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Original Article

Bacteriological profile and antibiotic susceptibility patterns of wound infections at Uttara Adhunik Medical College Hospital, Dhaka

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Abstract

Background and objectives: Wound infection is one of the most important causes of morbidity and mortality worldwide and antibiotic resistant bacteria are a great part of complications in treatment of the infection. The present study was conducted to isolate and identify the etiological agents of wound infection and to assess the antimicrobial susceptibility pattern of the isolates.

Methodology: The retrospective study was carried out on wound infection suspected patients for six months duration. The collected pus specimens were first observed macroscopically then streaked on MacConkey agar, blood agar and incubated at 35°C for 24 to 48 hours. The isolated bacteria were identified by macroscopic and microscopic observations and biochemical reactions. Antibiotic susceptibility pattern of the isolates were assessed by Modified Kirby Bauer disc diffusion technique.

Results: A total of 146 pus samples were collected; of which 84 (57.5%) showed bacterial growth. Out of a total 84 bacterial isolates; 47 (55.95%) were gram negative and 37 (44.05%) were gram positive bacteria. *Staphylococcus aureus* (42.86%) was the most common bacteria followed by *Pseudomonas* spp. (25%), *Escherichia coli* (15.48%), *Klebsiella pneumoniae* (5.95%), *Proteus* spp (4.76%), *Enterobacter* spp (3.57%) and *Streptococcus* spp. (1.19%). *S.aureus* isolates were sensitive to Linezolid (100%), Rifampicin (100%), Doxycycline (86.11%), Clindamycin (83.33%) and Cloxacillin (75%). Low sensitivity of *S.aureus* to Ciprofloxacin (33.33%) and Azithromycin (33.33%). Among gram negative isolates *Pseudomonas aeruginosa* were found highly sensitive to Imipenem (93.54%), Piperacillin-Tazobactum (87.09%), Cefepime (80.64%) and Amikacin (77.42%). Low sensitivity of *Pseudomonas aeruginosa* to Aztreonam (35.48%) and Ceftazidime (51.61%). Isolated *Escherichia coli* were highly sensitive (98%) to Imipenem, Amikacin and showed lowest sensitivity to almost all of the other drugs.

Conclusion: By this study it is recommended, culture of wound swab and antibiotic susceptibility testing should be done before starting antibiotics, which will guide medical practitioners for empirical treatment of wound infection, so as to reduce the spread of drug resistant bacteria.

Introduction

A wound is a breakdown in the protective function of the skin; the loss of continuity of epithelium with or without loss of underlying connective tissue. Wounds can be accidental, pathological or post-operative. These wounds range from minor cuts and burns to major surgical wounds and body ulcers. Pathogen infecting wounds can originate either from the external environment or from the patient's endogenous flora such as the patient's skin, mucous membranes or gastrointestinal tract. Wound infection is thus the presence of pus in a lesion as well as the general or local features of sepsis such as pyrexia, pain and induration. Wound infections usually occur when the virulence factors of the pathogen overcome the host

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immune system. Pyogenic infections are an important cause of sepsis[1]. It results in limb loss, long hospital stays, higher costs and is responsible for significant human mortality and morbidity worldwide. It is also important because it can delay healing and cause wound breakdown[2]. The causative agents of wound infections may vary with the geographical location, from hospital to hospital and with different surgical procedures performed. The commonest organism causing wound infections are *Staphylococcus aureus* followed by other gram-negative bacilli[1]. Bacterial wound infections are treated with different types of antibiotics. The selection of the suitable antibiotic depends on a number of factors including the causative agent, the site and severity of the infection. This means that physicians need to know the prevalent organisms and the resistance patterns existing in their localities. These infections are difficult to treat because of the pathogens with increasing antibiotic resistance[3]. The indiscriminate use of antibiotics has also led to increase in multidrug resistant organisms (MDRO)[4]. In the present era infections have become the leading cause of morbidity in patients of surgery, trauma etc[5].

Wound Infection

Wound infection is one of the most common hospital acquired infections[6]. Hence, the present study was carried out to identify the causative agent of wound infection and antibiotic susceptibility pattern of the isolates, which will be beneficial as guidance for medical practitioners to select empirical antimicrobial therapy and on the implementation of infection control measures that plays an important role in minimizing the emergence rate of antimicrobial resistance (MDR).

