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Antibiotic susceptibility pattern of bacterial isolates from soft tissues infections among patients visiting a tertiary care centre

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Abstract

Background: Pus is a thick, white to yellow fluid that consists of dead leucocytes, cellular debris, necrotic tissues, and bacteria characteristic of pyogenic infections that may be exogenous or endogenous. The resistant pathogens isolated from wound infections have become a global challenge and a grave threat to the public health worldwide.

Objectives: To characterise the bacterial isolates from clinical specimens of pyogenic wound infections and to determine the antibiotic susceptibility test.

Methods: A hospital-based, descriptive, cross-sectional study was carried out after ethical clearance in the department of microbiology, Nobel Medical College from 2021 May to 2022 January. Various clinical specimens were obtained by convenience sampling. Identification and antibiotic sensitivity test were done as per the standard microbiological procedures. Data were analysed by SPSS v.20.

Results: Out of 1704 sample collected, 901 (52.8%) showed growth in which 893 (52.4%) showed monomicrobial growth and 8 (0.46%) showed mixed growth. Among the samples, females outnumbered males (F: M-1.4:1) and majority of infection were observed in age group between 21-30 years. Most of the isolates were Gram-negative bacteria (462, 50.8%) which were mostly sensitive to amikacin. The most predominant organism was *Staphylococcus aureus* (427, 47%) which was 100% sensitive to vancomycin and highly resistant to ampicillin (341, 79.8%).

Conclusion: The dryness in the pipeline of new antibiotic and emergence of multidrug resistant strains have pointed the current need towards active microbial surveillance in all clinical settings and prudent use of antibiotics.

Key words: Antibiotic; Pus; *Staphylococcus aureus*.

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INTRODUCTION

Pus, a thick, white to yellow fluid, consists of dead leucocytes, cellular debris, necrotic tissues, and bacteria characteristic of pyogenic infections which may be exogenous or endogenous.[1, 2] Skin prevents entry of microbes into human body unless mechanism is breached due to injury, trauma or surgical intervention which is exogenous infections.[3] Endogenous infections may be associated with appendicitis, cholecystitis etc.[1] Skin and soft tissue infections have very similar symptoms like swelling, redness, warmth, smooth and shiny skin, blisters, and pimples that get formed in the area.[4] The common bacteria from wound infections are *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Streptococcus pyogenes*, *coagulase negative staphylococci*, *Acinetobacter species*, *Pseudomonas spp.*, *Escherichia coli*, *Klebsiella spp.*, *Proteus spp.*, *Enterobacter spp.*, and anaerobes.[5]

Pyogenic infections have become leading cause of morbidity and mortality in surgery patients, trauma

etc.[6] Nepali people are generally prone to injuries, unaware about prevention and disabilities that lead to complications due to poor management of wounds at initial stage.[7] Multidrug resistant organisms have become a global challenge and grave threat to public health worldwide due to indiscriminate use of antibiotics.[6,8] Therefore, this study was intended to characterise bacterial isolates from pyogenic wound infections and to determine the antibiotic susceptibilities.

METHDOLOGY

A teaching hospital-based, descriptive, cross-sectional study was conducted in the Department of Microbiology, Nobel Medical College and Teaching Hospital, Biratnagar, Morang, Nepal, a tertiary care centre for a period of nine months, from 2021 May to 2022 January. This study was started after acquiring approval from the institutional review committee of Nobel Medical College (Ref. 397/2021). The sample size was estimated to be 350 by using formula, $n = Z^2*P*(1-P)/e^2$, where Z = 1.96 at the confidence level of 95%; e = 0.05 was the error taken as 5%; and P = 0.65 (65%) was the expected prevalence of bacterial growth from literature.[8]

During this time period pus samples (N = 1704) and data like age, sex and a brief history of illness were obtained from patients attending outpatient departments (OPDs) using convenience sampling technique. Patients admitted in various wards and intensive care units (ICUs) of the hospital were included while the insufficient information of the patient history and patients with history of recent antibiotic therapies were excluded from the study population. The collected data were entered in Microsoft Excel 2007 and analysed using IBM SPSS Statistics for Windows, version 20 (IBM Corp., Armonk, N.Y., USA).

