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Original Research Article.

Antimicrobial Susceptibility Pattern of Bacterial Isolates Obtained From Wound Infections at a Tertiary Care Hospital of Nepal

Amrita Dhakal[1*], Ramendra Raman[2]

[1*]Lecturer, Devdaha Medical College and Research Institute, Kathmandu University, Devdaha, Nepal.
[2]Assistant Professor, KDC Dental & General Hospital, Kathmandu, Nepal.

ABSTRACT

Introduction: Wound infections are one of the most common hospital acquired infections and are an important cause of morbidity and account for 70-80% mortality.

Objectives: The present study was conducted to isolate and identify the etiological agents of wound infection and assess the antimicrobial susceptibility pattern of the bacterial isolates.

Materials and Methods: This prospective observational study was conducted in the department of microbiology of Devdaha Medical College and Research Institute during March 2015 to September 2017. Pus samples received from indoor and outdoor patients were processed according to the standard protocol. Antimicrobial susceptibility pattern of isolates were performed by Modified Kirby Bauer disc diffusion method.

Results: A total of 105 pus samples were collected of which 82 (78.1%) showed bacterial growth. Among 82 bacterial isolates, 46 (56.1%) were Gram negative and 46 (43.9%) were Gram positive bacteria. *Staphylococcus aureus* (41.5%) was the most predominant bacteria followed by *Escherichia coli* (25.6%) and *Klebsiella* species (20.7)

Conclusion: The present study implies that the wound infection is ongoing problem. The main threats of wound infections are *Staphylococcus aureus*, *Escherichia coli* and *Klebsiella* species. So we should minimize the wound contamination by using appropriate antibiotics with continuous surveillance to monitor antimicrobial susceptibility patterns of common isolates found in wound infection.

Keywords: Pus, Antimicrobial Susceptibility, *Staphylococcus aureus.*

***Correspondence to:**
Amrita Dhakal,
Department of Microbiology, DMCRI,
Kathmandu University, Devdaha, Rupandehi, Nepal.
Email: amudhakal3@gmail.com; Cell No.: +977-9851194867

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INTRODUCTION

A wound is a rupture in the skin along with the exposure of subcutaneous tissue following loss of skin integrity which provides a moist, warm and nutritive environment that is conducive to microbial colonization and proliferation.[1,2] Infection of wound results in the production of pus.[3] Pus is a whitish yellow discharge resulting in an accumulation of the body's defense mechanism which is generated during an inflammatory pyogenic infection due to bacteria.[4]

Wound can be contaminated by a variety of microorganisms ranging from bacteria to fungi and parasites as well as virus.[1,2] The most common pus generating bacteria are *Staphylococcus aureus (S.aureus)*, *Klebsiella* species, *Pseudomonas*, *Escherichia coli* and *Streptococci* among which *S. aureus* is the most common bacterial agent that induces pus.[5]

Wound infections are one of the most common hospital associated infections and are an important cause of morbidity and account for 70-80% mortality.[6,7] Improper and extended use of systemic and topical antimicrobial agents has provided the selective pressure resulting in the emergence of antimicrobial resistant strains.[8] Therefore, there is a requirement of regular bacteriological review of wound infections to provide the most qualitative health care facilities to the infected persons.[7]

The present study is aimed to determine the bacteriological agents causing wound infections and their antimicrobial susceptibility patterns, which is fruitful in the management of these infected wounds and formulating a Rational Antibiotic Policy in Rupandehi district of Nepal.

