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Research Article

Antimicrobial susceptibility pattern of bacterial isolates from wound infection and their sensitivity to antibiotic agents at super specialty hospital, Amravati city, India

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ABSTRACT

Background: Wound infection is one of the health problems that is caused and aggravated by the invasion of pathogenic organisms. Information on local pathogens and sensitivity to antimicrobial agent is crucial for successful treatment of wounds. So the present study was conducted to determine antimicrobial susceptibility pattern of bacterial isolates from wound infection and their sensitivity to antimicrobial agents.
Methods: A retrospective study was conducted among patients with wound infection in Suyash super speciality hospital, from January 2012 to December 2013. Wound swab was collected using sterile cotton swabs and processed for bacterial isolation and susceptibility testing to Systemic antimicrobial agents.
Results: In this study 78 bacterial isolates were recovered from 258 specimens showing an isolation rate of 31.2%. The predominant bacteria isolated from wounds were gram positive staphylococci 36 (46.2%), followed by gram negative streptococci 18 (23.1%) gram negative pseudomonas 12 (15.4 %) and gram negative proteus 8 (10.4%). The gram positive and gram negative bacteria constituted 68 (87.2%) and 10 (12.8%) of bacterial isolates; respectively.
Conclusion: In the present study most of the pathogens isolated from wound isolates showed high rate of resistance to most commonly used newer antibiotics used to treat bacterial infections. Therefore, rational use of antibiotics should be practiced.

Keywords: Bacterial isolates, Antimicrobial susceptibility pattern, Drug resistance, Wound infection

INTRODUCTION

Infection is an important arbiter of success or failure of surgical practice and it is the most common form of postoperative morbidity and a major cause of mortality in all surgical specialties. Surgical wound infection is clinically defined as purulent discharge from the surgical wound.[1] Surgical wound is characterized by inflammation around periwound area. Surgical wound infections are the second most common cause of nosocomial infections.[2] The high rate of surgical wound infections is associated with higher morbidity, mortality and increased medical expenses.[3] In spite of the new antibiotics available today, surgical wound infection still remains a threat due to secondary bacterial contamination and widespread use of prophylactic antibiotics that lead to emergence of multi-drug resistant bacteria.[4] The primary function of intact skin is to control microbial populations that live on the skin surface and to prevent underlying tissue from becoming colonized and invaded by potential pathogens.[5] Exposure of subcutaneous tissue following a loss of skin integrity (i.e. wound) provides a moist, warm, and nutritious environment that is conducive to microbial colonization and proliferation. Since wound colonization is most frequently poly-microbial, involving numerous microorganisms that are potentially pathogenic, any wound is at some risk of becoming infected.[6] Infection in wound constitutes a major barrier to healing and can have an adverse impact on the patient's quality of life as well as on the healing rate of the wound. Infected wounds are likely to be more painful, hypersensitive and odorous, resulting in increased discomfort and inconvenience for the patient.[7] The prevalent organisms that have been associated with wound infection include Staphylococcus aureus (S. aureus) which from various studies have been found to account for 20-40% and Pseudomonas aeruginosa (P. aeruginosa) 5-15% of the nosocomial infection, with infection mainly following surgery and burns. Other pathogens such as Enterococci and members of the Enterobacteriaceae have been implicated, especially in immune compromised patients and following abdominal surgery.[8] Wound healing needs a good healthy environment so that the normal physiological process will result in a normal healing process with minimal scar formation. One of the most important strategies to keep the process of healing ongoing is to sterilize damaged tissue from any microbial infection.[9] Continued use of systemic and topical antimicrobial agents has provided the selective pressure that has led to the emergence of antibiotic resistant strains which in turn, has driven the continued search for new agents. Unfortunately, the increased cost of searching for effective antimicrobial agents and the decreased rate of new drug discovery has made the situation increasingly worrisome.[10] Surgical wound infection has been a major concern among health care practitioners, not only in terms of increased trauma to the patient but also in view of its burden on financial resources and the increasing requirement for cost effective management within the health care system.

Hence the present study is designed to update profile of bacteria present in wounds, their sensitivity to antibiotics and sensitivity to alternative topical agents at super specialty hospital, Amravati city.

METHODS

Study design

This was a retrospective study.

Study duration

The study was conducted in a period of one year from January 2012 to December 2013.

