ORIGINAL ARTICLE

Identification of pathogenic bacteria isolated from tissues, bones infections and their antibiotic susceptibility pattern at Khyber Teaching Hospital, Peshawar

Abdus Samad, Muhammad Asghar, Muhammad Naeem, Noor Rehman, Naheed Asghar, Muhammad Haroon, Safia Rahman, Sadiq Azam, Resham Ali, Aaqib Khan, Ghadir Ali, Ibrar Khan, Farman Ullah

Received:
2nd February, 2018

Accepted:
13th September, 2018

Abstract

Objectives: To determine the frequency of SSIs causing pathogenic bacteria and their antibiotics susceptibility pattern in order to develop a drug of choice to treat SSIs (tissues and bone infection).

Study design: Cross-section study

Place and duration of study: This Cross-section study was designed in the Orthopedics unit and Department of Pathology at Khyber Teaching Hospital, Peshawar from May to November 2017.

Material and Methods: In this study a total of 433 patients with closed fractures of different age groups of both gender were selected. Samples were collected with sterile swabs and then cultured on different Media like Blood Agar, Mac Conkey Agar and Mannitol Salt Agar. Colonies were Gram stained for the identification of Gram positive and Gram-negative bacteria. Biochemical tests were performed to confirm the clinical bacterial isolates. Antibiotic sensitivity was conducted by "disc-diffusion" method on Mueller Hinton Agar using CLSI guidelines.

Results: Out of 433 cases of SSIs 27 (6.23%) yielded bacterial growth. Out of 27 bacterial infections; (66.66%) were deep and (33.33%) superficial infections. The increase prevalence (51.8 %) of Staphylococcus aureus observed among pathogenic micro-organism, followed by Escherichia Coli (25.9%), Pseudomonas aeruginosa(11.11%), Enterobacter spp(7.4%) and Salmonella spp(3.75%). S.aureus showed good response to Gentamycin (CN), Clindamycin (DA), Fusidic Acid (FD), Vancomycin (VAN) and Amikacin (AK) and resistant to Ampicilin (AMP), Ciprocin (CIP), Velocef (CE) and Amoxicilin (AMC). The E.coli was found to be sensitive to Amikacin (AK), Imipenem (IMP), Sulzone (SCF) and Cefoxitin (FOX) while resistant to Ampicilin (AMP), Fosfomycin (FOS), Levofloxacin (Lev) and Amoxicilin (AMC).

Conclusions: We observed a high rate of infection in our study as compare to the previous studies. We also concluded that in SSIs, other micro-organisms are also involved including Pseudomonas spp, Enterobacter spp and Salmonella spp.

Keywords: SSIs, Austin Moore prosthesis, humerus plating and femur plating, disc diffusion, Antibiogram, Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia Coli, Enterobacter spp.

Khyber Teaching Hospital, MTI, Peshawar
A Samad
M Asghar
M Naeem
N Rehman
M Haroon
S Rahman

MTI, Hayatabad Medical Complex, Peshawar
N Asghar

University of Peshawar, Peshawar
S Azam
I Khan

Correspondence:
Dr. Noor Rehman
Microbiologist, Khyber Teaching Hospital, MTI, Peshawar, Pakistan.
Cell: +92 333-9535336
Email: noorbangash61@yahoo.com

Introduction:

Surgical site infections (SSIs) consists of both infected bones and infected tissues and are the microbial contamination of surgical or operative wounds that occur within 30 days of a surgery or within 1 year post-surgery.[1] Infections associated with surgical incisions are called SSIs.[4] SSIs are one of the mostly occurring nosocomial (Hospital acquired) infections.[1] SSIs are considered as a common surgical complication that may be a disaster for the patient because it can compel a patient to use more Antibiotics and to stay at hospital for long.[2]

