Antibiotic Susceptibility Pattern of Bacterial Isolates from Soft Tissues Infection among Patients Visiting Birendra Military Hospital, Chhauni, Kathmandu

Kushaalta Giri[1], Sharada Gurung[1], Simrika Subedi[1], Alina Singh[3] and Nabaraj Adhikari[2*]

[1]Department of Microbiology, Sainik Awasiya Mahavidyalaya, Sallaghari, Bhaktapur

[2]Central Department of Microbiology, Tribhuvan University, Kirtipur

[3]Birendra Military Hospital, Chhauni, Kathmandu

***Corresponding author:** Nabaraj Adhikari, Central Department of Microbiology, Tribhuvan University: E-mail: adhikarinaba13@gmail.com

ABSTRACT

Objectives: To determine the rate of soft tissues infection and perform antibiotic pattern susceptibility test of bacterial pathogens isolated from soft tissue infected patients visiting Shree Birendra Hospital, Kathmandu, Nepal.

Methods: A total of 380 wound specimens (open and closed) including pus and wound swabs were processed in the laboratory of Birendra Military Hospital, Chhauni from August to November 2018. The specimens were cultured on Blood Agar blood agar and Mac-Conkey agar and incubated at 37°C for 24 hrs. Antibiotic Susceptibility Test was performed by using modified Kirby-Bauer disc diffusion method. Thus, multidrug resistant (MDR) bacteria and methicillin resistant *Staphylococcus aureus* (MRSA) were differentiated.

Results: Out of 380 bacterial isolates, 86(43.21%) were Gram positive and 113(56.78%) were Gram negative bacteria. Among all the Gram-positive isolates 43(53.09%) were found to be MRSA. Similarly, 62(54.86%) were found to be MDR among the Gram-negative bacteria. Gentamicin and Amikacin were found to be the most effective drug though the resistance pattern is not homogenous against all isolates.

Conclusion: Antibiotic susceptibility pattern of all bacterial isolates showed that, Gentamycin, Amikacin, Levofloxacin, Piperacillin/ Tazobactam, Doxycycline were the effective drug for Gram-negative bacteria and Amikacin, Teicoplanin, Linezolid, Doxycycline, Gentamycin and Azithromycin were the most effective drug for Gram-positive organisms. Thus it can be concluded that these antibiotics may be used for the empirical treatment of soft tissues infection.

Key words: Antibiotic susceptibility, bacteria, soft tissue, MRSA

INTRODUCTION

Soft tissues infections are infection of the skin and soft tissue and are usually caused by bacteria. The infection develops when there is a break in the skin, such as a wound or athlete's foot, which may be minor or even unnoticed. This allows bacteria to enter through the skin and grow, causing infection and swelling. People suffering from cut, scarps or other abrasion can get any of this infection. The symptoms of skin and soft tissue infections are all very similar and usually include swelling and redness of the skin as well as warmth radiating from the area. Other symptoms include smooth and shiny skin, small blisters and pimples that get formed in the area (Baddour 2019).

The most common pathogens in these infections are *Staphylococcus aureus* (including MRSA), *P. aeruginosa*, *Enterococcus* spp, *Escherichia coli* and other antibiotics resistant Enterobacteriaceae (Rosser et al. 2005).

The performance of antimicrobial susceptibility testing by the clinical microbiology laboratory is important to confirm susceptibility to chosen empirical antimicrobial agents or to detect resistance in individual bacterial isolates (Edelsberg et al. 2009). Multidrug resistant

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bacteria, bacteria that resist to more than three classes of antibiotics, are more problematic as compared to normal bacteria because infections with multidrug-resistant bacteria are hard to treat since few or even no treatment options remain (Magiorakos et al. 2012). In some cases, health care providers have to use antibiotics that are more toxic for the patient. Multidrug-resistance facilitates spread of antibiotic resistance. When multidrug-resistance plasmids are transferred to other bacteria, these become resistant to many antibiotics at once. In environments where bacteria are continuously exposed to antibiotics, like in hospitals or some large production animal farms, multidrug-resistance may be favorable and are therefore selected and spread further (Bessa et al. 2013). Multidrug-resistance complicates efforts to reduce resistance. When many different antibiotics are selected for the same resistant bacteria or plasmids, reducing use of one type of antibiotic is not enough to reduce resistance to that antibiotic. Thus, there is an increasing prevalence of pathogenic multidrug-resistant bacteria globally. An example is ESBL (extended spectrum beta lactamase)-producing Gram-negative bacteria like *E. coli* and *Klebsiella pneumoniae* (Woerther et al. 2013).

