Research Article

Bacteriological profile of wound infection and antibiotic susceptibility pattern of the isolates

Abstract

Introduction: Wound infection is the one of the most important causes of morbidity and mortality worldwide and antibiotics resistant bacteria are the great part of complications on treatment of the infection.

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

Methods: The study was carried out on wound infection suspected patients visiting B and B Hospital for six months duration. The collected pus specimen were first observed macroscopically then streaked on MacConkey agar and blood agar, incubated at 370C for 24 hours. The isolated bacteria were identified by macroscopic and microscopic observations and biochemical reactions. Antibiotic susceptibility pattern of the isolates was assessed by Modified Kirby Baur disc diffusion technique.

Results: A total of 503 pus samples were collected; of which 43.7% showed bacterial growth. Out of a total 220 bacterial isolates; 158 (71.82%) were Gram negative and 62 (28.18%) were Gram positive bacteria. *Pseudomonas spp.* (34.55%) was most common followed by *Staphylococcus aureus* (21.36%), *Escherichia coli* (11.82%), *Acinetobacter baumannii* (11.36%), *Enterobacter aerogens* (8.18%), Coagulase negative Staphylococci (CoNS) (5.45%), *Klebsiella pneumoniae* (4.55%), *Citrobacter freundii* (1.36%), *Streptococcus spp.* (0.91%) and *Enterococcus spp.* (0.46%). Most of Gram negative bacteria (63.9%) and Gram positive bacteria (93.5%) were susceptible to amikacin and chloramphenicol respectively. Among 220 isolates, 138 (62.73%) were found to be multi drug resistant (MDR). All the isolates of *A. baumannii* were isolated from in-patients and almost 96.4% (24/25) were MDR.

Conclusion: In this study, significant number of MDR strains was found as the causative agent of wound infection. So, routine microbiological analysis of the wound specimen and their antibiotic susceptibility testing are recommended that will guide medical practitioners for empirical treatment of wound infection, so as to reduce the spread of resistant bacteria.

Keywords: wound infection, antibiotic susceptibility, MDR

Mahat P,[1] Manandhar S,[1] Baidya Baidya[2]
[1]Department of Microbiology, National College, Nepal
[2]B and B Hospital, Nepal

Correspondence: Pushpa Mahat, Department of Microbiology, National College, Khusibu, Kathmandu, Email mahatpuspha1@gmail.com

Introduction

The exposure of subcutaneous tissue following a loss of skin integrity (i.e. a wound) provides a moist warm and nutritive environment that is conducive to microbial colonization and proliferation. The progression of a wound to an infected state is likely to involve a multitude of microbial and host factors.[1] Wound can be infected by a variety of microorganisms ranging from bacteria to fungi and parasites as well as virus.[2] The most common organisms are *S. aureus*, *P. aeruginosa*, *E. coli*, *Klebsiella spp.* and *Acinetobacter spp.*[3,4]

Antibiotics, although, have been of great value in treatment and in prophylaxis to prevent infections, the timing of administration, choice of antimicrobial agent, durations of administration have clearly defined the value of antibiotics in reducing wound infections.[5] Advance in control of infection have not completely eradicated the problem of the wound infection because of development of drug resistance.[6]

Wound infection results in sepsis, limb loss, long hospital stays, higher costs and is responsible for significant human mortality and morbidity worldwide.[7] It is one of the most common hospital acquired infections.[8] It remains an ongoing problem which although, cannot be completely eradicated however, by taking prompt control measures against the most commonly isolated organism and proper care of wound may lead to the minimum of wound infection.[1] 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).

Methods

A cross-sectional study was carried out in a total of 503 aseptically collected pus samples from wound infection suspected patients visiting B and B hospital, Lalitpur from October 2013 to April 2014. The pus specimen were macroscopically analyzed for their color and odor then were streaked on MacConkey agar and Blood agar plates and incubated at 370C for 24 to 48 hours. All the isolates were identified by colony morphology, staining reaction and the biochemical properties.[9] The antimicrobial susceptibility test of isolates was performed by Modified Kirby Bauer disc diffusion method using the standard guidelines of the[10] and MDR strain was identified as resistant to ≥3 antimicrobial classes.[11] The computer data were studied using

the statistical software SPSS version 17. Chi-square test was used to calculate probabilities and determine significance. A p-value of less than or equal to 0.05 was considered to be statistically significant p<0.05).