Additional disorders associated with SSIs may be tissue destruction, unsuccessful or long lasting of wound healing. SSIs develops in about 3% of all surgical processes and most common pathogenic micro-organism is Staphylococcus aureus, second common pathogenic microorganism. Is E.coli and then Pseudomonas spp.[2] The SSIs related to orthopedic implant operations results from the growth of pathogenic micro-organisms resides in the biofilm whose removal is difficult. 3 SSIs are categorized in three stages; an early stage lasts for less than two weeks; second delayed phase lasts for 2 to 10 weeks while late phase is more than 10 weeks.[3]

In 1896, Brewer GE reported the infection rates of 39% in post-surgical patients that was reduced to 0.2% with sophisticated preventive measures (Aseptic) and introduction of Antibiotics.[5]

Surgical site infections can be found as superficial infections in which only skin is infected. Tissues below the skin, organs, or implanted material can also be infected which are more lethal. Symptoms include; Erythema (Redness) and pain around the surgical site, Flow of muddy fluid from surgical wound and Temperature.[6]

According to center of disease control "CDC" there are three levels of SSIs; Superficial incisional, infection of the skin and subcutaneous tissue which is marked by erythema, pain, heat, swelling and flow of pus. Deep incisional, infection of the fascial and muscle layers which is marked by the presence of purulent material or an abscess, fever with tenderness of the wound, or a separation of the edges of the incision exposing the deeper tissues. Organ or space infection, which involves any part of the anatomy other than the incision that is opened or manipulated during the surgical procedure. These infections are marked by the flow of pus or abscess.[6]

Patients, surgeons, nurses and the operation theatre's (OT) environment and surgical instruments are main factors that may involve in SSIs.[7] Washing of hands before and after surgery, providing fundamental hygienic strategies, prophylactic (preventive) antibiotics use at the exact time and strength, surgical dressing, and limiting the number of staff (nurses or doctors) in the OT all these factors may lead to reduce the chances of SSIs.[7] Implementation of preventive strategies includes nasal decolonization, antibiotic prophylaxis, pre-operative showers, pre-operative oxygen supplementation, cleaning of surgical sites. Hospital practicians can also apply preventive measures to low the risk of SSIs like proper hand hygiene, sterile clothes and utensils.[13]

The current study was designed todetermine the frequency of infected tissues and infected bones at Surgical-site and knowing about the common micro-organisms of Surgical-site infected tissues and bones. This study will be helpful to design appropriate antibiogram profile of the isolated pathogenic micro-organisms in order to develop a pharmaco-therapeutic plan for clinicians to treat SSIs.

Materials and Methods:

This study was designed in Orthopedics unit and Pathology (Microbiology) department at Khyber Teaching Hospital, Peshawar from May to November. A total of 433 patients were studied having SSIs (Infected bones and tissues) associated with Austin Moore prosthesis, tibial plating and humerus plating. The collected data was processed in the Microbiology lab at Khyber Teaching Hospital, Peshawar. The collected SSIs samples were inoculated on different Media like; Blood agar, MacConkey agar and Mannitol Salt Agar (MSA) media. Growth was observed after 24-48 hours incubation at 37°C aerobically.[11] After incubation the agar plates were observed for bacterial growth. Gram staining of bacterial isolates was performed to differentiate between Gram positive and Gram-negative bacteria. Biochemical tests like urease, citrate, triple sugar iron (TSI) and oxidase were performed for the identification of bacterial isolates. The bacteria isolated were S. aureus, Pseudomonas spp, E. coli, Enterobacter spp and Salmonella spp. After identification, antibiotic sensitivity of the bacterial isolates was checked on Mueller Hinton Agar by using various antibiotics, based on 'disc diffusion' method (Kirby Bauer) according to the CLSI guidelines.[12]

Table-1: Prevalence of bacterial isolates from infected bones and infected tissues (n=433)

Total positive	Total negative	Total cases	Percentage (%)
27	406	433	6.23

Table-2: Frequency of bacterial isolates from infected bones and infected tissues (n=27)