MATERIALS AND METHODS

Sample Collection and Processing

This prospective observational study was conducted after the permission of ethical committee. A total of 105 pus samples were collected in the department of microbiology of Devdaha medical College and Research Institute. The study was conducted during March 2015 to September 2017 from the patients attending indoor and outdoor department of hospital. Two wound swabs on a sterile cotton swab (one for Gram stain and another for culture) or aspirated pus in a syringe were collected for the study. Each sample was inoculated on blood agar and MacConkey agar plate. Inoculated agar plates were incubated at 37 °C for 24 hours aerobically. After incubation, growth of positive cultures was identified using standard microbiological technique including characteristic colony morphology, gram staining, motility testing and biochemical tests. The isolates were classified into Gram positive and Gram negative bacteria accordingly on the basis of biochemical test such as catalase, coagulase, oxidase, triple sugar iron agar, sulphide indole medium, citrate utilization and urease production.[9]

Antimicrobial Susceptibility Test

The antibiotic sensitivity of isolated organism was carried out by using Modified Kirby Bauer disc diffusion method on Muller Hinton agar by using commercially available antibiotic discs (Hi Media. Mumbai, India).

Results were interpreted as sensitive or resistant and compared with standard chart as for standard strain. The ATCC standard strain of *Escherichia coli* (25922), *Staphylococcus aureus* (25923) and *Pseudomonas aeruginosa* (27853) were used as quality control throughout the study for culture and antimicrobial susceptibility test.[10]

Identification of Multidrug Resistant (MDR) Isolates

Multidrug resistant (MDR) bacterial isolates were identified according to the criteria recommended by international expert committee of the European Centre for Disease Prevention and Control (ECDC) and the Centers for Disease Control and Prevention (CDC).[11] In this study, the isolates resistant to at least one antimicrobial agent from three different groups of first line drugs tested were regarded as MDR.

Statistical Analysis

Data were analyzed using statistical software SPSS version 16. Chi-square test was applied to calculate probabilities and significance. A p-value of less than or equal to 0.05 was considered to be statistically significant ($p \leq 0.05$).

Table 1: Culture positivity of wound infection

Total no of sample	No of sample showing growth	Sterile sample	% of culture positivity
105	82	23	78.1%

Table 2: Age and gender distribution of positive cases of wound infection

Age group	Female (%)	Male (%)	Total (%)
1-15 year	5(6.1)	8(9.8)	13(15.9)
16-45 year	33(40.2)	12(14.8)	45(54.9)
46-60 year	8(9.8)	8(9.6)	16(19.5)
Above 60 year	6(7.3)	2(2.4)	8(9.7)
Total	52(63.4)	30(36.6)	82(100)

Table 3: Distribution of isolates collected from outdoor and indoor patients

Department	Frequency	Percentage
Outdoor	22	26.8
Indoor	60	73.2
Total	82	100

Table 4: Pattern of Gram negative and Gram positive isolates

Gram negative isolates		Gram positive isolates	
Name	**Number (%)**	**Name**	**Number (%)**
Escherichia coli	21(25.6)	***Staphylococcus aureus***	34(41.5)
Klebsiella species	17(20.7)	***Streptococcus pyogenes***	2(2.4)
***Pseudomonas* species**	8(9.8)		
Total isolates (%)	46(56.1)	**Total isolates (%)**	36(43.9)

Table 5: Antimicrobial sensitivity pattern of Gram negative bacteria

Antibiotics	No of samples	Sensitive (%)	Resistant (%)
Amikacin	39	34(87.2)	5(12.8)
Ciprofloxacin	21	5(23.8)	16(76.2)
Ceftriaxone	22	12(54.5)	10(45.5)
Ampicillin	31	5(16.1)	26(83.9)
Gentamicin	28	19(67.9)	9(32.1)
Cefixime	33	13(39.4)	20(60.6)

Table 6: Antimicrobial sensitivity pattern of Gram positive bacteria

Antibiotics	No of samples	Sensitive (%)	Resistant (%)
Ciprofloxacin	16	5(31.2)	11(68.2)
Ceftriaxone	10	4(40)	6(60)
Ampicillin	17	6(35.3)	11(64.7)
Gentamicin	31	20(64.5)	11(35.5)
Cotrimoxazole	24	10(41.7)	14(58.3)
Clindamycin	7	5(71.4)	2(28.6)