Results

Out of 503 pus samples from wound infection suspected patients, 220 (43.7%) showed bacterial growth whereas 283 (56.3%) were growth negative (Table 1). Out of 302 samples from in-patients, 160 (53.0%) samples and among 201 samples from out patients, 60 (29.9%) samples showed bacterial growth. The higher percentage of culture positivity seen among in-patients was found to be statistically significant (P<0.001) (Table 2).

Table 1 Growth pattern of Bacteria

S. No.	Growth	No. of samples	% of samples
1	Culture positive	220	43.7
2	Growth negative	283	56.3
	Total	503	100

Table 2 Distribution of growth positive cases in in-patient and out-patient

Patient type	Growth		No growth		Total	P-Value
	No.	%	No.	%		
In-patient	160	53	142	47	302	0.001
Out-patient	60	29.9	141	70.1	201	
Total	220	43.7	283	56.3	503	

Among 370 (73.56%) male patients and 133 (26.44%) female

patients, 172 (46.50%) and 48 (36.1%) were found growth positive respectively. The result was statistically significant (P=0.038) (Table 3). Among 220 positive cases the highest positive cases 67 (30.45%) was found in the patients of age group 21-30 years (Table 4).

Table 3 Gender-wise distribution of growth positive cases

Patient type	Growth		No Growth		Total	P-Value
	No.	%	No.	%		
Male	172	46.5	198	53.5	370	0.038
Female	48	36.1	85	63.9	133	
Total	220	43.7	283	56.3	503	

Among 220 bacterial isolates, Gram negative bacteria were predominant with 158 (71.82%) isolates, while Gram positive bacteria contributed 62 (28.18%) of total isolates. Altogether 10 different bacterial species were isolated, among which *Pseudomonas spp.* (34.55%) were predominant followed by *S. aureus* (21.36%) (Table 5). Out of 220 isolates, most of the isolates were isolated from in-patient (72.73%) than out-patient (27.27%). All the *A. baumannii* were from in-patients (Table 6).

Vancomycin was used only for the isolates resistant to cloxacillin. Most of the Gram positive bacterial isolates (more than 90%) were found to be sensitive to chloramphenicol. Penicillin G was ineffective (100%) to all the Gram positive isolates followed by amoxycillin (Table 7). Most of the Gram negative bacterial isolates (more than 48%) were found to be sensitive to amikacin, gentamicin and cefoperazone/ sulbactum whereas 73.4% of them were resistant to ciprofloxacin (Table 8).

Table 4 Age and Gender-wise distribution of growth positive cases

Age group(Yrs)	Male total	Positive	Female total	Positive	Total cases	Total positive cases
1≤10	21	7	13	4	34	11 (5%)
20-Nov	74	43	7	1	81	44(20%)
21-30	82	50	36	17	118	67(30.45%)
31-40	61	25	30	14	91	39(17.73%)
41-50	56	18	16	3	72	21(9.55%)
51-60	41	21	12	2	53	23(10.45%)
61-70	16	2	11	4	27	6(2.73%)
≥71	19	6	8	3	27	9(4.09%)
Total	370	172	133	48	503	220

Table 5 Distribution of bacterial isolates

Organism	No of isolates (%)	% of Total isolates
Gram Positive Bacteria		
S. aureus	47 (75.81)	21.36
CoNS	12 (19.35)	5.45
Streptococcus spp.	2 (3.23)	0.91
Enterococcus spp.	1 (1.61)	0.46
Total	62 (100)	28.18
Gram Negative Bacteria		
Pseudomonas spp.	76 (48.10)	34.55
E. coli	26 (16.46)	11.82
Acinetobacter baumannii	25 (15.82)	11.36
E. aerogens	18 (11.39)	8.18
K. pneumoniae	10 (6.33)	4.55
C. freundii	3 (1.90)	1.36
Total	158 (100)	71.82

