https://doi.org/10.22502/jlmc.v8i2.367

Original Research Article

Bacteriological Profile of Wound Infection and Antibiotic Susceptibility Pattern of Various Isolates in a Tertiary Care Center

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ABSTRACT:

Introduction: Wound infection due to various pathogenic microorganisms and the development of resistance to antibiotics is one of the major problems in medical sector. This study aimed to identify the etiological agents of wound infection along with their antibiotic susceptibility. **Methods**: A total of 400 wound swab specimens were collected from the patients visiting a tertiary center in western Nepal over a period of six months. Thus, collected specimens were processed in Microbiology laboratory for isolation of causative agents. Antibiotic susceptibility test was performed for entire isolates by Kirby Baur disc diffusion method. Methicillin-Resistant *Staphylococcus aureus* was detected by cefoxitin disc diffusion test and Extended-Spectrum Beta-Lactamases producing Enterobacteriaeae by Phenotypic confirmatory disc diffusion test as recommended by Clinical and Laboratory Standards Institute. **Results**: Two hundred and fifty-nine (64.7%) of specimens were infected, giving rise to 269 different isolates. Among these, 163 (60.6%) were gram positive and 104 (38.6%) were gram negative. *Staphylococcus aureus* (n = 130, 48.3%) was the most predominant bacteria followed by *Escherichia coli* (n=44, 16.3%), and *Klebsiella pneumoniae* (n=23, 8.5%). Gentamicin followed by co-trimoxazole was the most effective among the tested antibiotics for *Staphylococcus aureus*. Gentamicin and ciprofloxacin were shown effective for isolated gram-negative bacteria. **Conclusion:** Fifty-eight (44.6%) of total *Staphylococcus aureus* were Methicillin-Resistant *Staphylococcus aureus* positive and 16 (20.7%) of total Enterobacteriaceae were Extended-Spectrum Beta-Lactamases producers. The increased prevalence of Methicillin-Resistant *Staphylococcus aureus* and Extended-Spectrum Beta-Lactamase suggest rational use of antibiotics on the basis of antibiotic sensitivity results.

Keywords: **Antibiotic susceptibility test, Extended-Spectrum Beta-Lactamases, Methicillin-Resistant *Staphylococcus aureus***

INTRODUCTION:

A wound is any breach or damage in skin due to trauma, accident, surgical operation or burn providing route of entry for bacteria causing infection. Wound infection is the result of successful invasion and proliferation by one or more species of microorganisms, sometimes resulting in pus formation.[1] Skin is colonized by transient as well as resident commensal floras.[2] These floras will remain commensal until skin remains intact. Any abrasion in skin surface provides an open door for bacterial invasion leading to infection.[3] Both aerobic and anaerobic bacteria often join to form synergistic infections like gangrene, necrotizing fasciitis, and cellulitis of skin and soft tissue.[2]

Wound infection can be due to variety of microorganisms ranging from bacteria, fungi, parasites and virus.[4] The common responsible bacterial pathogens are *Staphylococcus aureus*,

Submitted: 02 June, 2020
Accepted: 14 October, 2020
Published: 29 October, 2020

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How to cite this article:
Maharjan N, Mahawal BS. Bacteriological Profile of Wound Infection and Antibiotic Susceptibility Pattern of Various Isolates in a Tertiary Care Center. Journal of Lumbini Medical College. 2020;8(2):218-224. DOI: https://doi.org/10.22502/jlmc.v8i2.367 Epub: 2020 October 29.