Table 7: Antibiotic sensitivity pattern of *Staphylococcus aureus*

Antibiotics	No of samples	Sensitive (%)	Resistant (%)
Ciprofloxacin	14	5(35.7)	9(64.3)
Ceftriaxone	10	4(40)	6(60)
Ampicillin	16	6(37.5)	1062.5)
Gentamicin	29	19(65.5)	10(34.5)
Cotrimoxazole	23	10(43.5)	13(56.5)
Clindamycin	7	5(71.4)	2(28.6)

Table 8: Antibiotic sensitivity pattern of *Escherichia coli*

Antibiotics	No of samples	Sensitive (%)	Resistant (%)
Amikacin	17	15(88.2)	2(11.8)
Ciprofloxacin	16	3(18.8)	13(81.2)
Ceftriaxone	13	8(61.5)	5(38.4)
Ampicillin	15	2(13.3)	13(86.7)
Gentamicin	15	9(60)	6(40)
Cefixime	15	7(46.7)	8(53.3)

Table 9: Distribution of MDR pathogens among indoor and outdoor patients

Department	Total isolates	MDR (%)	P value
Indoor	60	14(23.3)	
Outdoor	22	5(22.7)	0.954
Total	82	19(23.2)	

Table 10: Distribution of MDR pathogens among male and female patients

Department	Total isolates	MDR (%)	P value
Female	52	14(26.9)	
Male	30	5(16.6)	0.289
Total	82	19(23.2)	

RESULTS

Out of 105 samples, 82 (78.1%) were found to be culture positive. (Table 1)

Infection rate was higher in female (63.4%) as compared to male (36.6%) and in the age group 16-45 years (54.9%). (Table 2)

High rate of wound infection was observed in indoor patients (73.2%) than in outdoor patients (26.8%). (Table 3)

Gram negative bacteria were predominant with 46 (56.1%) isolates while Gram positive bacteria constituted 46 (43.9%) of total isolates. Altogether 5 species of bacteria were isolated among which, *Staphylococcus aureus* (41.5%) was predominant followed by *Escherichia coli* (25.6%) and *Klebsiella* species (20.7), (Table 4).

Most of the Gram negative bacterial isolates were found to be sensitive to amikacin (87.2%) followed by gentamicin (67.9%) and ceftriaxone (54.5%). Ampicillin (16.1%) was the least effective antibiotic among Gram negative bacterial isolates. (Table 5)

Most of the Gram positive bacterial isolates were found to be sensitive to clindamycin (71.4%) followed by gentamicin (64.5%). Ciprofloxacin (31.2%) was the least effective antibiotic among Gram positive bacterial isolates. (Table 6)

Among 34 isolates of *Staphylococcus aureus*, the most effective antibiotic was found to be clindamycin with (71.4%) sensitivity followed by gentamicin with (65.5%) sensitivity. (Table 7)

Among 21 isolates of *Escherichia* coli, the most effective antibiotic was found to be amikacin with (88.2%) sensitivity followed by ceftriaxone with (61.2%) and gentamicin (60%) sensitivity respectively. (Table 8)

Among 82 bacterial isolates, 19(23.2%) were found to be MDR isolates. Out of 60 isolates from indoor patients, 14 (23.3%) were found to be MDR strains, and out of 32 isolates from outdoor patients, 5 (22.7%) were found to be MDR strains. The results were statistically insignificant (p=0.954). (Table 9)

Out of 52 isolates from female patients, 14 (26.9%) were MDR strains and out of 30 isolates from male patients, 5 (16.6%) were MDR strains and the results were statistically insignificant (p=0.289). (Table 10)

DISCUSSION

Out of 105 samples, 82 (78.1%) were having culture positivity. Our study was in accordance with the study carried out by Kishor K et al (83.1%).[12] This inequality might be because of variations in the methods of collection of wound swab, as it needs proper cleaning of the wound surface before sample collection to avoid skin contaminants like coagulase negative staphylococcus.[8]

The incidence of wound infection was found higher in case of females (63.4%) than in males (36.6%). This was in contrast to studies done by many authors of Nepal (Mahat P et al, Yaka J. K. et al and Chaudhary P et al)[1,2,13] as well as other countries (Mama M et al[14] and Kamble P et al[15]). There is no explainable reason for such incidence.