Citation: Mahat P, Manandhar S, Baidya B. Bacteriological profile of wound infection and antibiotic susceptibility pattern of the isolates. *J Microbiol Exp.* 2017;4(5):119–122. DOI: 10.15406/jmen.2017.04.00126

Table 6 Distribution of isolates among in-patient and out-patient

Organisms	In patient		Out patient		Total
	Frequency	%	Frequency	%	
S. aureus	23	48.9	24	51.1	47
CoNS	8	66.7	4	33.3	12
Streptococcus spp.	2	100	0	0	2
Enterobacter spp.	1	100	0	0	1
Pseudomonas spp.	58	76.3	18	23.7	76
E. coli	20	76.9	6	23.1	26
Acinetobacter baumannii	25	100	0	0	25
E. aerogens	14	77.8	4	22.2	18
K. pneumoniae	7	66.7	3	30.3	10
C. freundii	2	66.7	1	33.3	3
Total	160	72.73	60	27.27	220

Table 7 Antibiotic susceptibility pattern of Gram positive isolates

Antibiotics	Sensitive		Intermediate		Resistant		Total
	No.	%	No.	%	No.	%	
Ciprofloxacin	27	43.5	7	11.3	28	45.2	62
Ofloxacin	27	43.5	7	11.3	28	45.2	62
Ceftiraxone	46	74.2	6	9.7	10	16.1	62
Gentamicin	41	66.1	1	1.6	20	32.3	62
Chloramphenicol	58	93.5	1	1.6	3	4.8	62
Cefoperazone/ Sulbactam	31	86.11	1	2.78	4	11.11	36
Penicillin G	0	0	0	0	62	100	62
Amoxycillin	11	17.7	0	0	51	82.3	62
Erythromycin	29	46.8	5	8.1	28	45.2	62
Clindamycin	54	91.53	0	0	5	8.47	59
Cloxacillin	56	94.92	0	0	3	5.08	59
Vancomycin	5	100	0	0	0	0	5

Table 8 Antibiotic susceptibility pattern of Gram negative isolates

Antibiotics	Sensitive		Intermediate		Resistant		Total
	No.	%	No.	%	No.	%	
Ciprofloxacin	40	25.3	2	1.3	116	73.4	158
Ofloxacin	41	25.9	3	1.9	114	72.2	158
Ceftriaxone	5	6.1	1	1.22	76	92.68	82
Ceftazidime	4	5.26	0	0	72	94.74	76
Gentamicin	78	49.4	2	1.3	78	49.4	158
Amikacin	101	63.9	14	8.9	43	27.2	158
Chloramphenicol	52	32.9	8	5.1	98	62.6	158
Cefoperazone/ Sulbactum	77	48.7	31	19.6	50	31.6	158
Meropenem	47	37.9	10	8.07	67	54.03	124
Imipenem	97	78.22	12	9.68	15	12.1	124
Piperacillin/ Tazobactam	42	33.87	23	18.55	59	47.58	124
Colistin	50	100	0	0	0	0	50

Out of 47 isolates of *S. aureus,* 95.7% showed sensitivity towards chloramphenical, clindamycin, and cloxacillin. Penicillin G was ineffective (100%), followed by amoxycillin (89.4%). Among the *S. aureus* isolates, two isolates were found to be methicillin resistant *S. aureus* (MRSA), sensitive to vancomycin (Table 9).

The effective antibiotics against *Pseudomonas spp.* were amikacin with (68.4%) sensitivity and gentamicin with (63.2%) respectively. The most ineffective antibiotic was ceftazidime (94.7%) (Table 10).

