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Research Article

Antibiotic Susceptibility Test Profiling of Bacterial Species Causing Wound Infection

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Abstract

Background: The present study was carried out for patients suffering from wound infection in Nobel Medical College and Teaching Hospital with a goal to find out the common type of wounds and the bacteria associated with them and their antimicrobial susceptibility testing.

Methods: Samples were collected for culture and sensitivity from the patients with an age range a month to 86 years. The types of wound included surgical wounds, breast abscesses, trauma, burns and other pyogenic wounds.

Results: In this study, a total of 460 pus samples from the infected wounds were collected and analyzed. The etiological agents were isolated, identified by culture and biochemical tests and their susceptibility pattern to commonly used antibiotics were determined using standard protocols. The male patients were high in number (n = 273) than females (n = 187) and the majority of patients belonged to age group 21 - 30 (n = 125). Out of total 460pus samples, 285 (62%) samples showed growth. The growth was found to be highest in the surgical wound (40.11%) followed by trauma (25.14%) and lowest was in burn wound (5.98%). In a total of 285 growth positive specimens, 13 different bacterial species were found of which 165 (58%) were Gram-positive and 74 (26%) were Gram-negative bacteria. Among Gram-positive bacteria, *S. aureus* (53%) was most common followed by CONS (36%), *Streptococcus viridian* (11%) and *Enterococcus faecalis*. Among Gram-negative bacteria, *E. coli* (52%) was most predominant followed by *Citrobacter fruendii* (17%), *Pseudomonas aeruginosa* (12%), *Acinetobacter* spp., *Proteus mirabilis* (5%), *Enterobacter cloacae* (3%), *Pseudomonas* sp. (2%), *Proteus vulgaris* (2%) and *Citrobacter diversus* (2%).

Conclusions: Among the antibiotics used, the most effective antibiotic for Gram-positive isolates was found to be Ciprofloxacin while among the Gram-negative isolates, Amikacin was the most effective antibiotic. Hence it was concluded that wound infections are the major health problem of Nepalese. Routine microbiological analysis of the wound specimens and their antibiotic susceptibility testing is recommended that will guide clinician for the treatment of wound infection.

Keywords: *Wound Infection; Bacterial Isolates; Antibiotics*

Introduction

Human skin acts as an excellent barrier to infection [1], once the barrier function of the skin is breached, it becomes far more likely to be attacked by bacteria. A wound occurs when the integrity of any tissue is compromised (e.g. skin breaks, muscle tears, burns, or bone fractures). The wound is a discontinuity or break off the surface of the body. A wound is a simple wound when only the skin is involved. It can be complex when underlying tissue, nerves, vessels, tendons etc. are involved. Any purulent discharge from a closed surgical incision together with the signs of inflammation of the surrounding tissue is considered as wound infection irrespective of whether microorganisms can be cultured or not [2].

Wound infection is very common in Nepal. People are prone to agricultural wounds, bite wound infection, accidental wounds, industrial wounds, burn wound, surgical wounds, etc. Later these wounds become complicated due to poor management of wounds in the initial stage and poor aseptic techniques used in hospitals [3]. Most commonly encountered organisms in wound infection are *Staphylococcus aureus*, *Streptococcus* species, *Escherichia coli*, *Proteus* species, *Pseudomonas* species, *Acinetobacter* species, *Clostridium* species, *Bacteroides fragilis* and *Candida* species etc. [4]. In this context, the present study was carried out for patients suffering from wound infection in Nobel Medical College and Teaching Hospital with a goal to find out the common type of wounds and the bacteria associated with them and their antimicrobial susceptibility testing.

Materials and Methods

The study was carried out in the Bacteriology section, pathology department, Nobel Medical College and Teaching Hospital, Biratnagar. In 10 months' study from May 2014 to February 2015, a total of 460 samples were collected for culture and sensitivity from the patients with an age range a month to 86 years. The types of wound included surgical wounds, breast abscesses, Trauma, burns and other pyogenic wounds.

