

Bacteriological profile and antimicrobial susceptibility patterns of wound infections among adult patients attending Gandaki Medical College Teaching Hospital, Nepal.

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ABSTRACT

Introduction: Wound infections are significant group of infections in the hospitals worldwide. The wide spread uses of antimicrobial agents lead to emergence of resistant pathogens contributing to increased morbidity and mortality. Accurate and prompt antimicrobial therapy is required to reduce the complications. This study was aimed to investigate pyogenic bacterial pathogens and their susceptibility patterns. **Methods:** A cross sectional study was carried out at Gandaki Medical College Teaching Hospital from July to December 2018. Wound specimens obtained from adult patients were inoculated onto appropriate media and pathogens were identified using standard microbiological methods. Antimicrobial susceptibility patterns were determined by Kirby-Bauer disc diffusion method following the guidelines of Clinical and Laboratory Standard Institute (CLSI). **Results:** A total of 264 specimens were included in the study of which 167 (63.3%) were positive for bacterial growth. Of these, polymicrobial growth was observed in two specimens. Gram positive bacteria (119, 70.4%) were the leading cause of infections, *Staphylococcus aureus* (102, 85.7%) being the most dominant. Among the Gram negative pathogens (50, 29.6%), *Escherichia coli* (31, 62%) was found to be the predominant followed by *Pseudomonas aeruginosa* (10, 20%). Overall, the isolates were resistance to Ampicillin (90.7%), Amoxicillin (64.9%), Cloxacillin (68%), Ofloxacin (61.5%) and Cotrimoxazole (55.6%). Lesser rates of resistance were observed to Doxycycline, Erythromycin, Amikacin, Gentamicin and Imipenem. **Conclusion:** This study revealed the most common pathogens causing pyogenic wound infections in our setting. Again, these pathogens are resistance to commonly used antibiotics. Therefore, this study could be helpful to develop proper guidelines of antibiotics to be used for prophylactic and empiric treatment.

Key words: Wound Infection, Bacterial Pathogens, Antimicrobial Resistance, MDR

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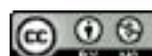
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INTRODUCTION

Any abrasion or breach in the skin surface provides an open door for bacterial entry. Wound provides moist and nutritive enriched environment for colonization and proliferation of bacteria to establish an infection.¹ Entry of bacterial pathogens and their lodgment provoke host immune system, bringing defensive immune cells into the area and eventually formation of pus. Therefore, infected wounds are characterized by inflammation and pus formation with bacterial burden.² Some of the common bacterial pathogens causing pyogenic wound infection include *S. aureus*, *S. pyogenes*, *Enterococcus* spp., *E. coli*, *Klebsiella* spp., *Pseudomonas aeruginosa*, *Proteus* spp. and *Acinetobacter* spp.^{3,4} However, the epidemiological and antimicrobial susceptibility pattern of these pathogens vary from one country to the other



and also in different geographical areas within the same country.⁵ Therefore, investigation of bacterial pathogens associated with wound infection and their susceptibility pattern should be carried out in each setting to establish empirical therapy for pyogenic infections.

In developing countries like Nepal, wound infections, although preventable and curable remained one of the major public health problems. Moreover, emergence of multidrug resistance pathogens pose extra burden on management of pyogenic infections.⁴ The crisis of antimicrobial resistance in developing countries has been attributed to misuse of antibiotic practices such as, over use, under use, and inappropriate use. Important drug resistant pyogenic infections causing pathogens in Nepal include Methicillin and Vancomycin resistance *S. aureus*, Vancomycin resistance *Enterococcus* and Extended-spectrum beta-lactamase (ESBL) producing Gram negative bacilli such as *E. coli*, *Klebsiella* spp. and *Pseudomonas aeruginosa*.⁶ Therefore, knowledge of causative agents and their resistance pattern would be helpful for management of pyogenic infections in each setting.

Although different studies, including in Kathmandu, had been conducted to access the bacterial profiles of pyogenic infections,^{4,7,8} those data might not be consistent enough to describe the current trend of our region. Therefore, this study aimed to characterize the bacterial etiological agents causing pyogenic wound infections and to determine the antimicrobial susceptibility patterns of those isolates to commonly used antimicrobial agents.

MATERIALS AND METHODS

Study design and population: A cross sectional study was carried out from July to December, 2018 at Clinical Laboratory of Gandaki Medical College Teaching Hospital, Pokhara Nepal. All patients more than 15 years of age fulfilling the criteria of wound infection,² who visited the hospital during the study period were enrolled in this study. Patients undergoing antimicrobial therapy were excluded. Ethical approval for the study was obtained from Gandaki Medical College Institution Review Committee.

Sample collection, processing and culture methods: All clinical samples such as pus, pus aspirates and wound swabs collected aseptically were processed immediately in the laboratory. The specimens were inoculated onto MacConkeys agar, Blood agar and Chocolate agar (HiMedia Laboratories, India) plates. Inoculated MacConkeys and Blood agar plates were incubated in aerobic condition while Chocolate agar plates were incubated in 5-10% CO₂ atmosphere environment at 37°C for 24-48 hours.

