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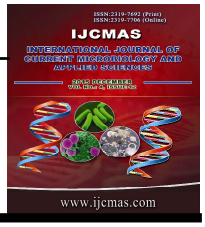

Original Research Article

Bacteriological Profile and Antimicrobial Sensitivity of Wound Infections

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Wound infection, Aerobic culture, *Staphylococcus aureus*, Antibiotic sensitivity

A B S T R A C T

Wound infections are one of the most common hospital acquired infections and are an important cause of morbidity and mortality. The objective of this study is to determine the causative aerobic bacteria and antimicrobial sensitivity of wound infections from pus specimen.The study population included patients admitted to different wards in the hospital and also those attending the out patient department. A total of 133 pus samples were received and were processed by doing Gram's stain and culture. Out of them 57% of the samples were obtained from surgical wound infections and 43 % of the samples were obtained from non-surgical wounds. Out of the 133 pus samples 66 samples (59.6%) were culture positive. *Staphylococcus aureus* was the most frequently isolated organism (30.88%) followed by *Pseudomonas aeruginosa* (25.02 %). Gram positive cocci were mostly sensitive to Amikacin (90.4%) followed by Levofloxaccin(76.1%).Among the Gram negative isolates Pseudomonas species were mostly sensitive to Amikacin (82.4%) followed by Ofloxacin (76.4%), *Escherichia coli* and Proteus species were 100% sensitive to Amikacin. *Klebsiella* species were sensitive to both Amikacin and Gentamicin (90.9%). A knowledge about the bacteriological profile and their antibiotic susceptibility pattern of wound infections will guide in appropriate treatment.

Introduction

Wound infections may be endogenous or exogenous. Endogenous infections or auto infections are caused by organisms that have been leading a commensal existence elsewhere in the patient's body.In exogenous infection the source of infecting organism is outside the body of patient who becomes infected. Infection may occur after accidental or intentional trauma of the skin or other tissue when it is called surgical or postoperative sepsis. A wound infection is defined by the US Centre for Disease Control and Prevention (CDC) as surgical site infection (SSI). This is further defined as:

Superficial incisional SSI – infection involves only skin and subcutaneous tissue of incision.

Deep incisional SSI – infection involves deep tissues, such as facial and muscle layers.

Organ/space SSI – infection involves any part of the anatomy in organs and spaces other than the incision, which was opened or manipulated during the operation.

Although this definition of wound infection is restricted to those arising from a surgical incision, a broader and more general definition would be infection of a wound caused by physical injury of the skin as a result of penetrating trauma from plants, animals, guns, knives or other objects. Wounds break the continuity of the skin and allow organisms to gain access to tissues and cause infection. Wound infections are one of the most common hospital acquired infections and are an important cause of morbidity and account for 70-80% mortality. Gottrup et al (2005) and Wilson et al (2004). Infections arising in surgical wounds are one of the most common hospital acquired infections and are an important cause of morbidity and mortality. Various studies in India have shown that overall postoperative infection rate, following clean surgeries ranged from 3.03% to 4.04%, while in those following clean contaminated surgeries ranged from 10.06 to 22.47%.

For any given type of operation, the development of a wound infection approximately doubles the cost of hospitalization. Nandi et al (1999) and Razavi et al (2005). A knowledge about the bacteriological profile and antibiotic sensitivity pattern helps in managing the wound infections better and reduces the hospital stay as well as cost to the patient. The objective of the present study is to determine the causative aerobic bacteria and antimicrobial sensitivity of wound infections from pus specimen.

Materials and Methods

The study was conducted at Bhaskar General Hospital over a period of one year from October 2014 to October 2015.The study population included patients admitted to different wards in the hospital and also those attending the out patient department with the mean ages from one month to 80 years.