All the specimens were visually examined for consistency, colour, turbidity, presence or absence of blood depending upon the type and site of wound. Additionally, pus swabs were observed whether they were labelled correctly or not and then the samples were processed as per standard bacteriological techniques for aerobic cultures. Each aseptically collected specimen was inoculated onto the Blood Agar (BA), Chocolate agar (CA), and MacConkey agar (MAC) plates (HiMedia Laboratories, India) by surface streaking method. The BA and MAC plates were incubated in aerobic atmosphere and CA plates were incubated in additional 5–10% carbon dioxide (CO_2) at 37° Celsius (C) for 24-48 hours. Identification of significant isolates associated with pyogenic infections was carried out following standard microbiological techniques including morphological appearance of the colonies: Gram's staining, catalase test, coagulase test, and oxidase test with other biochemical parameters indole, methyl red, Voges-Proskauer, citrate, urease, and Triple Sugar Iron Agar (TSI). Assurance of pure culture inoculums was done by setting purity plate along with the biochemical tests.[7,8] The susceptibility of bacterial isolates against different antibiotics was determined by modified Kirby-Bauer disk diffusion method on Mueller-Hinton agar following standard procedures recommended by the Clinical and Laboratory Standards Institute (CLSI).[7] The antibiotic discs and concentration (µg) used for both Gram-positive and Gram-negative bacteria were as follows: Ampicillin (AMP 10 mcg), amikacin (AK 30 mcg), ceftriaxone (CTR 30 mcg), cefotaxime (CTX 30 mcg), ceftazidime (CAZ 30mcg), ciprofloxacin (CIP 5 mcg), levofloxacin (LE 5 mcg), tobramycin (TOB 10 mcg), gentamicin (GEN 10 mcg), vancomycin (VA 30mcg), linezolid (LZ 30 mcg), teicoplanin (TEI 30 mcg), cotrimoxazole (COT 30 mcg), erythromycin (ERY 15mcg) and chloramphenicol (C 30mcg) from HiMedia Laboratories, India. *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923, and *Pseudomonas aeruginosa* ATCC 27853 were used as the control organisms for antibiotic sensitivity.[9]

RESULTS

Out of 1704 samples collected, 901 (52.8%) showed growth with 893 (52.4%) showing monomicrobial growth (Table 1). Among the total samples, 698 (41.9%) were obtained from the male patients (Table 2). Among the samples, females outnumbered males (F:M = 1.4:1). In this study, the highest rate of infection was observed in age group between 21-30 years (Table 3).

Out of total 908 bacterial isolates, 462 (50.8%) were Gram-negative and 446 (49.11%) were Gram-positive bacterial isolates. The most predominant isolate (427, 47%) was *Staphylococcus aureus* (Table 4).

Among all the antibiotics used, the highest number of *Staphylococcus aureus* were found to be sensitive (S) to vancomycin (427, 100%) followed by linezolid, teicoplanin, amikacin and resistant (R) to ampicillin (341, 79.8%), erythromycin (340, 79.6%) and cotrimoxazole (Table 5). Out of 18 isolates of *Enterococcus faecalis*, all isolates were sensitive to vancomycin, linezolid, and teicoplanin; and resistant to ampicillin (18, 100%) followed by erythromycin and cotrimoxazole. Among one isolates of *Staphylococcus epidermidis*, vancomycin, linezolid, teicoplanin, amikacin, levofloxacin, and ceftriaxone were sensitive and other all antibiotics were resistant (Table 5).

A total of 249 *Escherichia coli* were isolated from wound specimens and the most sensitive antibiotic was found to be amikacin (249, 96.3%) followed by levofloxacin, gentamicin, meropenem and resistant to cefuroxamine (191, 76.7%), cefotaxime, ceftriaxone (Table 6). Among 112 *Klebsiella pneumoniae* isolates, amikacin was the most sensitive antibiotic and resistant to cefuroxamine. All the isolates of *Klebsiella oxytoca* (100%) were sensitive to amikacin (Table 6).

Among different antibiotics used for *Pseudomonas aeruginosa* the most sensitive antibiotic was amikacin (61, 88.4%) while the most resistant antibiotic was cefotaxime (Table 7).