Different clinical isolates	Total Positive (n=27)	Percentage (%)
Staphylococcus aureus	14	51.8
Escherichia coli	7	25.9
Pseudomonas spp	3	11.11
Enterobacter spp	2	7.4
Salmonella spp	1	3.75
Total	27	6.23

Table-3: Age wise distribution of positive cases of SSIs (infected tissues and bones) (n=27)

Differences in age	No. of Positives Cases	Percentage (%)
4-13	8	29.6 %
14-23	2	7.4 %
24-33	3	11.11 %
34-43	7	25.9 %
44-53	6	22.22 %
>54	1	3.7 %
Total	27	-

Table-4: Frequency distribution of different types of infections in SSIs (n=27)

Infection types	Positive	Percentage %
Superficial Infection	9	33.33 %
Deep Infection	18	66.66 %
Total	27	-

Table-5: Different types of bone surgery vs SSIs

Implants	Positive	Percentage %
Austin Moore prosthesis	3	11.11 %
Humerus plating	8	29.6 %
Tibial plating	16	59.2 %
Total	27	-

Results:

A total of 433 SSIs clinical isolates was subjected for bacterial growth; only 27(6.2%) isolates yielded bacterial growth as shown in Table-1. Out of 27 positive isolates, 18(66.66%) cases were male while 9(33.33%) were female. S. aureus was the most frequent pathogenic microorganism i.e. (51.8%) followed by Escherichia Coli (25.9 %), Pseudomonas aeruginosa (11.11%), Enterobacter (7.4 %) while (3.75 %) yielded Salmonella spp growth as shown in Table 2. The highest prevalence 8(29.6%) was reported in the age group between 4-13 years, followed by 7(25.9%) in age group 34-43 years, 6(22.22%) in age group 44-53 years, 3(11.11%) in age group 24-33 years, 2(7.4%) in age group 14-23 years and 1(3.7%) was in age group > 54. Most of the positive clinical isolates were reported in age groups ranges 4-13, 34-43 and 44-53 respectively as shown in Table 3. The superficial infection was detected in 9(33.33%) cases out of the total positive (27) while 18(66.66%) cases were found in deep infectionas shown in Table 4. Three different types of implants i.e. Austin Moore prosthesis, Humerus plating and femur plating established different percentages of infections. The results revealed that 3(11.11%) positive cases were related to Austin Moore prosthesis followed by 8(29.6%) cases in Humerus plating while 16(59.2%) cases were associated to tibial plating as shown in Table 5. The antibiotics susceptibility pattern was observed in all positive isolates of SSIs. The S. aureus was observed to be sensitive to Gentamycin (CN), Clindamycin (DA), Fusidic Acid (FD), Vancomycin (VAN) and Amikacin (AK) while resistant to Ampicilin (AMP), Ciprocin (CIP), Velocef (CE) and Amoxicilin (AMC). The E. coli showed good response toAmikacin (AK), Imipenem (IMP), Sulzone (SCF) and Cefoxitin (FOX) while resistant to Ampicilin (AMP), Fosfomycin (FOS), Levofloxacin (Lev) and Amoxicilin (AMC). The Pseudomonas spp were sensitive to Amikacin (AK), Imipenem (IMP) and Gentamycin(CN) while resistant to Levofloxacin (Lev), Fosfomycin (FOS) and Amoxicilin (AMC). The Enterobacter spp were sensitive to Chloramphenicol (C), Cefoxtin (FOX), Cefixime (CEF) and Tygacil (TGC) while resistant to Sulzone (SCF), Amikacin (AK) and Ciprocin (CIP). The Salmonella spp were sensitive to Cotrimoxazole (SXT), Amoxicilin (AMC) and Cefoxitin (FOX) while resistant to Fosfomycin (FOS), Chloramphenicol (C), Cefixime (CEF) and Ampicilin (AMP) as shown in Figure 1.