Bacterial isolates were identified using standard protocols of the WHO.⁹ Antimicrobial susceptibility testing (AST) was carried out by Kirby-Bauer disc diffusion method on Mueller Hinton agar or Blood agar (HiMedia, India) as recommended by CLSI guideline.¹⁰ AST was performed against antibiotics (HiMedia, India), Ampicillin (10 µg), Amoxycillin (10 µg), Cloxacillin (5 µg), Cotrimoxazole (25 µg), Ofloxacin (5 µg), Doxycycline (10 µg), Ceftriaxone (30 µg), Erythromycin (15 µg), Amikacin (30 µg), Gentamicin (10 µg) and Imipenem (10 µg). The zone of inhibition was measured and the result was interpreted according to the guideline.¹⁰ *S. aureus* ATCC-25923 strain was used as reference organism for AST.

Determination of multidrug resistance and special resistance: Any bacterial isolate that showed resistant to at least one agent in three groups of antimicrobial drugs tested were considered as multidrug resistant.¹¹ To determine the prevalence of Methicillin resistance *S. aureus* (MRSA), phenotypic test was performed using Cefoxitin (30µg) disc as recommended by CLSI guideline.¹⁰ Data Analysis: Patient's details and all the data related with isolation and characterization of bacterial isolates were also recorded and analyzed using Excel data analysis tools, Microsoft Excel Spreadsheet 2007.

RESULTS

A total number of 264 patients (142 male and 122 female) with wound infection were included in this study. The age range was 16 to 81 years with majority of patients (68.6%) of age group 16-40 years. Of 264 specimens included in the study, 167 (63.3%) were positive for bacterial growth; 89 samples (53.3 %) were from male and 78 samples (46.7 %) were from female patients. Polymicrobial growth was observed with two specimens. A total of 169 bacterial pathogens were recovered with predominance of Gram-positive bacteria (119, 70.4%). *Staphylococcus aureus* (102, 60.4%) was the most frequent isolate followed by *E. coli* (31, 18.3%), Coagulase negative *Staphylococcus* (CoNS) (10, 5.9%) and *Pseudomonas aeruginosa* (10, 5.9%), respectively (Table 1).

The antimicrobial susceptibility patterns of the pathogens are presented in Table 2 and 3. The leading pathogen *S. aureus* were highly resistant to Ampicillin (95.1%), Cloxacillin (65.7%), Ofloxacin (64.7%) and Cotrimoxazole (56.9%). The second predominant pathogen, *E. coli* showed 71%, 64.5%, 58.1%, and 51.9% resistance to Amoxycillin, Cloxacillin, Ofloxacin and Cotrimoxazole respectively. Similarly, isolates of CoNS, *E. coli* and *Pseudomonas aeruginosa* also showed higher rate of resistance to those antibiotics (Table. 2). However, the bacterial

pathogens were highly susceptible to Doxycycline (68.6%), Erythromycin (73.3%), Amikacin (73.4%), Gentamicin (79.9%) and Imipenem (90.0%) (Table.3).

In this study, we demonstrate high rate of MDR pathogens associated with pyogenic infections. Among Gram positive pathogens, 60% CoNS and 56.9% *S. aureus* were MDR. Of total 102 *S. aureus* isolates, 31(30.4%) were Methicillin resistant (MRSA). Similarly, *E. coli* (58.1%) followed by *P. aeruginosa* (50.0%) constituted Gram negative pathogens with highest MDR strains. The additional finding, we observed was that the least common isolates like *E. faecalis*, *Acinetobacter* spp., *P. mirabilis*, and *Enterobacter* spp. were also resistant to commonly prescribed antibiotics (Table 2).

Table 1. Bacterial Isolates associated with wound infections in this study.

Bacterial Isolates	Frequency	Percentage (%)
Gram Positive isolates (n=119)		
<i>S. aureus</i>	102	60.4
CoNS	10	5.9
<i>S. pyogenes</i>	05	3.0
<i>E. faecalis</i>	02	1.2
Gram Negative isolates (n=50)		
<i>E. coli</i>	31	18.3
<i>P. aeruginosa</i>	10	5.9
<i>Klebsiella</i> spp.	4	2.3
<i>Acinetobacter</i> spp.	2	1.2
<i>Proteus mirabilis</i>	2	1.2
<i>Enterobacter</i> spp.	1	0.6
Total	169	100.0

Table 3. Overall susceptibility pattern of bacterial isolates to various antimicrobial agents in this study.