Table 1: Growth pattern of specimens

Growth pattern	Number (Percent)
Monomicrobial growth	893 (52.4)
Mixed growth	8 (0.4)
No growth	803 (47.1)
Total	**1704 (100)**

Table 2: Genderwise distribution of specimens

Gender	Number (Percent)
Male	698 (41.9)
Female	1006 (58.1)
Total	**1704 (100)**

Table 3: Distribution of samples according to age

Age (Years)	Number (Percent)
<10	137 (8)
11-20	204 (11.9)
21-30	596 (34.9)
31-40	187 (10.9)
41-50	172 (10)
51-60	100 (5.8)
>60	308 (18)
Total	**1704 (100)**

Table 4: Distribution of bacterial isolates from pus sample (N = 908)

Bacterial isolates	Number (Percent)
Staphylococcus aureus	427 (47)
Escherichia coli	249 (27.4)
Klebsiella pneumoniae	112 (12.3)
Pseudomonas aeruginosa	69 (7.6)
Enterococcus faecalis	18 (1.9)
Klebsiella oxytoca	11 (1.2)
Acinetobacter anitratus	8 (0.8)
Citrobacter koseri	7 (0.7)
Proteus species	6 (0.6)
Staphylococcus epidermidis	1 (0.1)
Total	**908 (100)**

Table 5: Antibiogram of gram-positive cocci (N = 446) expressed in n (%)

Antibiotics	*Staphylococcus aureus* (N = 427)		*Enterococcus faecalis* (N = 18)		*Staphylocccus epidermidis* (N = 1)	
	Sensitive (S)	Resistant (R)	S	R	S	R
Vancomycin	427 (100)	-	18 (100)	-	1 (100)	-
Linezolid	426 (99.7)	1 (0.2)	18 (100)	-	1 (100)	-
Teicoplanin	420 (98.3)	7 (1.6)	18 (100)	-	1 (100)	-
Amikacin	395 (92.9)	32 (7.5)	16 (88.8)	2 (11.1)	1 (100)	-
Levofloxacin	364 (85.2)	63 (14.7)	16 (88.8)	2 (11.1)	1 (100)	-
Chloramphenicol	352 (82.4)	75 (17.5)	15 (83.3)	3 (16.6)	-	1 (100)
Ceftriaxone	301 (70.4)	126 (29.5)	15 (83.3)	3 (16.6)	1 (100)	-
Cotrimoxazole	176 (41.2)	251 (58.8)	4 (22.2)	14 (77.7)	-	1 (100)
Erythromycin	87 (20.3)	340 (79.6)	4 (22.2)	14 (77.7)	-	1 (100)
Ampicillin	86 (20.1)	341 (79.8)	-	18 (100)	-	1 (100)

Table 6: Antibiotic sensitivity pattern of *Enterobacteriaceae* (N = 385)

Antibiotics	*Escherichia coli* (N = 249)		*Klebsiella pneumoniae* (N = 112)		*Klebsiella oxytoca* (N = 11)		*Citrobacter koseri* (N = 7)		*Proteus species* (N = 6)	
	S	R	S	R	S	R	S	R	S	R
Amikacin	240 (96.3)	9 (3.6)	97 (86.6)	15 (13.3)	11 (100)	-	6 (85.7)	1 (14.2)	6 (100)	-
Levofloxacin	225 (90.3)	24 (9.6)	84 (75)	28 (25)	8 (72.7)	3 (27.3)	6 (85.7)	1 (14.2)	5 (83.3)	1 (16.6)
Meropenem	210 (84.3)	39 (15.6)	87 (77.6)	25 (22.3)	7 (63.6)	4 (36.3)	7 (100)	-	3 (50)	3 (50)
Gentamicin	214 (85.9)	35 (14.1)	91 (81.2)	21 (18.7)	10 (90.9)	1 (9)	5 (71.4)	2 (28.5)	4 (66.6)	2 (33.3)
Ceftazidime	139 (55.8)	110 (44.1)	55 (49.1)	57 (50.9)	6 (54.5)	5 (45.4)	5 (71.4)	2 (28.5)	3 (50)	3 (50)
Ceftriaxone	120 (48.2)	129 (51.8)	83 (74.1)	29 (25.9)	7 (63.6)	4 (36.3)	3 (42.8)	4 (57.1)	3 (50)	3 (50)
Cefotaxime	82 (33)	167 (67)	48 (42.8)	64 (57.1)	4 (36.3)	7 (63.6)	5 (71.4)	2 (28.5)	3 (50)	3 (50)
Cefuroxamine	58 (23.3)	191 (76.7)	30 (26.7)	82 (73.2)	1 (9)	10 (90.9)	2 (28.5)	5 (71.4)	3 (50)	3 (50)