Collection of Material

Relevant clinical history of the patient was taken from patients in the wards with wound infection from different departments of the hospital like surgery, Gynaecology and Obstetrics, Orthopaedics, ENT, Medicine, Dermatology and also patients attending the out patient departments. History regarding the type of surgery,or any other injury or burn or any oozing skin lesion and its duration and other associated symptoms like pain, presence of discharge, redness and swelling. For patients with wounds with copious discharge, the area around the wound was cleaned with 70% ethyl alcohol and the exudates were collected from the depth of the wound using sterile syringe, if adequate amount of exudate was not present, sample was collected by two sterile cotton swabs by gently swabbing the surface of the wound.The swabs were used for Gram stain and culture, a third swab was collected and was put in nutrient broth at the bed side. All the specimens were processed immediately after transported to the laboratory.The nutrient broth was incubated at 37^0C.

A smear was made on a clean glass slide using one of the swabs and stained by Gram's staining. Gram stained smears were analyzed under oil immersion magnification. Presence of pus cells and microorganisms was determined. For each morphologically distinct microorganism seen, the Gram reaction (Gram-positive or Gram-negative), morphology (e.g., coccus, rod, yeast), other

distinguishing features (e.g., formation of chains or clusters) were determined.

The other swab was inoculated on nutrient agar, 5% blood agar and MacConkey agar by rolling the swab over the agar and streaking from primary inoculums using a sterile bacteriological loop. These plates were incubated aerobically at 37°C for 24-48 hours. Primary plates were observed for any visible growth after overnight incubation and if there was no growth after 24 hours, subcultures were done from nutrient broth. Primary plates were further incubated for another 24 hours. Plates were observed for growth. The isolates were identified following standard identification procedures like colony morphology, Gram stained smear from the colony, motility and biochemical tests.Antimicrobial susceptibility testing of the bacterial isolates was done by Kirby Bauer disc diffusion method. The strengths of antibiotic discs used (in μ) are as follows: Ampicillin 25mcg, Amikacin 10 mcg, Gentamicin 30mcg, Cotrimoxazole 25mcg, Levofloxacin 5mcg, Ofloxacin 2mcg, Ciprofloxacin 1mcg, Ceftriaxone 10mcg, Cefoperazone 75mcg, Cefotaxime 10mcg, Cefoxitin 30mcg, Cefuroxime 30mcg, Azithromycin 30mcg, Clarithromycin 15mcg

Results and Discussion

A total of 133 pus samples were received for processing to the Microbiology department. Out of them 57% of the samples were obtained from surgical wound infections and 43 % of the samples were obtained from non-surgical wounds. The age of the patients varied from 1 month (umbilical stump infection) to 80 yrs. Females were slightly 63(51.1%) more than the males 60(48.9).

Out of the 133 pus samples 66 samples (59.6%) were culture positive, two samples showed two bacteria on culture.Out of the 133 pus samples 112 (84.21%) were positive for microorganisms and pus cells by Gram stain but only 68 (51.1%) samples were positive by aerobic culture. 21 samples were negative for microorganisms by Gram stain. Table 1.

Out of the 66 samples that were positive for bacterial growth on aerobic culture, 21 were Staphylococcus aureus, 17 were Pseudomonas aeruginosa, 2 were Pseudomonas fluroscene, 8 were *Escherichia coli*, 11 were Klebsiella species, 7 were Proteus species, and 2 were Coagulase negative Staphylococci (CONS). Two samples showed mixed infection with Klebsiella species and Pseudomonas aeruginosa. Staphylococcus aureus was the most frequently isolated organism (30.88%) followed by Pseudomonas aeruginosa (25.02 %).Table 2.

Antimicrobial susceptibility testing of the bacterial isolates was done by Kirby Bauer disc diffusion method. Among the Gram positive cocci, Staphylococcus aureus and CONS were mostly sensitive to Amikacin (90.4%) followed by Levofloxaccin(76.1%). Table.3.

Among the Gram negative isolates Pseudomonas species were mostly sensitive to Amikacin (82.4%) followed by Ofloxacin (76.4%) Table 4.*Escherichia coli* isolates were 100% sensitive to Amikacin. Table5. Proteus species were also 100% sensitive to Amikacin. Table 6.Klebsiella species were sensitive to both Amikacin and Gentamicin (90.9%).Table 7. Almost all the isolates were mostly sensitive to Amikacin.