Methicillin resistant *Staphylococcus aureus* (MRSA) emerged as a cause of infection among patients exposed to the bacteria in health care centers. It is a common cause of hospital and community acquired infections worldwide (Barret et al. 1968). Treatment of *S. aureus* infections which has now become more challenging with the emergence of MRSA, are often multidrug resistant (Ciccarone et al. 2001).

MATERIALS AND METHODS

Study site and population

A hospital based descriptive cross-sectional study was conducted during August– November 2018 at Shree Birendra Hospital Chhauni, Kathmandu, Nepal. A total of 380 specimens (pus and swab) were processed from soft tissues infection during study period. The study populations were the patients irrespective of age and sex with soft tissue infection as referred by the physicians for routine clinical care.

Isolation and identification: Wound Swabs were collected and inoculated on Blood agar plates and Mac-Conkey agar plates. The blood agar plates were incubated at 37°C for 24 hrs enriched with CO_2 while Mac-Conkey agar plates were aerobically incubated in ordinary incubation at 37°C for 24 hrs. Blood agar was examined for haemolysis of the medium, colonial characteristic and gram staining was carried out. Mac-Conkey agar plates were examined for Gram's negative organism and lactose fermenter and non-lactose fermenter and colonial character of the organism (WHO 2003).

Isolates were identified using standard microbiological techniques as described by Cheesbrough (2006), comprising of colony morphology, Gram staining and various other biochemical tests such as catalase production test, coagulase production test, oxidase test, IMViC tests, Triple sugar iron agar tests, etc. and reported accordingly.

Antibiotic susceptibility testing: The antibiotic susceptibility testing of individual isolate was carried out by modified Kirby-Bauer disc diffusion method as per CLSI guidelines (2014) using Muller Hinton Agar (MHA). In this study antibiotics used were Ampicillin (10µg), Ceftriaxone (30µg), Ciprofloxacin (5µg), Cloxacillin (5µg), Cotrimoxazole (µ), Erythromycin (15µg), Gentamicin (10µg), Aztreonam (30µg), Amoxicillin (30µg), Ofloxacin (5µg), Cefepime (30µg), Amikacin (30µg), Amoxyclav (20/10µg), Clindamycin(2µg), Levofloxacin (5µg), Cefotaxime (30µg), Ceftazidime (30µg), Doxycycline (30µg), Azithromycin (15µg), Piperacillin (100µg), Piperacillin+Tazobactum (PTZ/100/10µg), Teicoplanin (30µg), Polymyxin B (300unit) and Linezolid (30µg). The organism's showing resistant to more than three different class of antibiotics was taken as Multi-drug resistant isolates (Magiorakos et al. 2012). Screening for methicillin resistance was performed by cefoxitin disc diffusion method and interpreted according to CLSI (2018).

RESULTS

Out of 380 samples collected, 199 (52.36%) sample showed growth and 181(47.63%) showed no growth. (Table 1).

Table 1: Growth pattern of the specimen

Growth	Number	Percentage
Growth	199	52.36
No growth	181	47.63
Total	**380**	**100**

Out of 380 patients, the rate of infection was found to be higher among the males (36.05%) in comparison to females (16.31%). (Table 2)

Table 2: Sex-wise distribution of the patients

Sex	Growth (%)	Total (%)
Male	137 (36.05)	247 (65)
Female	62 (16.31)	133 (35)
Total	**199 (52.36)**	**380 (100)**

As far as the age wise distribution is concerned, the highest rate of infection was observed in the age group 45 to 59 years as shown in table 3.

Table 3: Age-wise distribution of the patients

Age (Years)	Growth n (%)	Total (%)
≤ 14	15 (3.94)	23 (6.05)
15-29	42 (11.05)	82 (21.57)
30-44	45 (11.84)	94 (24.73)
45-59	58 (15.26)	88 (23.16)
60-74	31 (8.15)	78 (20.52)
75-89	7 (1.84)	14 (3.68)
90 above	1 (0.26)	1 (0.26)
Total	**199 (52.36)**	**380 (100)**