Table 7: Antibiotic sensitivity pattern of gram-negative non-fermenters, n (%)

Antibiotics	*Pseudomonas aeruginosa* (N = 69)		*Acinetobacter anitratus* (N = 8)	
	Sensitive	Resistant	Sensitive	Resistant
Amikacin	61 (88.4)	8 (11.5)	7 (87.5)	1 (12.5)
Levofloxacin	50 (72.4)	19 (27.5)	4 (50)	4 (50)
Meropenem	55 (79.7)	14 (20.2)	3 37.5)	5 (62.5)
Gentamicin	56 (81.1)	13 (18.8)	4 (50)	4 (50)
Ceftazidime	48 (69.5)	21 (30.4)	2 (25)	6 (75)
Ceftriaxone	41 (59.4)	28 (40.5)	2 (25)	6 (75)
Cefotaxime	23 (33.3)	46 (66.6)	1 (12.5)	7 (87.5)
Cefuroxamine	23 (33.3)	46 (66.6)	4 (50)	4 (50)
Ciprofloxacin	36 (52.1)	33 (47.8)	2 (25)	6 (75)
Tobramycin	60 (86.9)	9 (13)	7 (87.5)	1 (12.5)

DISCUSSION

Pyogenic infections were found to be prevalent at this tertiary care hospital. They can lead to increased morbidity, prolonged hospital stay and expensive treatment. Knowledge of common pathogens and their resistance status for commonly used antibiotics is essential to guide the clinician in treating these infections.[1] In this study the total number of samples collected were 1704 out of which 901 (52.8%) showed growth which was in accordance with study done by Giri et al. which was 52.4%.[4] However this number is low in comparison to other studies.[7-9] These variation in the growth rate from pyogenic wound specimens might be attributable to the quality of specimen processed, contamination with external microbiota, standard wound care practices in health care facilities, facilities of bacterial cultivation in the locality, and difficulty in growing of fastidious organism.[8,10]

In this study, monomicrobial growth was higher than polymicrobial growth. A total of 893 (52.4%) showed monomicrobial growth, eight (0.46%) showed mixed growth while 803 (47.12%) showed no growth. Multiple studies carried out in wound infection showed higher monomicrobial growth than polymicrobial growth.[5,6] Polymicrobial pyogenic infection might be associated with poor wound care, increased microbial survival and ineffective antimicrobial treatment.[6]

Among the total samples, 698 (41.9%) were obtained from the male patients and 1006 (58.1%) were from the female patients. Among the samples, females outnumbered males (F: M-1.4:1). Similar findings were documented by various studies where higher number of female cases than male.[5,10,11] The study done by Muluye et al. demonstrated that being female is a risk factor for getting an infection by bacteria than male. The authors observed that females were found to be 5.16 times at risk to get infected by bacteria than males.[12]

Age is one of the significant factors influencing the occurrence of infection.[13] In the present study highest rate of infection was observed in the age group between 21-30 years which agrees with the Chaudhary et al. and

Muluye et al.,[7,12] since the individuals in age group 21-30 years are the most active age and they are also involved in outdoor activity.[12] But study done by Upreti et al. showed higher number of cases in age group below 10 years.[5]

Gram-negative bacteria have been observed as the major cause for pyogenic wound infection in several studies.[1,2,4] Findings of current study also supported this fact, as majority of this study isolates were Gram-negative bacteria which accounts for 50.8% (n = 462) but *Staphylococcus aureus* which is a Gram-positive bacteria was most predominant one. However, Upreti et al. and Rijal et al. have documented the higher prevalence of Gram-positive bacteria. It is well known that *Staphylococcus aureus* and Gram-negative bacterial pathogens produce very potent virulence factors, responsible for maintaining the infection and delaying the process of wound healing.[5, 8]