The presence and profile of microorganisms in any wound will be influenced by factors such as wound type, depth, location, and quality, the level of tissue perfusion, and the

antimicrobial efficacy of the host immune response. A total of 133 pus samples from infected wounds received from different departments of the hospital were processed to know the bacteriological profile for aerobic bacteria. Out of them 57% of the samples were obtained from surgical wound infections and 43 % of the samples were obtained from non-surgical wounds. This is similar to the study done by Aizza Zafar et al (2008) were in the incidence of surgical wound infection (57%) followed by acute soft tissue infection (43%).

Out of the 133 pus samples 112 (84.21%) were positive for microorganisms and pus cells by Gram stain but only 66 (51.1%) samples were positive by aerobic culture. 21 samples were negative for microorganisms by Gram stain. This differs from the study done by Kaftandzieva et al.(2012) where in their study Gram stain compared to culture showed lower sensitivity(38%), but fair specificity (90%), and a positive predictive value (82.8%). Although the organisms seen on Gram stain were commonly isolated in culture, many specimens yielding a potential pathogen in culture had no organisms seen on the Gram stain. This situation mainly occurred when growth in culture was poor. Where as in our study though the organisms were seen in gram stain (84.21%) culture was positive in only (49.62%) the reason could be that the organisms were anaerobes and anaerobic culture was not not done in our study, hence they could not be isolated on culture.

Out of the 133 pus samples, 66 samples (59.6%) were culture positive, two samples showed two bacteria on culture (Polymicrobial). Staphylococcus aureus was the most frequently isolated organism (30.88%) followed by Pseudomonas aeruginosa (25.02 %).This is similar to the study done by Aizza Zafar et al(2008) were most frequently isolated organism was S. aureus 45 (41.28%) followed by Pseudomonas species 20 (18.35%). However our study differs from the study of Ramesh et al (2013) were most common organism isolated was *E. coli* (20.8%), followed by *S. aureus* (16.1%). This could be because in their study the sample was pus from postoperative wound infections only but we also included pus from infections of skin and soft tissues other than post operative wounds. Almost all the isolates were mostly sensitive to Amikacin.

Table.1 Direct Microscopy and Culture Positivity

Direct Microscopy	Microscopy positive	Culture Positive
Pus cells + Gram positive cocci	40	21
Pus cells + Gram negative bacilli	38	47
Puss cells + Gram positive cocci and bacilli	13	-
Pus cells+ no organisms	21	-
Total	112	68

Table.2 Frequency of Different Pathogens Isolated from Wound Infections

Pathogen	Frequency	Percentage
Staphylococcus aureus	21	30.88
Pseudomonas aeruginosa	17	25.02
Pseudomonas fluroscens	2	2.94
Escherichia coli	8	11.76
Klebsiella species	11	16.17
Proteus species	7	10.29
Coagulase negative Staphylococci	2	2.94
Klebsiella species +Psedomonas aeruginosa -2		
Total	68	100

Table.3 Antibiogram of Isolated Bacteria : Staphylocccus Aureus (n=21)

Antimicrobial agent	Disc concentration in mcg	Number of isolates % Susceptible	Resistant
Staphylocccus aureus (n=21)			
Ampicillin	25mcg	7(33.3%)	14(66.7%)
Amikacin	10 mcg	19(90.4%)	2(9.6%)
Ciprofloxacin	1mcg	11(52.3%)	10(47.7%)
Levofloxacin	5mcg	16(76.1%)	5(23.9%)
Ofloxacin	2mcg	10(47.6%)	11(52.4%)
Azithromycin	30 mcg	11(52.3%)	10(47.7%)
Clarithromycin	15mcg	11(52.3%)	10(47.7%)
Cefoxitin	30mcg	11(52.3%)	10(47.7%)

Table.4 Antibiogram of Isolated Bacteria : Pseudomonas Aeruginosa (n=17)

Antimicrobial agent	Disc concentration in mcg	Number of isolates % Susceptible	Resistant
Pseudomonas aeruginosa (n=17)			
Cefotaxime	10mcg	8(47%)	9(53%)
Cefoperazone	1mcg	7(41.2)	10(58.8)
Ceftriaxone	10mcg	3(17.6%)	14(82.4%)
Ciprofloxacin	1mcg	11(64.7%)	6(35.3%)
Levofloxacin	5mcg	12(70.5%)	5(29.5%)
Ofloxacin	2mcg	13(76.4%)	4(23.6%)
Gentamicin	30mcg	12(70.5%)	5(29.5%)
Amikacin	10mcg	14(82.4%)	3(17.6%)
Cotimoxazole	25mcg	5(29.5%)	12(70.5%)