Discussion:

SSIs are one of the most common nosocomial infections that may lead to tissue destruction and other post-surgical disorders. SSIs are categorized as second or third highly observed form of noso-comial infection. In 1896, Brewer GE reported the high infection rates of 39% in post-surgical patients that was reduced to 0.2% with sophisticated preventive measures (Aseptic) and introduction of antibiotics.[5]

The rate of infection according to our study is (6.23%) which is high as compare to the reported study (1%) regarding post-surgical infections.[8] According to the previous studies on SSIs held in Pakistan in 2014 and 2008 the rate of infection was 5.8% and 5.78% respectively.[4,2] The reason might be poor sterilization techniques during surgery procedures and unhygienic conditions after post-surgery in the respective wards.

The current study reported the frequency distribution of superficial infection (33.33%) and deep infection (66.66%). Other studies observed the low prevalence of superficial (5%) and deep infection (0.25%) in 413 total cases by Martson RA et al in 1996.[10] Another study conducted by Khan MS et al reported (2%) superficial and (3.8%) deep infection.[2] The higher results in our results might be due to geographical origin infections.

Staphylococcus Aureus was the prevalent pathogenic micro-organism in our study (51.8%) followed by E. coli (25.9%), Pseudomonas spp (11.11%), Enterobacter spp (7.4%) and Salmonella spp(3.75%) While according to the previous study of M Salman et al conducted in 2014[4] observed the prevalence of (94%) in S. aureus-followed by E.coli (6%). While Khan MS et al in 2008[2] reported the high prevalence of S. aureus (50%) followed by E.coli (33.33%) which results are on same line with our study.

The present study revealed that in SSIs, not only S. aureus and E.coli involved in infections of tissues and bones but other pathogenic micro-organisms are also involved like Pseudomonas spp, Enterobacter spp and salmonella spp while in previous study only E.coli and S. aureus were reported in Khyber Teaching Hospital, Peshawar in 2014 in (5.78 %) rate of infection.[4]

The present study revealed that the S. aureus was sensitive to Gentamycin (CN), Clindamycin (DA), Fusidic Acid (FD), Vancomycin (VAN) and Amikacin (AK), while most of the third-generation drugs were used as a prophylactic drug. The E.coli showed good response to Amikacin (AK), Imipenem (IMP), Sulzone (SCF) and Cefoxitin (FOX) andTygacil (TGC) while Pseudomonas spp, Enterobacter spp and Salmonella spp were sensitive to Amikacin (AK), Imipenem (IMP), Sulzone (SCF), Gentamycin(CN) andTygacil (TGC).

This is a very inspiring sign to see that sensitivity of the most drugs showed good response as these are cheap antibiotic drug. The same results were also reported by another study which are on same line with our study.[14] The combination of cefoperazone and sulbactum were also used in prophylactically. The results of other study also supported our study.[15] The results of the current study revealed that Vancomycin and Fusidic Acid showed good activity against S. aureus. Similar study was conducted by Salman et al, that Vancomycin is still effective against S. ureus. These are most helpful antibiotics for the treatment of SSIs.[16]

Conclusions:

It was concluded that S. aureus is most common organism causing SSIs followed by E. coli and Pseudomonas spp. We observed a high rate of infection in our study as compare to the previous studies in this regard which means that sophisticated preventive measures are not implemented. According to our study's rate of infection (6.23%) aseptic and preventive measures must be emphasized in order to reduce the rate of infection. Non-protective SSIs may lead to other disorders and may become a burden on patient in the form of high use of antibiotics and long-lasting stay at hospital.

Acknowledgement: This work was conducted at Pathology Department, Microbiology section, Khyber Teaching Hospital (KTH) Peshawar, Pakistan. The Authors are thankful to administration of KTH and Pathology Department, Peshawar for providing us research facilities.