Study area

Study was conducted among patients with wound infection in Suyesh super speciality hospital, study population**.** This included all patients with post-operative wound infections in the orthopedic/trauma ward at Suyesh super speciality hospital.

Inclusion criteria

a. Patients of all age groups except neonates

b. Presence of post-operative wound

c. Giving informed consent to participate

Exclusion criteria

a. Neonates

b. Infection occurring 30 days after operation if no implant is in place

c. Burn injuries and donor sites of split skin grafts

d. Procedures in which healthy skin was not incised such as opening abscess

e. Refusal to give consent for participating in the study

Case definition

Post-operative surgical site infection was defined according to CDC criteria (1, 48). Timing and classification of SSI was used; SSI was classified as superficial, deep incisional or organ/space infection (1, 48), with:

a. Purulent drainage with or without laboratory confirmation from the superficial or deep incision

b. Organism isolated from an aseptically obtained culture of fluid or tissue from superficial or deep incision or organ/space.

c. Sign or symptoms of infection: Pain and tenderness, localized swelling, or heat

d. Purulent drainage from the drain that is placed into the organ/space.

e. Diagnosis of SSI by surgeon.

Sampling procedure

A questionnaire was used to obtain data from the patient after obtaining an informed consent from the patient/guardians. Open wound swabs were aseptically obtained after the wound immediate surface exudates and contaminants were cleansed off with moistened sterile gauze and sterile normal saline solution. Dressed wounds were cleansed with sterile normal saline after removing the dressing. The specimen was collected on sterile cotton swab by rotating with sufficient pressure. Double wound swabs were taken from each wound at a point in time to reduce the chance of contamination. The samples were transported to the laboratory after collection using Amies transport media.

Culture and identification

Swabs collected were streaked on blood agar and MacConkey agar (oxoid) by sterile inoculation loop. The plates were incubated at 35-37°C for 24-48 hours. Preliminary identification of bacteria was based on colony characteristics of the organisms. Such as haemolysis on blood agar, changes in physical appearance in differential media and enzyme activities of the organisms. Biochemical tests were performed on colonies from primary cultures for identification of the isolates. Gram-negative rods were identified by performing a series of biochemical tests (oxoid). Namely: Kligler Iron Agar (KIA), Indole, Simon's citrate agar, Lysine Iron Agar (LIA), urea and motility. Gram-positive cocci were identified based on their gram reaction, catalase and coagulase test results.

Antibacterial susceptibility testing (AST)

Susceptibility testing was performed by Kirby-Bauer disk diffusion technique according to criteria set by CLSI 2011. The inoculum was prepared by picking parts of similar test organisms with a sterile wire loop and suspended in sterile normal saline. The density of suspension to be inoculated was determined by comparison with opacity standard on McFarland 0.5 Barium sulphate solution. The test organism was uniformly seeded over the Mueller-Hinton agar (oxoid) surface and exposed to a concentration gradient of antibiotic diffusing from antibiotic-impregnated paper disk into the agar medium, and then incubated at 37°C for 16-18 hours. Diameters of the zone of inhibition around the discs were measured to the nearest millimeter using a ruler and classified as sensitive, intermediate, and resistant according to the standardized table supplied by CLSI 2011.

The drugs tested for both gram negative and gram positive bacteria were ampicillin (10 µg), ciprofloxacin (5 µg), norfloxacin (10 µg), cephalexin (30 µg), gentamicin (10 µg), tetracycline (30 µg), chloramphenicol (30 µg), doxycycline (30 µg), nalidixic acid (15 µg) and ceftriaxone (30 µg), erythromycin (15 µg) and vancomycin (30 µg) only was used for gram positive bacterial isolates (oxoid). These antimicrobial selected based on the availability and prescription frequency of these drugs in the study area.

Data analysis

Data was edited, cleaned, entered and analyzed using Statistical Package for Social Science (SPSS) version 16.0. Descriptive analysis such as frequencies and mean were used. The Chi-square test was employed to compare the association of socio-demographic data, wound type, location with wound infection status of the patients. P value of <0.05 was considered to indicate statistically significant differences. The result was presented using tables and charts.

RESULTS

Socio-demographic characteristics

A total of 258 specimens were collected from patients with clinical evidence of wound infection (patients with complaints of discharge, pain, swelling, foul smelling and chronic wound) from January 2013 to December 2013. Of the 258 swabs 78 (30.23%) were culture positive for bacterial pathogens, while 180 (69.77%) were bacteriologically sterile. 14 (17.9%) were females and 64 (82.1%) were males. The majority of patients 22 (25.6%) were in age group 26 to 35 followed by 25.6% in 36 to 45 years of age. Thirty two (82.1%) were male and seven (17.9%) were female (Table 1).