The most effective antibiotics against *A. baumannii* isolates was colistin with 100% sensitivity, the opposite was ceftriaxone with 100% respectively. Out of the isolates, more than 92% were resistant to ciprofloxacin, ofloxacin, gentamicin, cefoperazone/ sulbactum and piperacillin/ tazobactam (Table 11).

Out of 220 bacterial isolates, 138 were found to be the MDR isolates. The higher MDR pattern was found in Gram negative isolates 106 (48.18%) than in Gram positive isolates 32 (14.55%). The highest percentage (96%) of MDR pattern was shown by *A. baumannii* followed by *K. pneumoniae* and the least (21.92%) by *E. coli* (Table 12).

Table 9 Antibiotic susceptibility of *S. aureus*

Antibiotic	Sensitive		Intermediate		Resistant		Total
	No	%	No	%	No	%	
Ciprofloxacin	16	34	6	12.8	25	53.2	47
Ofloxacin	16	34	6	12.8	25	53.2	47
Ceftriaxone	37	78.7	6	12.8	4	8.5	47
Gentamicin	29	61.7	1	2.1	17	36.2	47
Chloramphenicol	45	95.7	0	0	2	4.3	47
Cefoperazone/ Sulbactum	23	92	1	4	1	4	25
Amox Ycillin	5	10.6	0	0	42	89.4	47
Erythromycin	27	57.4	4	8.5	16	34	47
Clindamycin	45	95.7	0	0	2	4.3	47
Cloxacillin	45	95.7	0	0	2	4.3	47
Vancomycin	2	100	0	0	0	0	2

Table 10 Sensitivity pattern of *Pseudomonas spp.* to different antibiotics

Antibiotics	Sensitive		Intermediate		Resistant		Total
	No.	%	No.	%	No.	%	
Ciprofloxacin	24	31.6	0	0	52	68.4	76
Ofloxacin	25	32.9	0	0	51	67.1	76
Ceftazidime	4	5.3	0	0	72	94.7	76
Gentamicin	48	63.2	1	1.3	27	35.5	76
Amikacin	52	68.4	10	13.2	14	18.4	76
Chloramphenicol	14	18.4	3	3.9	59	77.6	76
Cefoperazone/ Sulbactum	37	48.7	22	28.9	17	22.4	76
Meropenem	19	31.67	2	3.33	39	65	60
Imipenem	55	91.67	2	3.33	3	5	60
Piperacillin/ Tazobactam	21	35	15	25	24	40	60
Colistin	24	100	0	0	0	0	24

Table 11 Antibiotic susceptibility pattern of *A. baumannii*

Antibiotics	Sensitive		Intermediate		Resistant		Total
	No	%	No	%	No	%	
Ciprofloxacin	1	4	0	0	24	96	25
Ofloxacin	1	4	0	0	24	96	25
Ceftriaxone	0	0	0	0	25	100	25
Gentamicin	2	8	0	0	23	92	25
Amikacin	3	12	3	12	19	76	25
Chloramphenicol	1	4	3	12	21	84	25
Cefoperazone/ Sulbactum	2	8	0	0	23	92	25
Meropenem	3	12	5	20	17	68	25
Imipenem	6	24	8	32	11	44	25
Piperacillin/ Tazobactam	1	4	1	4	23	92	25
Colistin	23	100	0	0	0	0	25

Table 12 Distribution of MDR pathogens among total isolates

Organisms	No. of Isolates	MDR Isolates	
		Frequency	%
S. aureus	47	24	50
CoNS	12	6	51.06
Streptococcus spp.	2	1	50
Enterococcus spp.	1	1	100
Pseudomonas spp.	76	52	68.42
E. coli	26	7	26.92
Acinetobacter Baumannii	25	24	96
E. aerogens	18	13	72.22
K. pneumoniae	10	9	90
C. freundii	3	1	33.33
Total	220	138	62.73

Citation: Mahat P, Manandhar S, Baidya B. Bacteriological profile of wound infection and antibiotic susceptibility pattern of the isolates. *J Microbiol Exp.* 2017;4(5):119–122. DOI: 10.15406/jmen.2017.04.00126

Among the 160 isolates from in-patients, 96 (60%) were MDR strains and out of 60 out-patient, 42 (70%) were found to be MDR strains, were statistically insignificant (P=0.172) (Table 13). Out of 172 isolates from male 103 (59.88%) were MDR strains and among 48 isolates from female patients, 35 (72.92%) were MDR strains, were statistically insignificant (P=0.99) (Table 14).

