Prevalence of Multidrug-Resistant and Extended-spectrum Beta-lactamase Producing Bacterial Isolates from Infected Wounds of patients in Kathmandu Model Hospital.

Kabita Adhikari¹*, Shaila Basnyat¹ and Basudha Shrestha²

¹Central Department of Microbiology, Tribhuvan University, Nepal
²Kathmandu Model Hospital, Kathmandu, Nepal

Abstract

The wound is an injury to living tissues caused by a cut, puncture, bite, blow, or other impacts. An infection is caused when germs enter wounds. This study was designed to isolate and identify the causative agents of wound infections and their antibiotic susceptibility pattern. A total of 339 samples were collected from January to June 2016 from Kathmandu Model Hospital, Kathmandu. Samples were inoculated on the Blood Agar and MacConkey agar plates were incubated at 37 °C for 24 hours. After incubation, all isolates were identified by using gram stain and biochemical methods. Antibiotic susceptibility tests were performed on Mueller Hinton agar plate by Kirby Bauer Disk Diffusion Technique. During the study period, altogether, 339 specimens were collected and processed as per the standard microbiological protocol. The overall prevalence of wound infection was 56.9%. Among 244 bacterial isolates, Escherichiacoli (24.2%) was most predominant bacteria followed by Staphylococcus aureus (19.7%), Coagulase-negative Staphylococcus (17.6%), Klebsiella pneumoniae (10.7%), Pseudomonas aeruginosa (8.6%), Acinetobacter spp (5.7%), Citrobacter freundii (4.9%) Proteus mirabilis (3.3%), Streptococcus viridans (2.0%), Klebsiella oxytoca (0.8%), Proteus vulgaris (0.4%), Serratia marcescens (0.4%), Enterobacter aerogens (1.2%), Enterobacter faecalis (0.4%). The most effective drug for Gram-negative bacteria and Gram-positive bacteria were amikacin and chloramphenicol, respectively. A total of Gram-negative bacteria, 77.55% were multidrug-resistant. The total Gram-negative bacteria most ESBL producers were E. coli (82.9%). We found S. aureus 33.3% of isolates were resistant to cefoxitin which indicates the increasing rate of Methicillin-resistant S. aureus (MRSA) in wound infection.

Keywords

E.coli, MRSA, S. aureus, wound, infections

Introduction

The wound infection is a breach in the skin, and exposure of the subcutaneous tissues provide suitable environment for microbial colonization and proliferation (Yakha et al., 2014). In wound infections, bacteria deposit and multiply in tissue an associated host reaction (Collier et al., 2004). The main reason of wound infection is the breach in the skin that let different cell types enter the wound that initiates an inflammatory response. Signs of redness, pain, swelling, and fever are characteristics of the inflammatory response in the wound (Shrestha et al., 2009).

Wound infections can be caused by different groups of bacteria, which including Gram-positive and Gram-negative. In Gram-positive bacteria S. aureus, coagulase-negative S. aureus, Enterococci and Gram-negative bacteria E. coli, P. aeruginosa K. pneumoniae, K. oxytoca, Enterobacter, P. mirabilis, P. vulgaris, Acinetobacter etc and other Streptococci and Candida (Gupta et al., 2002; Eselbehie et al., 2013).

Methicillin-resistant S. aureusis now endemic in most United States hospitals and long-term care facilities. Centres for Disease Control and Prevention of S. aureus isolates that are resistant
to methicillin has increased steadily in recent years (Mera et al., 2011). MRSA is a significant pathogen causing health-related problems in the world (Chan et al., 2014).

This study may help to select appropriate empirical antibiotic treatment and may help in minimizing the alarming trend of antibiotic resistance which would be helpful for the management of such infections in the respective hospital.

**Methodology**

This study was carried out by collecting wound swabs and pus samples from patients visiting Kathmandu Model Hospital, Kathmandu from January to June 2016. A total of 339 samples were cultured on Blood agar and Mac Conkey agar media incubated at 37 °C for 24 hours. Organisms were identified by a standard microbiological procedure including colony characters, Gram staining and biochemical reactions. The antibiotic sensitivity test of all isolates was performed by modified Kirby Bauer disc diffusion method on Mueller Hinton agar or Blood agar medium using antibiotic discs of Hi media. After this isolated *S. aureus* was screened for methicillin resistance using cefoxitin disc (30 µg) as per standard guidelines provided by CLSI, the zone of inhibition ≤ 21 mm is considered a positive result for MRSA strain. The test inoculum was matched with the Mac Farland tube 0.5 standard. Turbidity was prepared and carpet culture of Muller Hinton Agar (Cheesbrough 2006).