Table 1: Age & sex distribution of septicemia suspected patients.

Socio-demographic profile	Number n=78	Percentage
Age (years)		
15-25	16	20.5
26-35	22	28.2
36-45	20	25.6
46-55	14	17.9
56-65	6	7.7
Sex		
Male	64	82.1
Female	14	17.9

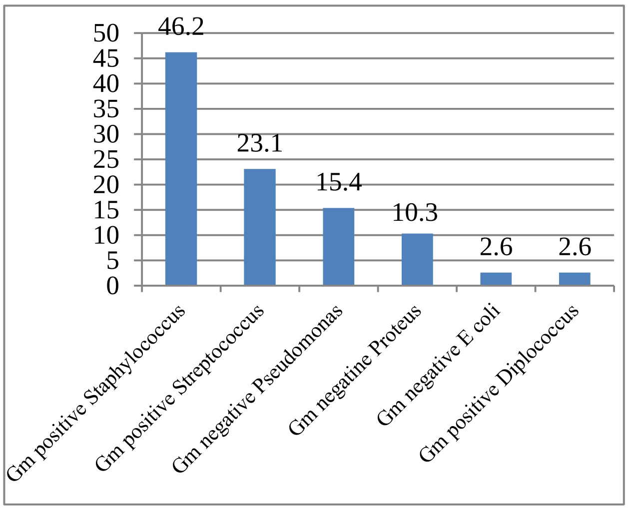

Figure 1: Frequency of bacterial isolates from septicemia suspected patients (Percentage of bacterial isolates).

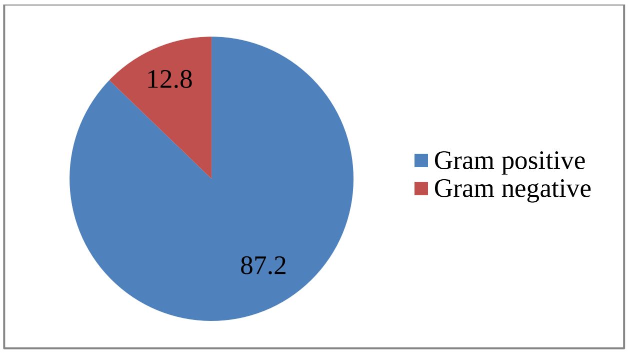

Figure 2: Prevalence of gram positive and gram Negative bacterial isolates. Bacterial isolates (%).

The predominant bacteria isolated from blood culture were gram positive staphylococci 36 (46.2%), followed by gram positive streptococci 18 (23.1%) gram negative pseudomonas 12 (15.4%) and gram negative proteus 8 (10.4%). The gram positive and gram negative bacteria constituted 68 (87.2%) and 10 (12.8%) of the culture isolates; respectively (Figure 1, 2).

Sensitivity patterns of antibiotic

Gram positive microorganisms were sensitive to chloramphenicol 44.4%, azithromycin 22%, cefotaxime 22%, amoxiclav 11.1%, ciprofloxacin 11.1%. Gram negative E. coli were sensitive to erythromycin, ciprofloxacin, chloramphenicol. Gram negative pseudomonas were sensitive to levofloxacin, azithromycin, ofloxacin, tetracycline, imipenem, sparfloxacin and amoxiclav. Gram negative diplococcic were sensitive to imipenem and ceftriaxone. Gram positive streptococci were sensitive to chloramphenicol, levofloxacin, sparfloxacin, gatifloxacin, azithromycin and ofloxacin (Table 2).

Table 2: Sensitivity pattern of the microorganisms.