For this study, pus samples were collected on a sterile cotton swab or aspirated into a syringe and labeled with date, time, method of collection and the patient's name, age, sex, inpatient number, bed number and ward. The sample was taken to the laboratory for processing as early as possible, to avoid desiccation of sample and to prevent the growth of some species at room temperature [5], which may obliterate the true pathogens. For laboratory investigation, two pus swabs were collected; one for the direct smear stains microscopy and the other for culture.

The sample was processed as soon as it reached the laboratory following standard laboratory procedures. Of two samples taken from each patient, one was used for Gram stain and other for culture [6].

The color, odour and whether it contained granules were noted. Specimens collected in a syringe were easy to evaluate but when obtained in swab were difficult to evaluate.

An even smear of the specimen was made on a clean slide. The smear was heat fixed and stained by the Gram stain method. The smear was examined for bacteria among pus cells using 40x and 100x objectives.

The fresh wound exudates (pus) were cultured for aerobic bacteria under aseptic technique. Since a swab was generally used for the inoculation, it was applied to a small area of the plates the rest of the surface was streaked out with the loop. For aerobic bacteria, Blood Agar, Nutrient Agar and Mac Conkey Agar plates were incubated at 37^0C for 24 to 48 hours in ordinary incubatory [7].

After overnight incubation, the culture plates incubated aerobically were examined for bacterial growth and identified using standard microbiological techniques which involve colony characteristics, staining reactions, and biochemical properties. To perform biochemical tests, the isolated organisms should be of pure culture. From the pure culture plate, catalase test, oxidize test and gram staining were performed. Isolated colony was then inoculated in peptone water and incubated at 37^oC for 4 hours to perform different biochemical tests (Catalase test, Oxidase test, Coagulase test, Oxidative-fermentative (OF) test, Motility test, Hydrogen sulphide (H2S) production test, Indole test, Methyl red (MR) test, Voges Proskauer (VP) test, Citrate utilization test, Triple sugar iron (TSI) test, Urea hydrolysis test (Urease test). Next day, organisms were identified on the basis of results of biochemical tests using standard bacteriological chart [7].

Result

In this study, a total of four hundred and sixty pus samples from patients visiting Nobel Medical College and Teaching Hospital, Biratnagar were studied for a period of ten months from May 2014 to February 2015. After culture, isolated bacteria were identified using standard bacteriological techniques [8]. The results obtained are shown below.

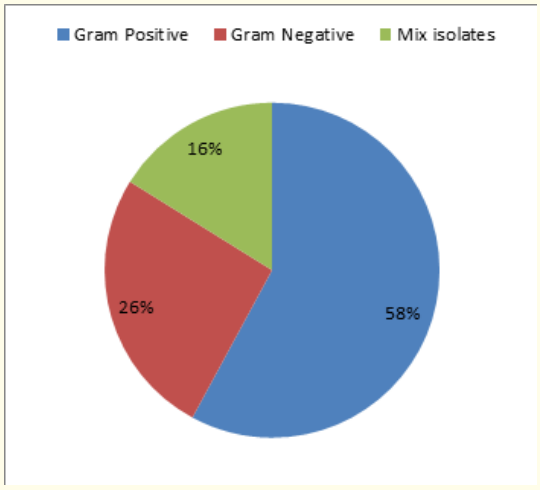

Figure1: *Pattern of Gram Positive and Gram Negative bacteria.*

Group	Total Patient		Growth		No growth	
	No	%	No	%	No	%
Male	273	59	174	64	99	36
Female	187	41	111	59	76	41
Total	460	100	285	62	175	38

Table 1: *Gender wise growth pattern.*

Age Group	Male		Female	
	No	%	No	%
1-10	31	11%	13	7%
11-20	59	22%	14	7%
21-30	59	22%	66	35%
31-40	34	12%	21	11%
41-50	37	14%	26	14%
51-60	18	7%	22	12%
61-70	19	7%	12	6%
71-80	14	5%	10	5%
81-90	2	1%	3	2%
Total	273	100%	187	100%