Screening of the suspected ESBL strains was performed according to the guidelines for screening issued by CLSI. According to this guideline, MDR isolates were screened for possible ESBL production using ceftriaxone (30µg), ceftazidime (30µg) and cefotaxime (30µg). Isolates were cefotaxime ≤27mm, ceftazidime ≤22mm and ceftriaxone ≤25mm were the possible ESBL producers. Regardless of the screening results, all the third generation cephalosporins resistant bacterial isolates were subjected to phenotypic confirmatory test using combined

| Types of organism | Sample       |          |          |          |          |          |
|-------------------|--------------|----------|----------|----------|----------|----------|
|                   | Wound swab   | Pus aspirates | Number | %    | Number | %    |
| E. coli          | 31           | 23.3     | 28       | 25.2    | 59       | 24.2    |
| S. aureus        | 22           | 16.5     | 26       | 23.4    | 48       | 19.7    |
| CoNS              | 24           | 18.0     | 19       | 17.1    | 43       | 17.6    |
| K. pneumoniae    | 15           | 11.3     | 11       | 9.9     | 26       | 10.7    |
| P. aeruginosa    | 14           | 10.5     | 7        | 6.3     | 21       | 8.6     |
| Acinetobacter spp| 7            | 5.3      | 7        | 6.3     | 14       | 5.7     |
| C. freundii      | 9            | 6.8      | 3        | 2.7     | 12       | 4.9     |
| P. mirabilis     | 6            | 4.5      | 2        | 1.8     | 8        | 3.3     |
| S. viridans      | 1            | 0.8      | 4        | 3.6     | 5        | 2.0     |
| K. oxytoca       | 1            | 0.8      | 1        | 0.9     | 2        | 0.8     |
| P. vulgaris      | 1            | 0.8      | 0        | 0.0     | 1        | 0.4     |
| S. marcescens    | 0            | 0.0      | 1        | 0.9     | 1        | 0.4     |
| E. aerogens      | 2            | 1.5      | 1        | 0.9     | 3        | 1.2     |
| E. faecalis      | 0            | 0.0      | 1        | 0.9     | 1        | 0.4     |
| Total            | 133          | 100      | 111      | 100     | 244      | 100     |
### Table 2. Antibiotic susceptibility pattern of *E. coli*

| Antibiotics         | Susceptibility pattern |          |          |          |          |          |
|---------------------|------------------------|----------|----------|----------|----------|----------|
|                     | Sensitive | Intermediate | Resistant |          |          |          |
|                     | No            | %          | No            | %          | No            | %          |
| Amikacin             | 49          | 83.1       | -            | -          | 10          | 16.9      |
| Amoxycillin          | 11          | 18.6       | -            | -          | 47          | 81.4      |
| Amoxyclav            | 11          | 18.6       | -            | -          | 47          | 81.4      |
| Ceftazime            | 18          | 30.5       | -            | -          | 41          | 69.5      |
| Ceftriaxone          | 18          | 30.5       | -            | -          | 41          | 69.5      |
| Chloramphenicol      | 54          | 91.5       | -            | -          | 5           | 8.5       |
| Colistin             | 59          | 100        | -            | -          | -           | -         |
| Co-trimoxazole       | 20          | 33.9       | -            | -          | 39          | 66.1      |
| Doxycycline-Hydrochloride | 49       | 83.1       | 1            | 1.7        | 9           | 15.3      |
| Gentamycin           | 42          | 71.2       | 3            | 5.1        | 14          | 23.7      |
| Imipenem             | 54          | 91.5       | 1            | 1.7        | 4           | 6.8       |
| Levofloxacin         | 22          | 37.3       | 4            | 6.8        | 33          | 55.9      |
| Meropenem            | 54          | 91.5       | 1            | 1.7        | 4           | 6.8       |
| Ofloxacin            | 22          | 37.3       | 4            | 6.8        | 33          | 55.9      |
| Piperacillin/Tazobactam | 54       | 91.5       | 1            | 1.7        | 4           | 6.8       |
| Polymixin-B          | 59          | 100        | -            | -          | -           | -         |
| Tigecycline          | 59          | 100        | -            | -          | -           | -         |