Sensitive antibiotic	Bacterial isolates					
	Gm +ve Staphylococci N (%)	Gm -ve E. coli N (%)	Gm -ve Psudomonas N (%)	Gm +ve Diplococci N (%)	Gm +ve Streptococci N (%)	Gm -ve Proteus N (%)
Chloramphenicol	16 (44.4)	2 (100)	0	0	6 (33.3)	0
Tetracycline	2 (5.5)	0	2 (16.6)	0	0	0
Cefoperazone	2 (5.5)	0	0	0	0	2 (25)
Levofloxacin	2 (5.5)	0	6 (50)	0	2 (11.1)	0
Erythromycin	4 (11.1)	2 (100)	2 (16.6)	0	0	0
Azithromycin	8 (22.2)	0	4 (33.3)	0	2 (11.1)	2 (25)
Ofloxacin	6 (16.6)	0	4 (33.3)	0	2 (11.1)	0
Ciprofloxacin	4 (11.1)	2 (100)	0	0	4 (22.2)	2 (25)
Imipenem	4 (11.1)	0	2 (16.6)	2 (100)	8 (44.4)	2 (25)
Gatiflox	2 (5.5)	0	0	0	2 (11.1)	2 (25)
Sparflox	0	0	2 (16.6)	0	4 (22.2)	0
Cefotaxime	8 (22.2)	0	0	0	0	2 (25)
Amoxclav	8 (22.2)	0	2 (16.6)	0	0	0
Ceftriaxone	0	0	0	2 (100)	0	0
Norfloxacin	2 (5.5)	0	0	0	0	0
Tobramycin	0	0	3 (33.3)	0	0	0
Bacitracin	2 (5.5)	0	0	0	0	0
kanamycin	2 (5.5)	0	0	0	0	0
Cotrimoxazole	0	0	0	0	0	2 (25)
Nitrofurantoin	0	0	0	0	2 (11.1)	0
Cefoporazone	4 (11.1)	0	0	0	0	0
Total	36 (46.2)	2 (2.6)	12 (15.4)	4 (2.6)	18 (23.1)	8 (10.3)

Resistance patterns of antibiotic

Antimicrobial resistance levels for the gram-negative organisms, causing wound infections were ranging from 5 to 88%. Gram positive groups were resistant to ceftriaxone (88.2%), cefazolin (85.29%), cefdinir (82.35%), cephalexin (58.82%), azithromycin and ofloxacin (11.76%). The range of resistance for Gram negative bacteria were from 20%-60%. 60% were resistant to amoxiclav, sparfloxacin 40%, ampicilin 40% and azithromycin 20% (Table-3).

Table 3: Resistance pattern of the microorganisms.

Resistance antibiotic	Gm +ve (%)	Gm -ve (%)
Cephalexin	40 (58.82)	2 (20)
Ceftriaxone	60 (88.2)	2 (20)
Nitrofurantoin	4 (5.88)	0
Cefixime	6 (8.82)	0
Ceftazidime	8 (11.76)	0
Cefazolin	58 (85.29)	0
Cefadroxyl	8 (11.76)	0
Cefdinir	56 (82.35)	0
Oflox	8 (11.76)	0
Azithromycin	8 (11.76)	2 (20)
Sparfloxacin	4 (5.88)	4 (40)
Amoxiclav	0	6 (60)
Tobramycin	0	0
Ampicillin	0	4 (40)

DISCUSSION

The risk of developing surgical wound infection depends on the number of bacteria that colonise the surgical wound.[11] While the operating wound following surgery is considered to be "clean", the surgical wound may be contaminated by air-borne bacteria in the operating room and intensive care units, by bacteria from endogenous sources such as the patient's mucous membrane, the hands of theatre personnel or by direct contamination by the patient's normal skin microflora.[12] The effect of specific types of microorganisms on wound healing has been widely published, and although the majority of wounds are polymicrobial involving both aerobes and anaerobes, aerobic pathogens such as S. aureus, P. aeruginosa and beta-hemolytic streptococci have been most frequently cited as the cause in delayed.

The present retrospective analysis revealed that incidence of positive culture was 30.23%.

The predominant bacteria isolated from wound swab were gram positive staphylococci 36 (46.2%), followed by gram negative streptococci 18 (23.1%) gram negative pseudomonas 12 (15.4%) and gram negative proteus 8 (10.4%). The gram positive and gram negative bacteria constituted 68 (87.2%) and 10 (12.8%) of the culture isolates respectively.