The highest growth rate of wound infection was documented at the age group of 16-45 years (54.9%). This was somehow similar to the observation done by Yaka JK et al.[2] Infection rate was higher in indoor patients (73.2%) as compared to outdoor patients (26.8%) which was in relation with the study conducted by Mahat P et al.[1] This may be because of factors acquiring nosocomial pathogens in patients with long term hospitalization and prior administration of antibiotics.

Among 82 bacterial isolates, 56.1% were gram negative bacteria and 43.9% were gram positive bacteria. Similar reports were found by Mahat P et al,[1] Yaka JK et al[2] and Acharya J et al.[17] Gram negative bacteria were found to be leading agent causing wound infection because they are more prevalent aerobes and facultative anaerobes in abscesses and skin wound .

In the present study, *Staphylococcus aureus* was the predominant organism (41.5%) causing wound infection. This was in agreement with the study done by Mantravadi HB et al,[4] Chaudhary P et al,[13]Amatya J et al[16] and Acharya J et al.[17] This may be because it is an endogenous source of infection and infection with *Staphylococcus aureus* may also be due to contamination of the wound from the environment, like from surgical instruments and health professionals.[8]

The most commonly isolated Gram negative bacterium was *Escherichia coli* followed by *Klebsiella* species in our study. Although *Staphylococcus aureus* was the most predominant organism found in pus sample, Gram positive cocci accounted for only 43.9% of the total isolates and 56.1% being Gram negative bacilli. Such similarity was shown in study reported by Mantravadi HM et al[4] and Raza MS et al.[18]

It is observed that the most effective antibiotic for Gram negative bacterial isolates was amikacin (87.2%) followed by gentamicin (67.9%). This observation was correlated with the study carried out by Parajuli P et al[6] and Chaudhary P et al.[13] Similarly, ampicillin (16.1%) was the least sensitive antibiotic among Gram negatives bacterial isolates which was in line with the documentation of Hailu D et al[8] and Acharya J et al.[17]

The most effective antibiotics for Gram positive bacterial isolates was clindamycin (71.4%) followed by gentamicin (64.5%) while ciprofloxacin (31.2%) was found to be the least effective antibiotic. Similar sensitivity pattern was shown by the isolates of *Staphylococcus aureus* also. In the study conducted by Mahat P et al,[1] clindamycin (91.53%) and gentamicin (66.1%) were more effective antibiotics for Gram positive isolates while ciprofloxacin (43.5%) was found to be somehow effective as compared to penicillin G and amoxycillin.

In the present study, the percentage of isolation of MDR strains was found to be very low (23.2%). This was in contrast to the study done by various authors like Yaka JK et al[2], Chaudhary P et al[13] and Manyahi J et al.[19] Exact reason behind the variation in the antibiotic resistance pattern might be the difference in study population including.

CONCLUSION

The present study implies that the wound infection is ongoing problem. The main threats of wound infections are *Staphylococcus aureus*, *Escherichia coli* and *Klebsiella* species. Wound infection cannot be eradicated easily from our environment. So we should implement certain preventive measures for the reduction of wound infection such as proper treatment protocols, good disinfection, clean surgical procedures, proper care of wound and good hygiene practices. Similarly, we should minimize the wound contamination by using appropriate antibiotics with continuous surveillance to monitor antimicrobial susceptibility patterns of common isolates found in wound infection. According to our study, higher incidence of wound infection was found in indoor patients, the prime focus should be given to the personal hygiene of the patients and health personnel as well as the entire hospital environment should be kept disinfected.

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