Pseudomonas aeruginosa, Acinetobacter spp. and bacteria belonging to the family Enterobacteriaceae.[1] Wound infections may be caused by only one pathogen known as mono-microbial or by more than one pathogen known as poly-microbial. The control of wound infection has become more challenging due to widespread bacterial resistance to antibiotics. Hospital acquired wounds are among the leading nosocomial cause of morbidity and increasing medical expense.[1] Infection caused by Methicillin Resistant *Staphylococcus aureus* (MRSA) and Extended Spectrum Beta Lactamase (ESBL) producers pose a major challenge in the treatment of wound infection.[5] So, appropriate drugs selected by antibiotic sensitivity testing have great importance. The aim of this study was to identify the etiologies of various wound infections along with their antibiotic susceptibility. Further, we also observed the prevalence of MRSA and ESBL producing gram negative bacilli involved in wound infections.

METHODS:

This prospective study was conducted in the Department of Microbiology of Lumbini Medical College and Teaching Hospital (LMCTH) over a period of six months from September 2019 to February 2020. Ethical approval was obtained from the Institutional Review Committee of the institute (IRC – LMC 22-G/O19).

The sample size was calculated by using the formula, $n = Z_{\alpha}^2 pq/d^2$. Taking the prevalence of wound infection (p)= 0.43,[6] and maximum tolerable error (d) as 0.05, the required minimum sample size calculated was 376. A total of 400 pus/wound swab samples collected from both sexes and all aged patients sent to the microbiology department from various departments for aerobic bacterial culture were included in the study.

Dry wound swabs and improperly labelled specimens were excluded.

Quality control:

Strain of *Escherichia coli* ATCC 25922 and *Staphylococcus aureus* ATCC 25923 were used as reference strains for quality control of antibiotic sensitivity test and biochemical test. The same strain of *Escherichia coli* and *Staphylococcus aureus* were used as negative control for ESBL and MRSA detection respectively.

Sample collection and processing:

Convenient sample technique was used for collection of samples. Sterile cotton swabs or sterile syringes were used to collect pus samples from infected wound and were labeled properly with patient's details along with date and time of sample collection. The collection and labeling of samples were done by trained nurses of respective departments. Collected samples were delivered to microbiology laboratory within an hour for microbiological tests. All the microbiological tests were carried out by researchers themselves.

Macroscopic examination was performed for aspirated pus samples to note color, consistency and presence of granules. Microscopic examination was done after gram stain for presumptive identification of gram positive and gram-negative bacteria.

Culture and identification of isolates:

Samples were inoculated into MacConkey agar and Blood agar. They were then incubated at 37ºC for 24 hours. After incubation, grown isolates were identified according to standard microbiological criteria such as colonies morphology, gram stain and biochemical properties.[7] Gram positive cocci were identified up to species level by Catalase test, Coagulase test, Bile Esculin Hydrolysis test and by using Optochin and Bacitracin disc whereas gram negative bacilli were identified by Catalase test, Oxidase test, Methyl Red test, Voges Prouskaure test, Indole test, Motility, Hydrogen sulfide production, Triple sugar iron test, Urease test and Citrate test.[7]

Antibiotic Susceptibility test:

Antibiotic susceptibility test was performed for all bacterial isolates by a modified Kirby – Bauer disk diffusion method according to the guidelines of Clinical and Laboratory Standard Institute on Mueller Hinton agar.[8] Antibiotic disc (Hi Media Laboratories, Pvt. Limited, India) such as ampicillin (10 mcg), cloxacillin (10 mcg), cefoxitin (30 mcg), ciprofloxacin (5 mcg), cefixime (5 mcg), gentamicin (10 mcg), co-trimoxazole (25 mcg), cefotaxime (30 mcg), ceftazidime (30 mcg), piperacillin (100 mcg), carbenicillin (100 mcg), tetracycline (30 mcg), imipenem (10 mcg), amikacin (30 mcg), vancomycin (30 mcg), piperacillin-tazobactam (100/10 mcg) and linezolid (30 mcg) were used for antibiotic susceptibility tests.