As reported by other study in present study, S. aureus 36 (46.2%) was the predominant organism isolated from wound infection. Similar finding was found in Onche and O. Adedeji study (2004) Staphylococcus aureus was the most commonly isolated micro-organism in this study accounting for 44%.[13]

The Study by Shittu et al.[14] reported that S. aureus was predominant (25%) followed by E. coli (12%), Pseudomonas aereginosa (9%) and S. epidermidis (9%). Also Sonawane et al.[15] showed that Staphylococcus aureus (29.26%) the commonest isolate followed by Escherichia coli (18.7%) and Pseudomonas spp (15.37%). It has been shown that majority of surgical wound infections are caused by Staphylococcus aureus, and other species of the patients own microflora.[16] A number of reports done previously on wound infection from different parts of the world indicated that S. aureus was the most frequent isolates.[17-21]

Madsen et al.,[22] Thu et al.,[23] Maksimovic et al.,[24] and Markovic et al.,[25] reported that S. aureus were the most common pathogens associated with cases of surgical site infection in orthopedic surgery units. The study by Etok, et al.,[26] 66.7% gram negative and 33.3% gram positive were isolated. Proteus spp (33.3%), Staphylococcus aureus (20%) and E. coli (20%) were the three predominant isolates. This result is in conformity with the findings of Oguachuba[27] who found Proteus spp to be the most common isolate (41.9%) followed by Staphylococcus aureus (25.6%). This finding is different from Gayne et al.[28] in which Pseudomonas spp had the highest prevalence of 33.3%. Onchne[29] found that Staphylococcus aureus accounted for 71.4% of the total isolates; while Mbamali[30] isolated Staphylococcus aureus in 60% of the patients.

The high prevalence of S. aureus infection may be because it is an endogenous source of infection. Infection with this organism may also be due to contamination from the environment e.g. contamination of surgical instruments. With the disruption of natural skin barrier S. aureus, which is a common bacterium on surfaces, easily find their way into wounds.

Gram positive microorganisms were sensitive to chloramphenicol 44.4%, azithromycin 22%, cefotaxime 22%, amoxiclav 11.1%, ciprofloxacin 11.1%. Gram negative E coli were sensitive to erythromycin, ciprofloxacin, chloramphenicol. Gram negative pseudomonas were sensitive to levofloxacin, azithromycin, ofloxacin, tetracycline, imipenem, sparfloxacin and amoxiclav. Gram negative diplococcic were sensitive to imipenem and ceftriaxone. Gram positive streptococci were sensitive to chloramphenicol, levofloxacin, sparfloxacin, gatifloxacin, azithromycin and ofloxacin.

Antimicrobial resistance levels for the Gram-negative organisms, causing wound infections were ranging from 5 to 88%. Gram positive groups were resistant to ceftriaxone (88.2%), cefazolin (85.29%), cefdinir (82.35%), cephalexin (58.82%), azithromycin and ofloxacin (11.76%). The range of resistance for Gram negative bacteria were from 20%-60%. 60% were resistant to amoxiclav, sparfloxacin 40%, ampicillin 40% and azithromycin 20%.

The susceptibility data collected in this study suggests that multidrug resistance is a common problem in hospital pathogens such as Staphylococcus aureus, E.

coli, Klebsiella spp, Pseudomonas spp & Proteus spp etc. Surgical wound infection isolates were found to be resistant to ceftriaxone, cefazolin, cefdinir, cephalexin, azithromycin and ofloxacin. The gram negative bacteria were resistant to amoxiclav, sparfloxacin, ampicillin and azithromycin. This has important implication as patients in a super speciality hospital receiving combinations of these drugs as empirical therapy.

CONCLUSIONS

The most common isolate in wound infection was staphylococci 36 (46.2%), followed by gram negative streptococci 18 (23.1%) gram negative pseudomonas 12 (15.4%) and gram negative proteus 8 (10.4%). The gram positive and gram negative bacteria constituted 68 (87.2%) and 10 (12.8%) of the culture isolates respectively. Gram positive microorganisms were sensitive to chloramphenicol 44.4%, azithromycin 22%, cefotaxime 22%, amoxiclav 11.1%, ciprofloxacin 11.1%. Gram negative E. coli were sensitive to erythromycin, ciprofloxacin, chloramphenicol. These isolates showed high frequency of resistance ceftriaxone, cefazolin, cefdinir, cephalexin, azithromycin and ofloxacin.

The susceptibility data suggests that multidrug resistance is a severe problem in local area. Therefore, rational use of antibiotics should be practiced. Also the pathogen shows susceptibility to certain older drugs suggesting a cyclical wave pattern of susceptibility to antimicrobial agents.

We advocate a rational use of antimicrobial agents rather than the empirical administration of systemic antimicrobials. The in vivo susceptibility of the antimicrobials agents correlates well with the in vitro susceptibility reports and in our majority of the patients achieved a good therapeutic response.

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