Out of total 199 bacterial isolates, 113 were Gram negative and 86-Gram positive bacterial isolates. The most predominant isolate was *Staphylococcus aureus* 81(40.70%), *Escherichia coli* accounting for 37 (18.59%) followed by *Pseudomonas* spp 30(15.07%), *Klebsiella pneumoniae* 18(9.04%), *Acinetobacter* spp 13(6.53%) and *Enterobacter* spp 6(3.01%). The least frequently isolated ones were CoNS 3(1.50%), *Proteus mirabilis* 2(1.005%), *Citrobacter freundii* 2(1.005%), *Serratia marcescens* 2(1.005%), *Citrobacter koserii* 2(1.005%), *Klebsiella oxytoca* 1(0.50%), *Enterococcus* spp 1(0.50%) and *Streptococcus* spp 1(0.50%). (Table 4)

Table 4: Distribution patterns of Gram positive and Gram-negative bacteria among growth

Organism	Number	Percentage
Staphylococcus aureus	81	40.70
Escherichia coli	37	18.59
Pseudomonas aeruginosa	30	15.07
Klebsiella pneumonia	18	9.04
Acinetobacter spp	13	6.53
Enterobacter spp	6	3.01
CoNS	3	1.50
Proteus mirabilis	2	1.01
Citrobacter freundii	2	1.01
Citrobacter koserii	2	1.01
Serratia marcescens	2	1.01
Klebsiella oxytoca	1	0.50
Enterococcus spp	1	0.50
Streptococcus spp	1	0.50
Total	199	100

Among all the antibiotics used, the highest number of *E. coli* (n=37) were found to be sensitive to gentamicin 29 (78.37%) followed by doxycycline 18(48.64%), amikacin 17(45.94%), levofloxacin 15(40.54%), Cotrimoxazole 13(35.13%), Piperacillin+Tazobactum 12(32.43%) as shown in table 5.

Out of 19 isolates of *Klebsiella* spp, 18 isolates were *Klebsiella pneumoniae* and 1 isolate were *Klebsiella oxytoca.* Among which the highest number of isolates were most sensitive to doxycycline 9(47.36%) followed by amikacin 8(42.105%), and others as shown in table 5

Among 13 isolates of *Acinetobacter* spp, was subjected

to AST against 14 antibiotics. Among which the highest isolate was found to be most sensitive to co-trimoxazole 3(23.07%), levofloxacin 1(7.69%), gentamicin 1(7.69%). All the isolates 13(100%) were resistant to Amoxycilin, Amoxyclav, Ceftriaxone, Cefotaxime, Amikacin, Ciprofloxacin, Ofloxacin, Piperacillin and PTZ.

Six isolates of *Enterobacter* spp, was subjected to AST against 14 antibiotics among which the isolate was found to be most sensitive to levofloxacin 5(83.33%), gentamicin 5(83.33%) and ofloxacin 4(66.66%).

Table 5: Antibiotic susceptibility pattern of *E. coli*, *Klebsiella* spp, *Acinetobacter* spp and *Enterobacter* spp.

Isolates	*E. coli* (n=37)		*Klebsiella* spp (n=19)		*Acinetobacter* spp (n=13)		*Enterobacter* spp (n=6)	
Antibiotics	Sensitive N (%)	Resistant N (%)	S ensitive N (%)	Resistant N (%)	Sensitive N (%)	Resistant N (%)	Sensitive N (%)	Resistant N (%)
Amoxycilin	3(8.10)	34(91.89)	0(0)	19(100)	0(0)	13(100)	0(0)	6(100)
Amoxyclav	8(21.62)	29(78.37)	1(5.26)	18(94.73)	0(0)	13(100)	0(0)	6(100)
Ceftriaxone	6(16.21)	31(83.78)	4(21.05)	15(78.94)	0(0)	13(100)	2(33.33)	4(66.66)
Cefotaxime	5(13.51)	32(86.48)	3(15.78)	16(84.21)	0(0)	13(100)	1(16.66)	5(83.33)
Cotrimoxazole	13(35.13)	24(64.86)	4(21.05)	15(78.94)	3(23.07)	10(76.92)	3(50)	3(50)
Gentamycin	29(78.37)	8(21.62)	6(31.57)	13(68.42)	1(7.69)	12(92.30)	5(83.33)	1(16.66)
Amikacin	17(45.94)	20(54.05)	8(42.11)	11(57.89)	0(0)	13(100)	2(33.33)	4(66.66)
Ciprofloxacin	7(18.91)	30(81.08)	4(21.05)	15(78.94)	0(0)	13(100)	1(16.66)	5(83.33)
Ofloxacin	9(24.32)	28(75.67)	7(36.84)	12(63.15)	0(0)	13(100)	4(66.66)	2(33.33)
Levofloxacin	15(40.54)	22(59.45)	7(36.84)	12(63.15)	1(7.69)	12(92.30)	5(83.33)	1(16.66)
Piperacillin	4(10.81)	33(89.18)	0(0)	19(100)	0(0)	13(100)	1(16.66)	5(83.33)
PTZ	12(32.43)	25(67.56)	3(15.78)	16(84.21)	0 (0)	13 (100)	3(50)	3(50)
Ampicilin	7(18.91)	30(81.08)	0(0)	19(100)	0(0)	13(100)	0(0)	6(100)
Doxycycline	18(48.64)	19(51.35)	9(47.36)	12(63.15)	0(0)	13(100)	0(0)	6(100)