Table 2: *Age and gender wise distribution of patients.*

Gram positive isolates	No in single isolates	No in mixed growth	MDR	MRSA	Total	Percentage
S. aureus	92	11	1	2	106	53%
CoNS	55	13	4	0	72	36%
S. viridans	12	10	0	0	22	11%
E. feacalis	1	0	0	0	1	0.004%
Total	160	34	5	2	201	100%

Table 3: *Types of Gram Positive isolates in wound specimen.*

Gram negative isolates	No in single isolates	No in mixed growth	MDR	Total	Percentage
E. coli	41	23	3	67	52%
C. fruendii	6	12	4	22	17%
P. aeruginosa	9	6	0	15	12%
Pseudomonas sps	2	0	0	2	2%
Acinetobactersps	3	1	3	7	5%
Proteus mirabilis	3	3	1	7	5%
Enterobacter cloacae	1	3	0	4	3%
Proteus vulgaris	1	2	0	3	2%
C. diversus	1	0	1	2	2%
Total	67	50	12	129	100%

Table 4: *Types of Gram Negative isolates in wound specimen.*

Type of Wound	Growth		No Growth		Total
	No	%	No	%	
Surgical	67	77%	20	23%	87
Burn	10	77%	3	23%	13
Trauma	42	52%	39	48%	81
Breast Abscess	11	69%	5	31%	16
Other Pyogenic wound	37	67%	18	33%	55
Total	167		85		252

Table 5: *Growth pattern in different types of wound specimen.*

Type of Wound	Single Isolate		Multiple Isolate		Total
	No	%	No	%	
Surgical	50	75%	17	25%	67
Burn	6	60%	4	40%	10
Trauma	40	95%	2	5%	42
Breast Abscess	10	91%	1	9%	11
Other Pyogenic wound	29	78%	8	22%	37
Total	135	81%	32	19%	167

Table 6: *Growth pattern of single and multiple isolates in different types of wound.*

Type of Wound	Gram positive		Gram negative		Mix		Total
	No	%	No	%	No	%	
Surgical	35	52%	26	39%	6	9%	67
Burn	3	30%	3	30%	4	40%	10
Trauma	30	71%	11	26%	1	2%	42
Breast Abscess	7	64%	3	27%	1	9%	11
Other Pyogenic wound	20	54%	16	43%	1	3%	37
Total	95		59		13		167

Table 7: *Pattern of Gram Positive and Gram Negative bacteria in different types of wound.*

Antibiotics	Sensitive		Intermediate		Resistant		Total
	No	%	No	%	No	%	
Amoxycillin	23	22%	0	0%	83	78%	106
Cefotaxime	52	49%	21	20%	33	31%	106
Ciprofloxacin	64	60%	30	28%	12	11%	106
Cotrimoxazole	61	58%	19	18%	26	25%	106
Gentamicin	85	80%	5	5%	16	15%	106
Erythromycin	53	50%	36	34%	17	16%	106
Cephalexin	18	17%	13	12%	75	71%	106
Cloxacillin	97	92%	0	0%	9	8%	106

Table 8: *Antibiotic sensitivity pattern of S. aureus.*

Antibiotics	Sensitive		Intermediate		Resistant		Total
	No	%	No	%	No	%	
Amoxycillin	23	32%	0	0%	49	68%	72
Cefotaxime	44	61%	11	15%	17	24%	72
Ciprofloxacin	55	76%	4	6%	13	18%	72
Cotrimoxazole	40	56%	7	10%	25	35%	72
Gentamicin	52	72%	8	11%	12	17%	72
Erythromycin	22	31%	16	22%	34	47%	72
Cephalexin	29	40%	7	10%	36	50%	72
Cloxacillin	53	74%	1	1%	18	25%	72