Table 13 Distribution of MDR pathogens among in-patient and out-patient

Types of patient	Total isolates	MDR isolates		P-Value
		Frequency	%	
In-patient	160	96	60	0.172
Out-patient	60	42	70	
Total	220	138	62.73	

Table 14 Gender-wise distribution of MDR pathogens

Gender	Total isolates	MDR isolates		P-Value
		Frequency	%	
Male	172	103	59.88	0.99
Female	48	35	72.92	
Total	220	138	62.73	

Discussion

Out of 503 samples, 220 (43.7%) showed bacterial growth. In similar studies 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.

The occurrence of higher number of male patients 370 (73.56%) than female patients for the collection of pus samples may be due to the higher involvement of males in physical outdoor works for earning livelihood as compared to females and more chances of accidents during the activities. The higher growth positive cases in male patients (78.18%) than in female (21.82%) was observed in this study and was supported by the similar studies carried out by KC et al.[7] & Shrestha.[14]

The higher number of bacterial growth was observed in in-patients 160 (72.73%) than in out-patients 60 (27.27%). Similarly, Acharya[15] & Bhattrai[16] also reported the higher number of growth positive cases in in-patients. This may be due to the factors associated with acquisition of nosocomial pathogens in in-patients and immunologically weak health status facilitates the wound infection in them.

The highest positive cases 30.45% (67/226) were observed in the age group of 21-30 years might be due to the active participation of this age group of people in different physical and mechanical works and during they may get injured.

Among the total 220 bacterial isolates, 71.82% were Gram negative and 28.18% were Gram positive bacteria. In similar study conducted by Yakha et al.[17] & Acharya[15] Gram negative bacteria were found 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 in open as well as in visceral infection, which increases their incidence in both open and closed types of wound. Isolation of Gram negative bacteria also increases in the cases of Hospital acquired infections (HAIs). A study conducted by Banjara[18] at TUTH showed the high rate of Gram negative bacteria in HAI.

Altogether, 10 different bacterial species were isolated with *Pseudomonas spp.* being the predominant one (34.55%) followed by *S. aureus* (21.36%), *E. coli* (11.82%), *A. baumannii* (11.36%), *E. aerogens* (8.18%) Coagulase negative Staphylococci (CoNS) (5.45%), *K. pneumoniae* (4.55%), *C. freundii* (1.36%), *Streptococcus spp* (0.91%) and *Enterococcus spp* (0.46%). Similar studies carried out by Hani and Adnan[19] & Ranjan et al.[20] also showed *Pseudomonas spp.* was the most prevalent bacteria among the total cases with 27.8% and 29.6% respectively. But, the study conducted by Zafar et al.[21] showed *Pseudomonas spp.* the second commonest bacteria with 18.35% and *S. aureus* as the most predominant isolates with 41.28% of the total cases. *Pseudomonas spp* was predominant (36.25%) among in-patients followed by *A. baumannii* (15.63%) but *S. aureus* was the most prevalent (40%) among out-patients. All the isolates of *A. baumannii* isolated from in-patients.

Regarding antibiotic susceptibility testing, chloramphenicol (93.5%) and amikacin (63.9%) were the most effective antibiotics, in general to the Gram positive and Gram negative bacteria respectively. Among 47 S. aureus, two were found to be methicillin resistant (MRSA). 89.4% of *S. aureus* were resistant to amoxycillin. The resistance of *S. aureus* to β- lactam antibiotics is due to the production of penicillinas that hydrolyzed the β-lactam ring. MRSA resistance to β-lactam antibiotics is conferred primarily by the production of a bacterial cell wall penicillin binding protein, PBP2a. This has low binding to β-lactams which allows the organism to survive and multiply. PBP2a production is mecA gene encode.[9] 51.06% (24/47) *S. aureus* isolates were MDR. The studies by Puyal[13], Acharya[15] and Bhattarai[16] support this result.