Name of drug	Susceptible	Resistant
Ampicillin	11 (9.3%)	108 (90.7%)
Amoxycillin	13 (35.1%)	37 (64.9%)
Cloxacillin	54 (32.0%)	115 (68.0%)
Cotrimoxazole	75 (44.4%)	94 (55.6%)
Ofloxacin	65 (38.5%)	104 (61.5%)
Doxycycline	116 (68.6%)	53 (31.4%)
Amikacin	124 (73.4%)	45 (26.6%)
Gentamicin	135 (79.9%)	34 (20.1%)
Ceftriaxone	106 (62.7%)	63 (37.3%)
Erythromycin	87 (73.3%)	32 (26.9%)
Imipenem	45 (90.0%)	05 (10%)

DISCUSSION

Among 264 specimens included in this study, 167 (63.3%) were positive for bacterial growth. The isolation rate of the pathogens was higher than previous study carried out by Acharya et al. (50.7%)¹² but comparable with the previous studies of Rijal et al. (64.9%)⁴ and Rai et al, (59.0%).⁷ However, it was lower than the study done in Ethiopia (83.9%).³ The observed variations might be due to the quality of specimens and prompt specimen processing facility in the laboratories.

Our study found *S. aureus* (60.4%) to be the predominant organism followed by *E. coli* (18.3%). This is in agreement with many previous studies on wound infection in different parts of the world including Nepal.^{3,4,12,13} The high prevalence of *S. aureus* and *E. coli* could be attributed to acquisition from the patients' own endogenous flora.

Table 2. Antibiotic susceptibility pattern of bacterial isolates in this study.

Organisms Isolated	Total Isolates	*Ampicillin/ **Amoxycillin		Cloxacillin		Ofloxacin		Doxycycline		Amikacin		Gentamicin		Ceftriaxone		*Erythromycin/ **Imipenem		Cotrimoxazole		
		S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	
Gram Positive Isolates																				
<i>S. aureus</i>	102	4.9%	95.1%	34.3%	65.7%	35.3%	64.7%	64.7%	35.3%	73.5%	26.5%	79.4%	20.6%	60.8%	39.2%	73.5%	26.5%	43.1%	56.9%	
CoNS	10	20%	80%	30%	70%	40%	60%	80%	20%	70%	30%	70%	30%	50%	50%	60%	40%	40%	60%	
<i>S. pyogenes</i>	05	80%	20%	20%	80%	40%	60%	80%	20%	60%	40%	80%	20%	60%	40%	80%	20%	40%	60%	
<i>E. faecalis</i>	02	-	100%	-	100%	50%	50%	100%	-	100%	-	100%	-	50%	50%	100%	-	50%	50%	
Gram Negative Isolates																				
<i>E. coli</i>	31	29%	71%	35.5%	64.5%	41.9%	58.1%	67.8%	32.3%	77.4%	22.6%	80.6%	19.4%	67.7%	32.3%	90.3%	9.7%	48.1%	51.9%	
<i>P. aeruginosa</i>	10	30%	70%	30%	70%	30%	70%	70%	30%	60%	40%	80%	20%	70%	30%	80%	20%	50%	50%	
<i>Klebsiella</i> spp.	4	25%	75%	25%	75%	75%	25%	75%	25%	50%	50%	75%	25%	75%	25%	100%	-	50%	50%	
<i>Acinetobacter</i> spp.	2	-	100%	-	100%	-	100%	100%	-	100%	-	100%	-	50%	50%	100%	-	-	100%	
<i>Proteus mirabilis</i>	2	-	100%	-	100%	100%	-	100%	-	100%	-	100%	-	100%	-	100%	-	50%	50%	
<i>Enterobacter</i> spp.	1	-	100%	-	100%	100%	-	100%	-	100%	-	100%	-	100%	-	100%	-	100%	-	
Total	169	*AST performed to Gram positive isolates only. ** AST Performed to Gram negative isolates only.																		

In our study the predominant bacterial isolates showed high rate of resistance to drugs such as Amoxicillin, Cloxacillin, Ofloxacin and Cotrimoxazole, which are commonly used for treatment of pyogenic infections. The prevalence of MRSA was found to be 30.4%. Moreover, the study also observed a high rate of emergence of multidrug resistance among the isolates associated in pyogenic infection. Although the prevalence of MRSA and other drug resistance pathogens is higher than that of isolates from developed countries,^{14,15} it is comparable to the previous studies performed in Nepal,^{4,12} and other developing countries like Ethiopia.^{3,13} Our findings revealed the existence of high rate of drug resistance pathogens in our setting which could be due to massive use of antimicrobials as a prophylactic purpose in surgical cases, longer duration and irrational use of antimicrobials in hospitals and use of antimicrobials without prescription in our country.^{6,16} Importantly, drug resistance pathogens in pyogenic wound infection may pose a risk of spread within hospital as well as community emphasizing the rational use of antibiotics.

CONCLUSION

The study revealed high frequency of drug resistance among isolates from pyogenic wound infections. There was an alarmingly high rate of resistance to the antibiotics commonly used for treatment of pyogenic infections. Continuous monitoring and surveillance are required to guide most appropriate therapy for wound infections and to prevent the emergence of drug resistance pathogens. This study could be helpful to formulate the proper guideline of antibiotics to be used for empiric treatment of pyogenic infection in our setting.

LIMITATIONS: Our study is based on characterization and evaluation of drug resistance among the isolates from wound infection. The risk factors associated with development of pyogenic infection and the development of drug resistance among the isolates were not evaluated. Moreover, anaerobic pathogens that could cause wound infections were not explored.

CONFLICTS OF INTEREST: None declared

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