Materials and method

A retrospective analysis of pus samples were done at Microbiology department of Uttara Adhunik Medical College Hospital from various departments (General surgery, Orthopedics surgery) between the months of 1st April to 30th September 2017. A total of 146 pus samples were cultured. The pus specimens were macroscopically analyzed for their colour and odour, streaked on MacConkey agar and Blood agar plates and incubated at 35°C for 24 to 48 hours. All the isolates were further processed according to the standard operating procedure (SOP) of the laboratory for its complete identification. Pure cultures of bacterial isolates were subsequently subjected to species identification and confirmation. Gram-positive isolates were identified using catalase and coagulase tests. Isolates of members of the *Enterobacteriaceae* family were identified biochemically by means of a series of tests: catalase, indole, citrate, urease, H_2S production and triple-sugar iron. Non-lactose fermenting Gram-negative bacteria was identified by indole, triple-sugar iron, urease, oxidase and catalase tests[7]. Antimicrobial susceptibility tests were performed by using the modified Kirby-Bauer disc diffusion method and susceptibility patterns were determined following CLSI guidelines[8]. Diameters of the zone of inhibition were measured to the nearest millimetre and categorized as sensitive, intermediate and resistant according to CLSI guidelines[9]. Isolates were classified as either susceptible or resistant to an antibiotic and all the isolates with intermediate resistance were classified as resistant. Culture media and antibiotic discs used in the study were obtained from Oxoid Ltd, UK. Demographic data regarding the pathogen isolated and its antimicrobial susceptibility were collected and analyzed.

Quality Control

Reference strains of *Escherichia. coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853) and *Staphylococcus aureus* (ATCC 25923) were used as a control reference strains for identification and drug susceptibility testing. Quality control for media was done by randomly taking the prepared culture media and incubating over night to see for any growth. Isolates of *Staphylococcus aureus* were further tested for methicillin resistance according to the CLSI guidelines by using Cefoxitin disc.

Results

Out of 146 pus samples from wound infection suspected patients, 84 (57.5%) showed bacterial growth, whereas 62 (42.5%) showed no growth (Table 1).

Table-1: Culture positivity of the study populations (n=146)

Culture	Frequency	Percentage
Growth	84	57.5
No growth	62	42.5
Total	146	100

Among 84 bacterial isolates, gram-negative bacteria were predominant with 47(55.95%) isolates, while gram-positive bacteria contributed 37 (44.05%) of total isolates. Altogether seven different bacterial species were isolated, among which *S. aureus* (42.86%) were predominant, followed by *Pseudomonas aeruginosa* (25%), *E.coli*

(15.48%), *K.pneumoniae* (5.95%), *Proteus* spp. (4.76%), *Enterobacter* spp (3.57%) and *Streptococcus pyogenes* (1.19%) (Table 2).

Table-2: Organisms isolated from wound swab (n=84)

All the bacterial isolates were tested for antimicrobial susceptibility. *S. aureus* was found highly sensitive to Linezolid (100%), Rifampicin (100%), Doxycycline (86.11%), Clindamycin (83.33%) and Cloxacillin (75%). Low sensitivity of *S. aureus* to Ciprofloxacin (33.33%) and Azithromycin (33.33%) was found. (Table-3)

Isolated organisms	Number	Percentge (%)
Staphylococcus aureus	36	42.86
Pseudomonas aeruginosa	21	25
Escherichia coli	13	15.48
Klebsiella pneumoniae	5	5.95
Proteus spp.	4	4.76
Enterobacter spp.	3	3.57
Streptococcus pyogenes	1	1.19
Total	84	100

Table-3: Antibiotic sensitivity pattern of *S.aureus* (n=36) in wound infections.

Antibiotics	S.aureus, 36(%)
Linezolid	36 (100)
Rifampicin	36 (100)
Doxycycline	31 (86.11)
Clindamycin	30 (83.33)
Cloxacillin	27 (75)
Cotrimoxazole	26 (72.22)
Azithromycin	12 (33.33)
Ciprofloxacin	12 (33.33)

Among isolated gram-negative bacteria, *Pseudomonas aeruginosa* were highly sensitive to Imipenem (93.54%), Piperacilli-Tazobactum (87.09%), Cefepime (80.64%) and Amikacin (77.42%). Low sensitivity of the bacteria to Aztreonam (35.48%) and Ceftazidime (51.61%). Isolated *Escherichia coli* were found 98% sensitive to Imipenem, Amikacin and showed lowest sensitivity to almost all of the other drugs (Table-4).