In present study, out of 76 *Pseudomonas spp.* isolates, 52 (68.42%) were found to be MDR isolates. Colistin was absolutely effective antibiotic against *Pseudomonas spp.* followed by imipenem (91.67%), amikacin (68.4%) and gentamicin (63.2%) respectively. ceftazidime was found to be the most ineffective (94.7%) antibiotics. Similar study by Acharya[15] found 62.16% MDR among *Pseudomonas spp.* isolates and amikacin was found to be 84.61% effective antibiotics. The study by Hani and Adnan[19] showed amikacin and gentamicin effectiveness, 78% and 72% respectively against *Pseudomonas spp.* But the study carried out by Falagas et al.[22] isolated the organism from ICU patients which was almost resistant to amikacin, gentamicin and other anti-pseudomonal antibiotics. The study conducted by Li et al.[23] showed that active efflux played role in the resistance to various non-β-lactam agents by *Pseudomonas spp.* strains and de-energization by the addition of a proton conductor increased the accumulation level to that expected for equilibration across the cytoplasmic membrane.

In this study, *A. baumannii* constituted 25 (15.82%) of the Gram negative isolates and 11.82% among the total bacterial isolates. Among the isolated *A. baumannii* 24 (96%) were found to be MDR strains. In the study conducted by Kelper et al.[24], out of 237 samples 38 (16.03%) were found to be *Acinetobacter spp.* out of which 29 (76.31%) isolates were found to be MDR. Almost all the antibiotics used in this study were found to be resistant to the organism. However, the imipenem and meropenem were effective 24% and 12% respectively against the *A. baumannii*. MDR *A. baumannii* is an important nosocomial pathogen. It has the capacity to survive in dry environments, which increases the risk for nosocomial transmission. *A. baumannii* can cause infection in any organ system, including bacteremia, pneumonia, endocarditis, meningitis, urinary tract infection, intra-abdominal abscess, osteomyelitis, soft tissue infection, surgical site infections.[25]

The prevalence of MDR isolates was found independent of gender and types of patients. Imipenem was found to be the most effective antibiotic for Gram negative bacteria from in-patients. *P.*

aeruginosa and *S. aureus* were found as the most frequent etiological agents from pus samples. The prevalence of MDR was found to be 62.7% (138/220) among the total bacterial isolates. All the *A. baumannii* were isolated from in-patients and almost 96% (24/25) were found to be MDR. The result obtained from this study will be helpful for policy makers in evaluating the infection control measures in hospitals. The routine antimicrobial susceptibility testing before antibiotic administration is highly recommended since it can play key role to limit the wound infections to minimum as the emergence of drug resistant bacteria is less likely when there is empirical drug therapy, fasten the process of wound healing hence the appropriate use of drug decreases the cost of wound infection treatment.

Conflicts of interest

There is no conflict of interest.

Acknowledgements

Funding

References

1. Bowler P, Durden B, Armstrong D. Wound Microbiology and Associated Approaches to Wound Management. *Clin Microbiol Rev*. 2001;14(2):244–269.

2. Church D, Elsayed S, Reid O, et al. Burn wound infection. *Clin Microbiol Rev*. 2006;19:403–434.

3. Gupta N, Gautam V, Saini S, et al. Prevalence of multi drug resistant organism in wound infection. *J Infect Dis Antimicrobial Agent*. 2002;19:111–117.

4. Esebelahie NO, Esebelahie FO, Omoregie R. Aerobic bacterial isolates from wound infection. *Afr J Cln Exper Microbial*. 2013;14(3):155–159.