Identification of MRSA:

Cefoxitin (30 mcg) was used for identification of MRSA. *Staphylococcus aureus* which showed a zone of inhibition ≤ 21 mm with cefoxitin on Mueller Hinton Agar after overnight incubation at 35º C were considered as MRSA.[8]

Screening and confirmation of ESBL:

Enterobacteriace showing zone of inhibition ≤27 for cefotaxime (30 µg) and/or ≤22 for ceftazidime (30 µg) and/or ≤ 25 for ceftriaxone (30 µg) and/or ≤ 27 for aztreonam (30 µg) respectively, the strain was suspected as a potential ESBL produce.

The isolates that were selected as potential ESBL producers using the screening method were confirmed when inhibition zones of ceftazidime, clavulanic acid and cefotaxime clavulanic acid were greater or equal to 5 mm compared with ceftazidime and cefotaxime alone.[8]

Data were analyzed by Statistical Package for Social Science (SPSS™) software version 18. Data were presented as frequency and percentage. Chi Square test was calculated for categorical variables to analyze significant difference at 95% confidence interval. A p value < 0.05 was considered statistically significant.

RESULTS:

Out of 400 samples collected from suspected wound infection, 259 (64.7%) showed growth of aerobic organism whereas 141 (35.3%) showed no growth. Ten (2.5%) specimens showed growth of two different bacteria so total bacterial isolates was 269. Among 269 isolates, 163 (60,6%) were gram positive and 104 (38.6%) were gram negative and two (0.7%) were *Candida albicans*.

Among total specimens, 142 (35.5%) were aspirated pus and 258 (64.5%) were wound swabs. Ninety-two (64.8%) of aspirated pus and 167 (64.7%) of wound swab showed growth. One hundred and eighty-nine (47.3%) of total specimens were collected from male patients and 211 (52.7%) were collected from female patients. Most specimens were collected from the age group 21-40 years (33.2%) as presented in Table 1.

Out of 400 cases, 276 (69%) were collected from In-patient Departments; 130 cases were from Surgery, 74 from Orthopedics, 36 from Gynecology, 16 from Ear, Nose and Throat (ENT), nine from Intensive Care Unit (ICU), five from Pediatrics and six from Internal Medicine. Sixteen (4%) were from Emergency Department (ED) and 108 (27%) from Out-Patient Department (OPD). Out of 276 in-patients, 171 (61.9%) were growth positive, out of 108 out-patients, 77 (71.2%) were growth positive and out of 16 ED patients, 11 (68.7%) were growth positive.

Organisms isolated from wound infections are shown in Figure 1. The antibiotic sensitivity patterns of gram positive and gram-negative bacteria are shown in Table 2 and Table 3 respectively.

Out of 130 *Staphylococcus aureus*, 58 (44.6%) were MRSA positive. Of them, 18 (31.1%) were isolated from Surgery department, 13 (22.4%) were from Orthopedics department, six (10.3%) were from Obstetrics and Gynecology department, 12 (20.6%) from OPD, three (5.2%) from ICU, three (5.2%) from ENT department, two (3.5%) from Pediatrics department and one (1.7%) from ED. Out of 77 gram negative bacilli belonging to Enterobacteriaceae family, 16 (20.7%) were ESBL positive. Eleven (68.7%) were *Escherichia coli*, four (25%) were *Klebsiella pneumoniae*, one (6.3%)

Table 1. Type of specimen, sex and age wise distribution of wound infection (N = 400)

Variables		Growth (%)	No growth (%)	Statistics
Specimen	Aspirated pus	92 (64.8%)	50 (35.2%)	$X^2 = 0.008$, df =1, p = 0.990
	wound swab	167 (64.7%)	91 (35.3%)	
Sex	Male	122 (64.6%)	67 (35.4%)	$X^2= 0.006$, df = 1, p = 0.937
	Female	137 (64.9%)	74 (35.1%)	
Age (years)	<20	79 (73.1%)	29 (26.9%)	$X^2 = 5.53$, df = 3, p = 0.137
	21 - 40	84 (63.2%)	49 (36.8%)	
	41 - 60	52 (57.8%)	38 (42.2%)	
	>60	44 (63.8%)	25 (36.2%)	

Table 2. Antibiotic sensitivity pattern of gram-positive bacteria.