Among 2 isolates of *Proteus mirabilis*, was subjected to AST against 14 antibiotics among which all isolates were found to be resistant to Amoxycilin 2(100%) and Cefotaxime 2(100%). Among four isolates of *Citrobacter* spp, two isolates were *Citrobacter freundii* and two were *Citrobacter freundii*. These bacterial isolates were subjected to AST against 14 antibiotics among which all isolates 4(100%) were found to be resistant to Amoxycilin, Amoxyclav, Ceftriaxone, Piperacilin, and Cefotaxime. Among 2 isolates of *Serratia marcescens*, was subjected to AST against 14 antibiotics among which both 2 isolates was found to be resistant to Amoxyclav and Doxycycline.

Out of 30 isolates of *Pseudomonas* spp, all were subjected to AST against 9 antibiotics. Among which the highest number of isolates were most sensitive to Polymyxin B 27(90%), followed by Gentamicin 24(80%), Amikacin 22(73.33%), PTZ 22(73.33%), Aztreonam 22(73.33%). The lowest sensitivity was towards Cefepime 12(40%).

Among 199 positive isolates, 113 were Gram negative organisms. Out of total Gram-negative organism isolates 62(54.86%) were multi drug resistant (MDR) and 51(45.13%) were not MDR.

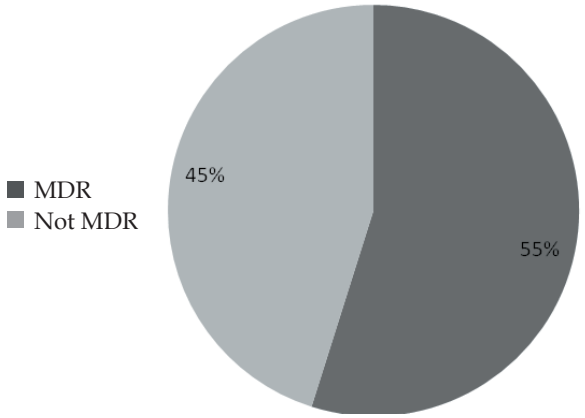

Figure 3: Distribution of MDR among Gram negative isolates

Altogether 81(94.17%) *Staphylococcus aureus* were isolated among 86 Gram positive cocci (GPC)GPC. Among *S. aureus*, 43 were MRSA and 38 were MSSA. These all *S. aureus* were subjected towards 12 antibiotics and highest sensitive towards Amikacin was found 79(97.53%) followed by Teicoplanin 75(92.59%) and Linezolid 73(90.12%) and the lowest sensitive to Ampicillin 5(6.17%).

Three CoNS were isolated among 86 GPC. Only one isolate was Coagulase Negative *Staphylococcus aureus* but other 2 were Methicillin resistant Coagulase Negative *Staphylococcus aureus* and subjected towards 12 antibiotics and found highest sensitivity towards Ampicillin 3(100%) followed by Cotrimoxazole 3(100%), Erythromycin 3(100%), and Azithromycin 3(100%).

Single *Enterococcus* spp was found and was subjected AST pattern against 12 antibiotics. It was sensitive against Cotrimozazole, Gentamicin, Amikacin, Ofloxacin, Cloxacillin, Erythromycin, Linezolid and resistant towards others.