5. Nichole RE. Preventing surgical site infection: a surgeon's perspective. *Emerg Infect Dis*. 2001;7(2):220–224.

6. Thomas KH. Surgical Wound Infection, an overview. *Am J Med*. 1981;70(3):712–718.

7. KC R, Shrestha A, Sharma VK. Bacteriology Study of Wound Infection and Antibiotic susceptibility Oattern of Isolates. *Nepal Journal of Science and Technology*. 2013;14(2):143–150.

8. Gottrup F, Melling A, Hollander D. An overview of surgical site infections: aetiology, incidence and risk factors. *EWMA Journal*. 2005;5(2):11–15.

9. Cheesbrough M. District Laboratory Practice in Tropical Countries. Part 2, Cambridge University Press, India. 2000:124–132.

10. CLSI. Performance standards foe antimicrobial susceptibility testing twenty–first informational supplement. 2011;31(1):M100–S21.

11. Magiorakos AP, Srinivasan A, Carey RB, et al. Multidrug–resistant, extensively drug–resistant and pandrug–resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. *Clin Microbiol Infect*. 2012;18(3):268–281.

12. Maharjan S. Bacteriology of Wound Infection Among patients Visiting B &B Hospital and Antibiotic Sensitivity Profile of the Isolates. M.Sc. dissertation submitted to the department of Microbiology, Nepal. 2009.

13. Phuyal K. Bacteriology of Wound Infection with Reference to Multi Drug Resistant Isolates. M.Sc. dissertation submitted to the Department of Microbiology, Nepal. 2008.

14. Shrestha P. Bacteriology of Wound Infection and their Antibiotic Susceptibility Pattern. M.Sc. dissertation submitted to the Department of Microbiology, Nepal. 2013.

15. Acharya S. Multi Drug Resistant of bacterial Isolates from wound Infection. M.Sc. dissertation submitted to the Department of Microbiology, Nepal. 2012.

16. Bhattarai S. Microbiological Profile of Wound Infection and their Antibiotic Susceptibility pattern. M.Sc. dissertation submitted to the Department of Microbiology, National College, Kathmandu Nepal. 2013.

17. Yakha JK, Sharma AR, Dohal N, et al. Antibiotic Susceptibility Pattern of Bacterial Isolates Causing Wound Infection Amongthe Patients Visiting B & B Hospital. *Nepal Journal of Science and Technology*. 2014;15(2):91–96.

18. Banjara MR. Study of Air, Water and Wound Infection in Different Wards of TUTH. M.Sc. dissertation submitted to the Central Department of Microbiology, Nepal. 2002.

19. Hani AM, Adnan SJ. Incident of *Pseudomonas aeruginosa* in Post–perative Wound Infection. *Am J Infect Dis*. 2009;5(1):1–6.

20. Ranjan KP, Ranjan N, Bansal SK, et al. Prevalence of *Pseudomonas aeruginosa* in Post–operative Wound Infection in a Referral Hospital in Haryana, India. *J Lab Physicians*. 2011;2(2):74–77.

21. Zafar A, Anwar N, Ejaz H. Bacteriology of Infected Wounds– A Study Conducted at Children Hospital Lahore. *Biomedicavol*. 2011;23:1.

22. Flagas ME, Ioannis AB, Sofia KK, et al. Outcome of Infection Due to Pandrug–resistant (PDR) Gram Negative Bacteria. *BMC infect Dis*. 2005;5:24.

23. Li XZ, Ma D, Livermore DM, et al. Role of Efflux Pump(s) in Intrinsic Resistance of *Pseudomonas aeruginosa*: Active Efflux as a Contributing Factor to β–Lactam Resistance. *Antimicrob Agents Chemother*. 1994;8(8):1742–1752.

24. Davis KA, Moran KA, McAllister CK, et al. Multidrug Resistant Acinetobacter Extremity Infections in Soldiers. *Emerg Infect Dis*. 22005;11(8):1218–1224.

25. Brooks GF, Carroll KC, Butel JS, et al. Jawez, Melnick and Adelberg's Medical Microbiology. (24th edn), MC Graw Hill Companies, New York, USA. 2007;263–270.