Bacteria	Frequency (%) of sensitivity to various antibiotics, N(%)						
	AMP	CIP	COX	CX	CFM	GEN	COT
Staphylococcs aureus (n = 130)	23 (17.7)	60 (46.2)	90 (69.2)	72 (55.4)	29 (22.3)	96 (73.8)	82 (63.1)
Enterococus spp (n =10)	9 (90)	10 (100)	Nt	Nt	6 (60)	6 (60)	9 (90)
Streptococcus spp (n = 8)	7 (87.5)	6 (75)	Nt	Nt	7 (87.5)	7 (87.5)	4 (50)
Coagulase negative staphylococcus (n = 15)	2 (13.3)	7 (40.7)	9 (60)	6 (40)	3 (20)	8 (53.3)	6 (40)

AMP – ampicillin, CIP – ciprofloxacin, COX- cloxacillin, CX- cefoxitin, CFM- cefixime, GEN- gentamicin, COT- cotrimoxazole, Nt- Not tested

Table 3. Antibiotic sensitivity pattern of gram-negative bacteria.

Bacteria	Frequency (%) of sensitivity to various antibiotics, N(%)									
	AMP	CIP	CFM	GEN	COT	CTX	CAZ	CB	PI	PIT
Citrobacter spp (n = 5)	3 (60)	5 (100)	4 (80)	5 (100)	5 (100)	5 (100)	4 (80)	Nt	Nt	Nt
Escherichia coli (n = 44)	16 (36.4)	29 (65.9)	18 (40.9)	32 (72.7)	19 (43.2)	29 (65.9)	22 (50)	Nt	Nt	Nt
Klebsiella pneumoniae (n = 23)	0 (0)	16 (69.6)	13 (56.5)	15 (65.2)	13 (56.5)	13 (56.5)	13 (56.5)	Nt	Nt	Nt
Pseudomonas aeruginosa (n = 17)	1 (5.9)	13 (76.5)	Nt	12 (70.6)	4 (23.5)	Nt	9 (52.9)	11 (64.7)	10 (58.8)	16 (94.1)
Proteus spp (n = 3)	1 (33.3)	2 (66.7)	3 (100)	3 (100)	2 (66.7)	3 (100)	2 (66.7)	Nt	Nt	Nt
Acinetobacter spp (n = 9)	2 (22.2)	4 (44.4)	2 (22.2)	4 (44.4)	3 (33.3)	2 (22.2)	2 (22.2)	Nt	Nt	Nt
Enterobacter spp (n = 2)	1 (50)	1 (50)	0 (0)	0 (0)	0 (0)	1 (50)	1 (50)	Nt	Nt	Nt
Chromobacterium voilacium (n = 1)	0 (0)	1 (100)	0 (0)	1 (100)	1 (100)	1 (100)	1 (100)	Nt	Nt	Nt

AMP - Ampicillin, CIP - Ciprofloxacin, CFM - Cefixime, GEN - Gentamicin, COT - Cotrimoxazole, CTX - Cefotaxime, CAZ - Ceftazidime, CB - carbenicillin, PI - piperacillin, PIP - piperacillin - tazobactam

was *Enterobacter* spp. Antibiotic susceptibility test of both MRSA and ESBL producers are shown in Table 4.

DISCUSSION:

We conducted this study to identify the etiologies of various wound infections along with their antibiotic susceptibility. In this study, 259 (64.7%) specimens were found to be infected which was almost similar to the study carried out by Sah et al. and Upreti et al. that reported 62%.[9, 10] KC et al. reported 60.2% growth positive rate.[11] This reflects that wound infection is a major clinical challenge.

