapeutic option. Among Extended spectrum beta lactamase producing isolates imipenem and piperacillin/tazobactam were found to be the most sensitive antibiotics followed by amikacin, gentamicin and ofloxacin. The presence of metallo beta lactamase producing Pseudomonas aeruginosa was not detected in our study and warrants further studies to detect their presence in this hospital.

The presence of MRSA and ESBL in our study calls for intensive infection control practices, and surveillance of infection is to be encouraged. In addition, surgeons are well informed about the most common organisms causing SSI and their sensitivity.

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