Table 9: *Antibiotic sensitivity pattern of CoNS.*

Antibiotics	Sensitive		Intermediate		Resistant		Total
	No	%	No	%	No	%	
Amoxycillin	13	59%	0	0%	9	41%	22
Cefotaxime	15	68%	1	5%	6	27%	22
Ciprofloxacin	17	77%	1	5%	4	18%	22
Cotrimoxazole	14	64%	0	0%	8	36%	22
Gentamicin	16	73%	1	5%	5	23%	22
Erythromycin	12	55%	1	5%	9	41%	22
Cephalexin	17	77%	2	9%	3	14%	22
Cloxacillin	15	68%	1	5%	6	27%	22

Table 10: *Antibiotic sensitivity pattern of S. viridans.*

Antibiotics	Sensitive		Intermediate		Resistant		Total
	No	%	No	%	No	%	
Amoxycillin	9	13%	2	3%	56	84%	67
Cefotaxime	36	54%	1	1%	30	45%	67
Ciprofloxacin	37	55%	3	4%	27	40%	67
Cotrimoxazole	32	48%	1	1%	34	51%	67
Amikacin	62	93%	2	3%	3	4%	67
Gentamicin	49	73%	8	12%	10	15%	67
Ceftriaxone	34	51%	1	1%	32	48%	67
Ceftazidime	23	34%	8	12%	36	54%	67

Table 11: *Antibiotic sensitivity pattern of E. coli.*

Antibiotics	Sensitive		Intermediate		Resistant		Total
	No	%	No	%	No	%	
Amoxycillin	2	9%	0	0%	20	91%	22
Cefotaxime	4	18%	3	14%	15	68%	22
Ciprofloxacin	13	59%	2	9%	7	32%	22
Cotrimoxazole	7	32%	0	0%	15	68%	22
Amikacin	16	73%	0	0%	6	27%	22
Gentamicin	13	59%	0	0%	9	41%	22
Ceftriaxone	6	27%	1	5%	15	68%	22
Ceftazidime	3	14%	3	14%	16	73%	22

Table 12: *Antibiotic sensitivity pattern of C. fruendii.*

Antibiotics	Sensitive		Intermediate No %		Resistant No %		Total
	No	%					
Amikacin	12	80%	1	7%	2	13%	15
Cefotaxime	3	20%	7	47%	5	33%	15
Ciprofloxacin	12	80%	3	20%	0	0%	15
Cotrimoxazole	0	0%	0	0%	15	100%	15
Cefixime	1	7%	1	7%	13	87%	15
Gentamicin	11	73%	2	13%	2	13%	15
Ceftriaxone	8	53%	2	13%	5	33%	15
Ceftazidime	10	67%	0	0%	5	33%	15

Table 13: *Antibiotic sensitivity pattern of P. aeruginosa.*

Antibiotics	Sensitive		Intermediate		Resistant		Total
	No	%	No	%	No	%	
Amoxycillin	0	0%	1	14%	6	86%	7
Cefotaxime	2	29%	0	0%	5	71%	7
Ciprofloxacin	3	43%	0	0%	4	57%	7
Cotrimoxazole	3	43%	0	0%	4	57%	7
Amikacin	4	57%	0	0%	3	43%	7
Gentamicin	2	29%	0	0%	5	71%	7
Ceftriaxone	2	29%	0	0%	5	71%	7
Ceftazidime	1	14%	0	0%	6	86%	7

Table 14: *Antibiotic sensitivity pattern of Acinetobacter species.*

Antibiotics	Sensitive		Intermediate		Resistant		Total
	No	%	No	%	No	%	
Amoxycillin	2	29%	0	0%	5	71%	7
Cefotaxime	6	86%	1	14%	0	0%	7
Ciprofloxacin	6	86%	0	0%	1	14%	7
Cotrimoxazole	3	43%	0	0%	4	57%	7
Amikacin	5	71%	0	0%	2	29%	7
Gentamicin	4	57%	1	14%	2	29%	7
Ceftriaxone	3	43%	1	14%	3	43%	7
Ceftazidime	4	57%	0	0%	3	43%	7