In our study, 71.2%, 68.7% and 61.9 of specimens collected from OPD, ED and in-patient

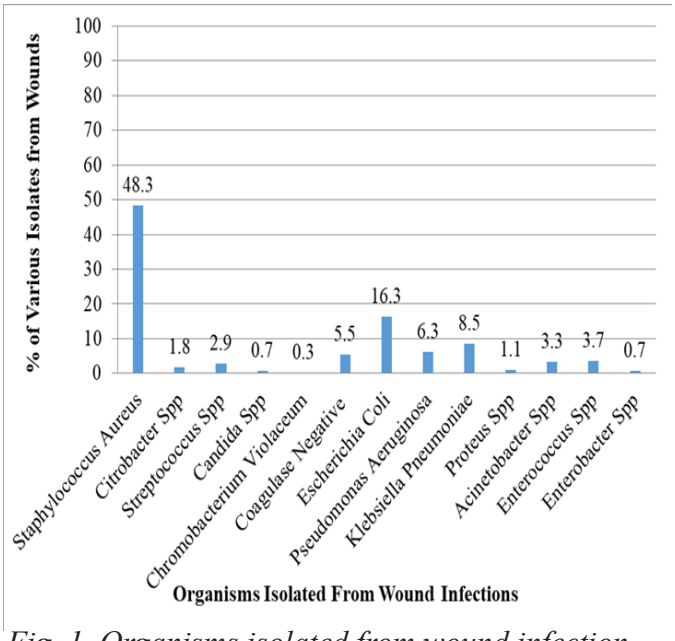

Fig. 1. Organisms isolated from wound infection.

department respectively showed growth. The study done by Yakha et al. showed 54.9%, 38.8% and 20.3% growth in in-patient, out-patient and ED respectively.[4] In our study, low growth rate in in-patient may be due to collection of specimens after antibiotic treatment or may be due to improper collection and transport up to laboratory.

Table 4. Antibiotic sensitivity pattern of MRSA and ESBL producers.

Antibiotic	MRSA (n = 58)	ESBL (n = 16)
	Frequency (%)	Frequency (%)
Ampicillin	0 (0)	0 (0)
Ciprofloxacin	13 (22.4)	04 (25)
Cefoxitin	0 (0)	Nt
Cefixime	0 (0)	0 (0)
Gentamicin	29 (50)	09 (56.3)
Cotrimoxazole	36 (62.1)	01(6.3)
Cefotaxime	Nt	0 (0)
Ceftazidime	Nt	0 (0)
Imipenem	Nt	15 (93.8)
Vancomycin	58 (100)	Nt
Amikacin	46 (79.3)	11 (68.8)
Tetracycline	Nt	08 (50)
Piperacillin tazobactam	Nt	14 (87.5)
Linezolid	58 (100)	Nt

The growth of bacteria in both male and female specimens was almost similar in our study; 64.6% in male and 64.9% in female. Whereas, the most studies showed higher growth of bacteria in specimens of male compared to female.[11, 14] The reason for this contrast finding may be the number of female patients included in our study is slightly higher than male patients. Monomicrobial wound infection (96.13%) was higher than polymicrobial (3.8%). This was agreed by Upereti et al and KC et al.[10, 11] The patient belonging age group < 20 (73.1%) were found to be highly infected followed by > 60 (63.8%). This may be due to weak immune system of old patients, and relatively younger patients and children.