Table-4: Antibiotic sensitivity pattern of the isolated gram-negative bacteria in wound infections.

Antibiotics	*P.aeruginosa* N=31	*E.coli* N=13	*K.pneumoniae* N=5	*Enterobacter* spp N=3	*Proteus* spp N=4
Cefepime	25 (80.64)	3 (24)	2 (40)	2 (67.5)	4 (100)
Ceftriaxone		2 (15.3)	2 (40)	2 (67.5)	4 (100)
Imipenem	29 (93.54)	12 (98)	4 (80)	1 (33.33)	2 (50)
Amikacin	24 (77.42)	12 (98)	2 (40)	3 (100)	4 (100)
Piperacilli-Tazobactam	27 (87.09)				
Ciprofloxacin		3 (23.71)	1 (20)	1 (33.33)	1 (25)
Amoxyclav		3 (23.71)	1 (20)	0 (00)	2 (50)
Aztreonam	11 (35.48)	3 (23.71)	2 (40)	2 (67.5)	4 (100)
Ceftazidime	16 (51.61)	4 (30.76)	2 (40)	1 (33.33)	4 (100)
Cefixime		4 (30.76)	2 (40)	2 (67.5)	3 (67)
Gentamicin	18 (58.06)	7 (53.84)	2 (40)	2 (67.5)	2 (50)

Discussion

Pyogenic infections are characterized by inflammation with pus formation. These infections may be endogenous or exogenous[9]. Loss of skin integrity by various factors would provide an environment for the colonization and growth of microorganisms. The growth of the pathogens depends on the type of wound such as in clean wounds the growth would be minimal where as in traumatic wounds there would be an increased chance of infection requiring an aggressive management[10,11]. In this study out of 146 samples, 84 (57.5%) showed bacterial growth. A study by Maharjan[12] showed similar result; 50.95% growth but by Puyal[13] showed 71.84% growth. The lesser percentage of growth positive cases may be due to the collection of samples from patients taking antibiotics.

Among the total 84 bacterial isolates, 55.95% isolates were gram negative, while gram positive bacteria contributed to 44.05%. Similar study was conducted by Acharya[14] & Yakha et al[15] where gram negative bacteria were predominant. Isolation of gram negative bacteria, during this study was higher, as they are more prevalent aerobes and facultative anaerobes in abscesses and skin wound. These bacteria have well recognized property for abscess formation.

Altogether, 7 different bacterial species were isolated with *S. aureus* (42.86%) being the predominant one followed by *Pseudomonas aeruginosa* (25%), *E. coli* (15.48%), *K. pneumoniae* (5.95%), *Proteus* spp. (4.76%), *Entero-*

bacter spp (43.57%) and *Streptococcus pyogenes* (1.19%). Similar studies carried out by Tarana MN et al, showed *S.aureus* (35.79%) was the most prevalent bacteria among the total cases[16] *Staphylococcus aureus* infection is usually associated with patient's own endogenous flora and it is a skin and nasal microbial flora, acquired also from contaminated hospital environment, surgical devices or from hands of health care workers[17].

In this study, *Staphylococcus aureus* showed 100% sensitive to Linezolid and Rifampicin and low sensitive to Azithromycin and Ciprofloxacin (33.33%). Similar study was found by Tarana MN et al and showed that *Staphylococcus aureus* 94.11% sensitive to Linezolid but dissimilar with Azithromycin 57.35%[16]. Remarkable susceptibility of *Staphylococcus aureus* to Linezolid, Rifampicin, Doxycycline and Clindamycin may be due to less use of these antibiotics as a result of their less availability, cost and toxic effect[18]. *Pseudomonas aeruginosa* showed susceptible to Imipenem (93.54%), Piperacillin+tazobactum (87.09%), Cefepime (80.64%) and Amikacin (77.42%). Similar study was done by Anbumani et al[19] that has shown susceptibility pattern to Imipenem 100%, Piperacillin+ tazobactum (87.71%). The resistance to Amikacin typically results from drugs inactivation by plasmid or chromosome-encoded enzymes, although enzyme-independent resistance result from defect in uptake and accumulation[20].

Conclusion

The most likely causative organisms and prevailing drug susceptibility pattern of this study may be helpful in deciding empirical therapy to reduce mortality and morbidity in wound infections. Therefore, periodic review of the bacteriological profile and antibiotic susceptibility pattern should be done at regular intervals to evolve the control strategies and reduce the infection.

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