Table.5 Antibiogram of Isolated Bacteria : *Escherichia coli* (n=8)

Antimicrobial agent	Disc concentration in mcg	Number of isolates % Susceptible	Resistant
Escherichia coli (n=8)			
Cefotaxime	10mcg	0	8(100%)
Cefoperazone	1mcg	0	8(100%)
Ceftriaxone	10mcg	0	8(100%)
Ciprofloxacin	1mcg	1(12.5%)	7(87.5%)
Levofloxacin	5mcg	1(12.5%)	7(87.5%)
Ofloxacin	2mcg	2(25%)	6(75%)
Gentamicin	30mcg	4(50%)	4(50%)
Amikacin	10mcg	8(100%)	0
Cotimoxazole	25mcg	0	8(100%)

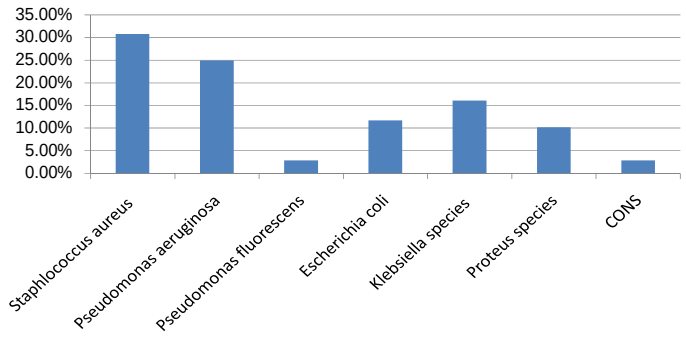
Table.6 Antibiogram of Isolated Bacteria : Proteus Species (n=7)

Antimicrobial agent	Disc concentration in mcg	Number of isolates % Susceptible	Resistant
Proteus Species (n=7)			
Cefotaxime	10mcg	2(28.6%)	5(71.4%)
Cefoperazone	1mcg	3(42.9%)	4(57.1%)
Ceftriaxone	10mcg	2(28.6%)	5(71.4%)
Ciprofloxacin	1mcg	5(71.4%)	2(28.6%)
Levofloxacin	5mcg	5(71.4%)	2(28.6%)
Ofloxacin	2mcg	1(14.3%)	6(85.7%)
Gentamicin	30mcg	2(28.6%)	5(71.4%)
Amikacin	10mcg	7(100%)	0
Cotimoxazole	25mcg	0	7(100%)

Table.7 Antibiogram of Isolated Bacteria : Klebsiella Species (n=11)

Antimicrobial agent	Disc concentration in mcg	Number of isolates % Susceptible	Resistant
Klebsiella species Species (n=11)			
Cefotaxime	10mcg	3(27.2%)	8(72.7%)
Cefoperazone	1mcg	2(18.1%)	9(81.8%)
Ceftriaxone	10mcg	3(27.2%)	8(72.7%)
Ciprofloxacin	1mcg	5(45.4%)	6(54.5%)
Levofloxacin	5mcg	6(54.5%)	5(45.4%)
Ofloxacin	2mcg	4(36.3%)	7(63.6%)
Gentamicin	30mcg	10(90.9%)	1(9.1%)
Amikacin	10mcg	10(90.9%)	1(9.1%)
Cotimoxazole	25mcg	5(45.4%)	6(54.5%)

Frequency of Different Pathogens Isolated from Wound Infections



In conclusion, wound infections are one of the most common hospital acquired infections and are an important cause of morbidity and mortality. Depending on the site of wound infection and clinical symptoms, the role of the microbiology laboratory is to determine the clinically significant isolates, perform antimicrobial susceptibility testing, and subsequently provide guidance on the most appropriate treatment. This will help in successful wound management and will also assist in the control of antibiotic usage and hence curtail the spread of antibiotic-resistant bacteria.

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