The isolation rate of gram-positive bacteria was greater (60.6%) than gram negative bacteria (38.6%) in our study. Khanam et al. and Pandey et al. showed similar results.[12,13] But Giri et al. and Sherchan et al. showed high isolation rate of gram negative bacteria.[14,15] *Staphylococcus aureus* was the most predominant (48.3%) bacteria followed by *Escherichia coli* (16.3%), similar to the studies conducted by KC et al. and Pandey et al.[11,13] Mahat et al. showed predominance of *Pseudomonas* species.[6] The high rate of isolation of *Staphylococcus aureus* in wound infection may be due to its presence in nasal cavity, as a normal flora, of most of the individuals. The unhygienic behavior like contact of wound site with the hand contaminated with the nasal discharge may be the possible reason. The carriers are two to nine times more likely to acquire infection than non-carriers. [16]

Isolation rate of gram-negative bacteria was found to be more (35.7%) than gram positive bacteria (29.6%) in the Surgery department. This finding was just opposite in the Orthopedics department where gram positive rate was 20.1% and gram negative was 14.3%. This was agreed by study done by KC et al.[11] Gastrointestinal tract is a source of Gram negative bacteria to contaminate wound so abdominal surgery without much precautions can be the reason whereas gram positive bacteria were generally acquired from skin surface itself to contaminate wound.

The most effective antibiotic for *Staphylococcus aureus* was gentamicin (73.8%) whereas ampicillin (17.7%) was least effective antibiotic. Giri et al. also showed gentamicin

(77.78%) as effective drug and ampicillin (6.17%) as the least effective antibiotic.[14] *Escherichia coli* was also highly sensitive to gentamicin (72.7%) followed by cefotaxime (65.9%) and ciprofloxacin (65.9%). Again, ampicillin (36.4%) was the least effective. Piperacillin tazobactam was an effective antibiotic for *Pseudomonas aeruginosa* (94.1%) followed by ciprofloxacin (76.5%), and gentamicin (70.6%). Similar study done by Sherchan et al. showed all these three antibiotics were effective equally (80%).[15]

Fifty-eight (44.6%) of total *Staphylococcus aureus* specimens were MRSA positive. This was slightly lower than the studies done by Balchandra et al. (67.6%) and Giri et al. (53.06%).[1,14] and was slightly higher than the study by Pant et al.(30.70%).[5] This shows prevalence of MRSA is in increasing trend. The most effective drugs for MRSA were linezolid and vancomycin with 100% sensitivity followed by amikacin (79.3%). This was similar to the study done by Harshan et al.[17]

Sixteen (20.7%) of Enterobacteriaceae specimens were ESBL positive similar to the study done by Upreti et al. (22.7%).[10] But it was slightly lower than that reported by Balchandra et al. (38.12%).[1] This slight difference may be because the prevalence of ESBL producing isolates varies geographically. The effective antibiotic for ESBL producers was found to be imipenem (carbapenems) (93.8%) followed by Piperacillin-tazobactam (87.5%). Sherchan et al. also showed highest sensitivity (97.14%) to meropenem (carbapenems).[15]

There are a few limitations of the study. In this study ESBL test was done only for Enterobacteriaceae and not for other bacterial isolates. Similarly, genetic level test, that identify the gene sequence responsible for MRSA and ESBL producers, was also not performed. Besides this, single centered study conducted in small sample size for small duration was also the limitation.

CONCLUSION:

Staphylococcus aureus was the main bacterial causative agent of wound infection followed by *Escherichia coli* and *Klebsiella-pneumoniae*. The antibiotic sensitivity pattern showed decreased sensitivity to most of the commonly used antibiotics like beta-lactams. Proper care of wounds and early microbial analysis along with their antibiotic sensitivity test are therefore crucial for wound management. On the basis of the result, treatment of infection should be done with appropriate antibiotics like in case of MRSA, vancomycin and linezolid is the best antibiotic and in case of ESBL producers carbapenem is the best antibiotic. The emergence of carbapenem resistant gram negative bacteria suggests performing Metallo Beta lactamase test to check whether the resistance is due to production of Metallo Beta Lactamase or not.

ACKNOWLEDGEMENT: Menuka Maharjan (laboratory assistant) and Kamal Thapa (laboratory technician) of Clinical Laboratory of Microbiology Department.

Conflict of Interest: The authors declare that no competing interests exist.

Financial Disclosure: No funds were available for the study.

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