On total growth-positive pus samples, 10 different bacterial species were isolated. The most predominant isolate was *Staphylococcus aureus* (427, 47%) followed by *Escherichia coli* (249, 27.4%), *Klebsiella pneumoniae* (112, 12.4%). Similar study done by Rijal et al. also found that *Staphylococcus aureus* (49.2%) as a predominant pathogen in wound infections followed by *Escherichia coli* (16.2%) and *Klebsiella pneumoniae* (10.5%). The predominance of *Staphylococcus aureus* in wound infections is supported by most of the studies as being a normal flora of human skin, it get access into the wound easily and also *Staphylococcus aureus* causes clinically relevant infections mostly because of its virulence factors such as coagulase, catalase, clumping factor A, and leucocidines.[4,5,7,10] However Nirmala et al. found *Klebsiella* species as the most predominant bacterial pathogen (635, 35%) followed by *Staphylococcus aureus* (334, 18%) and *Escherichia coli* (253, 14%).[3] Also Trojan et al. reported *Escherichia coli* as the most frequent pathogen as revealed by 51.2% followed by *Staphylococcus aureus* (21%), *Klebsiella pneumoniae* (11.6%).[14]

The major concern of this study was associated with high rate of antimicrobial resistance among pathogenic bacteria with the pyogenic infections. The prevalence of antimicrobial resistance changes with geographical areas, climatic conditions and endemicity of resistant pathogen in the locality.[8] No bacterial isolates was found to be sensitive to all antibiotics tested. Isolated bacteria showed multidrug resistance to the commonly used antibiotics in the hospital. Among all the antibiotics used, the highest number of *Staphylococcus aureus* were found to be sensitive to vancomycin (427, 100%) followed by linezolid (426, 99.7%). Similar studies done by Kumar et al. and Biradar et al. found that vancomycin and linezolid were 100% sensitive.[1,9] In this study, resistance to ampicillin was found to be the highest (341, 79.8%) followed by erythromycin (340, 79.6%) and cotrimoxazole (251, 58.8%). This finding is in agreement with the previous reports of Giri et al. and Rijal et al.[4, 8]

Out of 18 isolates of *Enterococcus faecalis*, all isolates were sensitive to vancomycin, linezolid, and teicoplanin and highly resistant to ampicillin (18, 100%). Similar findings were observed by Rijal et al where all the isolates were sensitive to vancomycin and teicoplanin.[8] But isolates of *Enterococcus* spp. were least susceptible to ampicillin, the drug of choice for enterococcal infections.[15]

A total of 249 *Escherichia coli* were isolated from wound specimens where amikacin, levofloxacin and gentamicin were found to be most sensitive which is in accordance with the study done by Giri et al.[4] In this study, *Escherichia coli* was highly resistant to cephalosporin group of drugs which accounts for 62.8% (n = 249). This finding was similar to the study done by Rijal et al. which accounts for 68%.[8]

Among 112 *Klebsiella pneumoniae* isolates, amikacin was the most sensitive antibiotic which accounts for 86.6% (n = 97). Finding from this study agree with the study done by Rijal et al. where amikacin was found 86.7% sensitive.[8]

All the isolates of *Klebsiella oxytoca* (11, 100%) were sensitive to amikacin as Pandey et al. have similar result with the present study, which showed 100% sensitivity towards amikacin.[10] In this study, 10 (90.9%), 8 (72.7%), 7 (63.6%), and 7 (63.6%) were sensitive to gentamicin, levofloxacin, meropenem, and ceftriaxone respectively. In contrast, *Klebsiella oxytoca* were highly resistant to cephalosporin group. All the isolates of *Citrobacter koseri* (7, 100%) were sensitive to meropenem. *Citrobacter koseri* and *Proteus* species were highly sensitive to amikacin and levofloxacin and resistant to cephalosporin group. This is in agreement with various studies.[7,8] Among different antibiotics used for *Pseudomonas aeruginosa* and *Acinetobacter anitratus* the most sensitive antibiotic was amikacin and tobramycin while the most resistant antibiotic was cefotaxime. Resistance to cephalosporins by Gram-negative bacteria is most commonly due to the production of β-lactamases, either chromosomally encoded or plasmid mediated. Other important mechanisms of resistance include decreased penetration of the antibiotics to the bacterial cell or active efflux pumps in Gram-negative bacteria which execrate drugs including multidrug efflux pumps, can also confer to resistance to β-lactam.[10]

CONCLUSION

In this study more than half samples showed growth, mostly monomicrobial growth. Among the samples, females outnumbered males and the highest rate of infection were observed in age group between 21-30 years. Most of the isolates were Gram-negative bacteria which were mostly sensitive to amikacin. The most predominant organism was *Staphylococcus aureus* which was highly sensitive to vancomycin and highly resistant to ampicillin. The data regarding the prevalence of microorganisms and their resistance patterns are beneficial to prescribe appropriate antibiotics.

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