Only 2 *Streptococcus* spp were isolated from 86 GPC isolates and subjected against 12 antibiotics and it was found highest sensitive towards Amikacin 2(100%), Ofloxacin 2(100%), Teicoplanin 2(100%), Linezolid 2(100%) followed by Gentamycin 1(50%), Clindamycin 1(50%), Doxycycline 1(50%). (Table 6)

Table 6: Antibiotic susceptibility pattern of Gram-positive cocci

Isolates	*Staphylococcus aureus* (n=81)		Coagulase Negative *Staphylococcus aureus* (n=3)		*Enterococcus* spp. (n=1)		*Streptococcus* spp.(n=2)	
Antibiotics	**S n(%)**	**R n(%)**	**S n(%)**	**R n(%)**	**S n(%)**	**R n(%)**	**S n(%)**	**R n(%)**
Cotrimoxazole	29(35.80)	52(64.197)	3(100)	0(0)	0(0)	1(100)	2(100)	0(0)
Gentamicin	63(77.78)	18(22.22)	1(33.33)	2(66.67)	0(0)	1(100)	1(50)	1(50)
Amikacin	79(97.53)	2(2.47)	0(0)	3(100)	0(0)	1(100)	0(0)	2(100)
Ofloxacin	31(38.27)	50(61.73)	0(0)	3(100)	0(0)	1(100)	0(0)	2(100)
Cloxacillin	41(50.62)	40(49.38)	3(100)	0(0)	0(0)	1(100)	2(100)	0(0)
Erythromycin	12(14.81)	69(85.19)	3(100)	0(0)	0(0)	1(100)	2(100)	0(0)
Azithromycin	49(60.49)	32(39.51)	3(100)	0(0)	1(100)	0(0)	2(100)	0(0)
Clindamycin	47(58.02)	34(41.98)	2(66.67)	1(33.33)	1(100)	0(0)	1(50)	1(50)
Teicoplanin	75(92.59)	6(7.41)	0(0)	3(100)	1(100)	0(0)	0(100)	2(100)
Doxycyclin	68(83.95)	13(16.05)	1(33.33)	2(66.67)	1(100)	0(0)	1(50)	1(50)
Linezolid	73(90.12)	8(9.88)	0(0)	3(s100)	0(0)	1(100)	0(0)	2(100)
Ampicillin	5(6.17)	76(93.83)	3(100)	0(0)	1(100)	0(0)	2(100)	0(0)

Antibiotic susceptibility pattern of *S. aureus*, CoNS, *Enterococcus* spp and *Streptococcus* spp.

Among 119 isolates, 81 were *S. aureus*. Out of total *S. aureus* isolates Methicillin sensitive *Staphylococcus aureus* were 38(46.91%) and Methicillin Resistant *Staphylococcus aureus* were 43(53.09%). (Figure: 4)

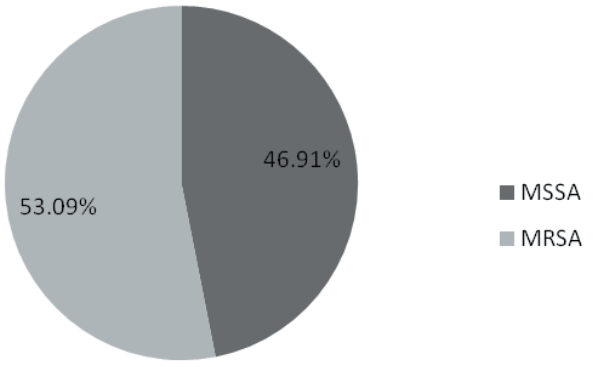

Figure 4: Distribution of MRSA among *S. aureus*

DISCUSSION

In this study the overall rate of bacterial Soft tissues infection among the study population was found to be 199 (52.36%). The result was in agreement with the study carried out by Sah et al. (2013) that reported 62% growth rate and close to the result reported by Acharya et al. (2008), accounting 50.7%. The predominance of male patients was seen in this study with male: female ratio of 65/35 and this finding was similar to the other studies where a much higher number of male patients have been reported Sharma et al. (2013) and Gurung et al. (2018). The patients with age >30 years had a much higher incidence of STIs (42.09%) in comparison to an incidence of 14.99% among the patients who were ≤29 years of age. Similarly, the study carried out by Murphy et al. (2001) also had a much higher incidence of STIs (89.41%) at age group >30 years. Advancing age is an important factor for the development of STIs, as in old age patients there is low healing rate, low immunity, increased catabolic processes and presence of co-morbid illness like diabetes, hypertension, etc. (Sharma et al. 2015).