Table 15: *Antibiotic sensitivity pattern of Proteus mirabilis.*

Discussion

The present study was conducted for a period of 10 months from May 2014 to February 2015 with an aim to identify the etiological agents causing wound infection along with their antibiotic susceptibility pattern among patients in Nobel Medical College and Teaching Hospital. In this study, a total of 460 pus samples from the infected wounds were collected and analyzed. The etiological agents were isolated, identified by culture and biochemical tests and their susceptibility pattern to commonly used antibiotics were determined using standard protocols. The male patients were high in number (n = 273) than females (n = 187) and the majority of patients belonged to age group 21 - 30 (n = 125). Out of total 460 pus samples, 285(62%) samples showed growth. The growth was found to be highest in the surgical wound (40.11%) followed by trauma (25.14%) and lowest was in burn wound (5.98%). In a total of 285 growth positive specimens, 13 different bacterial species were found of which 165 (58%) were Gram-positive and 74 (26%) were Gram-negative bacteria. Among Gram-positive bacteria, *S. aureus* (53%) was most common followed by CONS (36%), *Streptococcus viridans* (11%) and *Enterococcus feacalis*. Among Gram-negative bacteria, *E. coli* (52%) was most predominant followed by *Citrobacter fruendii* (17%), *Pseudomonas aeruginosa* (12%), *Acinetobacter* sp, *Proteus mirabilis* (5%), *Enterobacter cloacae* (3%), *Pseudomonas* sp. (2%), *Proteus vulgaris* (2%) and *Citrobacter diversus* (2%).

Conclusion

The growth of isolates was found to be higher in male patients than in female patients. The results statistical analysis revealed no significant differences (p = 0.9) between the gender and the occurrence of wound infection. The highest number of patients belonged to age group 21 - 30 followed by age group 11 - 20 and 31 - 40. For Gram-positive isolates, Gentamicin was found to be the most effective antibiotic while for Gram-negative, Amikacin was found to be the most sensitive drug. Among the antibiotics used, the most effective antibiotic for Gram-positive isolates was found to be Ciprofloxacin while among the Gram-negative isolates, Amikacin was the most effective antibiotic. Hence it was concluded that wound infections are the major health problem of Nepalese. Routine microbiological analysis of the wound specimens and their antibiotic susceptibility testing is recommended that will guide clinician for the treatment of wound infection.

However, the correct choice of antibiotics should be made only after the antibiotic susceptibility testing of the isolate and further extensive study need to be done to understand the exact pattern of antibiotics for the treatment.

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Bibliography

1. Abussaud MJ. "Incidence of wound infection in three different departments and the antibiotic sensitivity pattern of the isolates in a Saudi Arabian hospital". *Acta Microbiologica et Immunologica Hungarica* 43.4 (1996): 301-305.

2. Agarwal SL. "Study of post-operative infections". *Indian Journal of Surgery* 34 (1972)*:* 314-320*.*

3. Banjara MR. "Study of air, water and wound infection in different wards of TUTH". A dissertation presented to the Central Department of Microbiology, Tribhuvan University, Kathmandu, Nepal (2002).

4. Armstrong DG., *et al*. "Prevalence of mixed infections in the diabetic pedal wound. In: A retrospective review of 112 infections". *Journal of American Pediatric Medical Association* 85.10 (1995): 533-537.

5. Augustin M and Maier K. "Psychosomatic aspects of chronic wounds". *Dermatology and Psychosomatics* 4.1 (2003): 5-13.

6. Bowler PG. "The anaerobic and aerobic microbiology of wounds: a review". *Wounds* 10.6 (1998):170-178.

7. Cheesbrough M. "District laboratory practice in tropical countries". Part 2, Cambridge University Press, London (2000): 26-196.

8. Bauer AW., *et al*. "Antibiotic susceptibility testing by a standardized single disc method". *American Journal of Clinical Pathology* 45.4 (1996): 493-496.

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