Conflict of interest: None

Funding source: None

Role and contribution of authors:

Dr Abdus Samad, Idea conception, data collection & initial drafting

Dr Muhammad Asghar, Data collection & data analysis

Dr Muhammad Naeem, Data collection & Final drafting

Dr Noor Rehman, did reference collection

Dr Naheed Asghar, Data collection

Dr Muhammad Haroon, Data collection

Dr Safia Rahman, Data collection

Dr Sadiq Azam, Data collection

Dr Resham Ali, Data collection

Dr Aaqib Khan, Data collection

Dr Ghadir Ali, reference collection

Dr Ibrar Khan, Data collection

Dr Farman Ullah, Data collection

References:

1. Dr. Amaradeep G, Dr. Shiva Prakah SS and Dr. Manjappa CN. Surgical site infections in orthopedic implant surgery and its risk factors: A prospective study in teaching hospital. MIMS, Mandya, Karnataka, India 2017;3(3):169-172.
2. Khan MS, Rehman SU, Ali MA, Sultan B, Sultan S. Infection in orthopedic implant surgery, its risk factors and outcomes. J Ayub Med Coll Abbott abad 2008; 20(1):23-5.
3. Trampuz A, Zimmerli W. Diagnosis and treatment of infections associated with fracture fixation devices. Injury 2006;37(2):S59–66.
4. Muhammad Salman, Muhammad Ayaz Khan, TamjeedGul, Muhammad Bilal, Waheed Kamran. Frequency of Surgical Site Infection in orthopaedic implants surgery with its common bacteria and antibiotic sensitivity. Khyber Teaching Hospital, Peshawar, Pakistan 2014; 30(2):167-171.
5. Brewer GE. Operative surgery at the City Hospital with preliminary report on the wound infection. New York Med J. 1896.
6. R.Coello, A.Charlett, J.Wilson, V.Ward, A.Pearson, P.Borriellod. Adverse impact of surgical site infections in English hospitals 2005; 93-103.
7. Fahad A. Al-Mulhim, Mohammed A. Baragbah,Mir Sadat-Ali, Abdallah S. Alomran, and Md Q. Azam.Prevalence of Surgical Site Infection in Orthopedic Surgery: A 5-year Analysis2014;99(3): 264–268.
8. Cordero J. Infection of Orthopaedic Implants – Theory and Practice. In European Instructional Course Lectures 1999; 4:165-73.
9. National Nosocomial Infections Surveillance (NNIS) System. National Nosocomial Infections Surveillance (NNIS) System report: data summary from January 1992 to June 2002, issued August 2002. Am J Infect Control 2002;30:458-75.
10. Martson RA, Cobb AG, Bantley G. Stammor compare with Charnely total hip replacement. J Bone J Surg1996;78:178–84.
11. WondemagegnMulu ,GebreKibru, GetenetBeyene , Meku-Damtie. Postoperative Nosocomial Infections And Antimicrobial Resistance Pattern Of Bacteria Isolates Among Patients Admitted At FelegeHiwot Referral Hospital, Bahirdar, Ethiopia 2011.
12. CLSI. Performance Standards for Antimicrobial Susceptibility Testing. 26th ed. CLSI supplement M100S. Wayne, PA: Clinical and Laboratory Standards Institute; 2016.
13. Back to Basics: Preventing Surgical Site Infections 2014; 600-611.
14. Shah, MQ, Zardad, MS. et al. "surgical site infection in orthopedic implants and its common bacteria with their sensitivities to antibiotics, in open reduction internal fixation." Journal of Ayub Medical College Abbottabad2017;29(1): 50-53.
15. Khan, MS, Ahmed,Z. et al. "Common trend of antibiotics usage in a tertiary care hospital of Peshawar, Pakistan." J Ayub Med Coll Abbottabad 2010;22(1).
16. Slama, TG, Amin, A.et al. A clinician's guide to the appropriate and accurate use of antibiotics: the Council for Appropriate and Rational Antibiotic Therapy (CARAT) criteria, Elsevier 2005.