In this study, the frequency of Gram-negative bacteria was higher than Gram positive bacteria. However, a similar study carried out by Surucuoglu et al. (2005) showed the higher prevalence of Gram-positive bacteria (69%) than Gram negative bacteria (29%). The higher prevalence of Gram-positive bacteria was also depicted in researches carried out by Kaftandzieva et al. (2012). Practically, *S. aureus* was the major pathogenic Gram positive organism and *E. coli* was the major pathogenic Gram negative organisms for STIs, as in the study carried out by Fazii et al. (2013), and Ranabhat et al. (2013) shows the most common bacterial species detected was *Staphylococcus aureus* (37.50%) and *E. coli* (25%). In the study carried out by Karkee (2008) reported similar results that the most common bacteria (46.58%) were *S. aureus, E. coli* (12.38%) emerged as the next common organism causing wound infection in this study as in the other previously reported studies which is followed by, CoNS (11.40%) and *P. aeruginosa* (7.49%). The least common bacteria isolated were *C. freundii* (0.65%). In Saudi Arabia, Abussaud (1996) isolated *S. aureus* (35%), *P. aeruginosa* (25%) and *Klebsiella* spp (10%) as the major causative agents.

However, different studies showed that *P. aeruginosa* was the leading cause of wound infections. In a study conducted by Mousa (1997) to assess the rate of wound infection by aerobic bacteria and found that 19.1% of the wound infection was caused by *P. aeruginosa*. Similar study on wound infection by Nasser et al. (2003) showed *P. aeruginosa* (21.6%) as the most common isolate which in compare to our result was similar as the rate of infection by *P. aeruginosa* was fond to be 15.07%.

In antibiotic susceptibility pattern of Gram negative organism, gentamycin was most sensitive (62.83%) followed by amikacin (47.78%), Levofloxacin (39.76%), PTZ (38.05%), Doxycycline (34.94%),Cotrimoxazole (32.53%), Ofloxacin (30.12%), Ciprofloxacin (28.32%), Piperacillin (22.12%), Ceftriaxone (18.07%), Cefotaxime (13.25%), Ampicillin (12.05%), Amoxyclav (12.04%) and Amoxycillin (4.81%). However, the study carried out by Timalsina et al. (2015) for Gram negative isolates, Amikacin (45, 93.75%) was found to be the most sensitive antibiotic followed by Gentamycin (42, 89.36%), Ciprofloxacin (27, 56.25%) while Amoxycillin (13, 32.5%) and Cotrimoxazole (14, 29.16%) being the least sensitive antibiotic respectively. In our study, among Gram positive isolates, the most effective antibiotic was Amikacin (91.86%) followed by Teicoplanin (88.37%), Linezolid (84.88%), Doxycycline (82.56%), Gentamycin (75.58%), Azithromycin (63.95%), Clindamycin (59.30%), Cloxacillin (53,49%), Cotrimoxazole (39.53%), Ofloxacin (36.04%), Erythromycin (19.77%) and Ampicillin (12.79%). However, Tuladhar (1999) reported that Gentamicin was found to be most effective (89.53%) drug followed by Ciprofloxacin (83.72%) while only 16.27% of Gram-positive cocci were sensitive to Ampicillin.

The patterns of MDR among Gram negative bacterial isolates were 100% in *Acinetobacter* spp, 83.33% in *Enterobacter* spp, 77.77% in *Klebsiella pneumoniae,* 64.86% in *Escherichia coli,* 50% in *Proteus mirabilis,* 50% in *Citrobacter freundii,* 50% in *Citrobacter koserii,* 10% in *Pseudomonas* spp and no any MDR isolates in *Klebsiella oxytoca* and *Serratia marcescens* which was in contrast to results shown by Bhandari (2014) that reported that higher number of *E. coli* isolates, 64 (72.7%) were multi drug resistant followed by *Pseudomonas aeruginosa* 11 (91.7%) and *K. pneumoniae* 6 (75%). Out of all GPC, 81(94.17%) were *S. aureus* in which 43(53.06%) were MRSA and 38(46.94%) were MSSA which was similar to the study performed by Khanal and Jha (2010) which showed 68% MRSA and 32% MSSA. The study performed by Edelsberg et al. (2009) also showed 35.9%

MRSA which is also contrast to our study. Though a great array of bacteria is involved in wound infections, we were able to trace limited pathogens due to lack of adequate laboratory facilities and time boundary.

CONCLUSION

The rate of wound infection is higher among the patients visiting the tertiary care hospital in Kathmandu. The antibiotic susceptibility pattern of the pathogens causing wound infections in the study population revealed higher rate of multidrug resistant, indicating the limited therapeutic alternatives for the management of wound infected patients.

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CONFLICT OF INTEREST

The author declares no conflict of interest.

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