### Table 3. Antibiotic susceptibility pattern of *S. aureus*

| Antibiotics         | Susceptibility pattern |          |          |          |
|---------------------|------------------------|----------|----------|----------|
|                     | Sensitive | Intermediate | Resistant |          |
|                     | No            | %          | No            | %          | No            | %          |
| Amoxycillin         | 3          | 6.2        | -            | -          | 45          | 93.8      |
| Amoxyclav           | 3          | 6.2        | -            | -          | 45          | 93.8      |
| Cephalexin          | 31         | 64.6       | -            | -          | 17          | 35.4      |
| Chloramphenicol     | 45         | 93.6       | -            | -          | 3           | 6.4       |
| Ciprofloxacin       | 20         | 40.4       | -            | -          | 28          | 59.6      |
| Co-trimoxazole      | 15         | 29.8       | -            | -          | 33          | 70.2      |
| Doxycycline-Hydrochloride | 43       | 89.4       | 1            | 2.1        | 4           | 8.5       |
| Erythromycin        | 33         | 68.1       | -            | -          | 15          | 31.9      |
| Gentamycin          | 43         | 89.4       | -            | -          | 5           | 10.6      |
| Teicoplanin         | 48         | 100.0      | -            | -          | -           | -         |
| Tigecycline         | 48         | 100.0      | -            | -          | -           | -         |
| Vancomycin          | 48         | 100.0      | -            | -          | -           | -         |
disks test (CDT). ESBLs set consisting of Set 1:
cefazidime (30 μg) and cefazidime (30 μg) plus
clavulanic acid (10 μg), Set 2: cefotaxime (30 μg)
and cefotaxime (30 μg) plus clavulanic acid (10
μg). The zone of inhibition for the cefazidime
and cefotaxime discs were compared to that of
the cefazidime and cefotaxime plus clavulanic
acid combination discs. An increase in the zone
diameter of ≥5mm in the presence of clavulanic
acid, from any or all of the kit sets, was concluded
as confirmed ESBL producers.

Results and Discussion

From a total of 339 wound samples, 193 (56.9%)
samples showed aerobic bacterial growth and
43.1% were growth negative (figure 1).

Out of a total of 168 wound swab, 102 (52.8%)
were positive and also out of 171 pus aspirates 91
(47.2%) were positive. In this study, 187 (58.1%)
samples from male patients and among them, 112
(56.9%) were positive. 142 (41.9%) samples were
from female patients, and among them, 81 (57%)
were positive. Out of 193 positives samples, 146
(75.6%) showed monomicrobial growth, and 47
(24.4%) showed polymicrobial growth.

Out of 244 bacterial isolates obtained from
the 193 positive samples, 97 (39.8%) bacterial
isolates were Gram-positive, and 147 (60.2%)
bacterial isolates were Gram-negative. The most
common bacterial isolates were E. coli, followed
by S. aureus. Among Gram-positive S. aureus 48
(19.7%) were most common isolates followed
by CoNS 43 (17.6%), S. viridans 5 (2.0%) and
E. faecalis 1 (0.4%). From a total of 48 S. aureus
were isolated from wound samples, 16 (33.3%)
were MRSA. Among total positive isolates,
147 were Gram-negative, of which 59 (24.2%)
were the most common isolates followed by K.
pneumoniae 26 (10.7%), P. aeruginosa 21 (8.6%),
Acinetobacter spp 14 (5.7%), C. freundii 12 (4.9%),
P. mirabilis 8 (3.3%), K. oxytoca 2 (0.8%), P.
vulgaris 1 (0.4%), S. marcescens 1 (0.4%) and E..
aerogens 3 (1.2%) (Table 1).

The most susceptible first-line antibiotic for E. coli
(n=59) was amikacin. Among the total isolated
E. coli, 83.1% were susceptible to amikacin and
levofloxacin. Ofloxacin was the second most
effective antibiotic against the 37.3% E. coli.
Similarly, 18.6% of E. coli were least susceptible
to amoxyclillin and amoxyclav. Among the
isolated E. coli, 91.5% were susceptible to
meropenem, imipenem, piperacillin/tazobactam,
and chloramphenicol and 100% of isolated E. coli
were susceptible to third-line antibiotics colistin,
polymixin-B, and tigecycline (Table 2).

The most effective antibiotic for S. aureus
(n=48) was chloramphenicol (93.6%) followed
by gentamycin and doxycycline (89.4%). The
least susceptible to amoxyclillin and amoxyclav

| Bacteria       | NO | MDR | %   | MDR Bacteria | No | No MDR | %  |
|----------------|----|-----|-----|--------------|----|--------|----|
| E. coli        | 46 | 78.0|     |              | 13 | 22.0   |    |
| K. pneumoniae | 23 | 88.2|     |              | 3  | 11.5   |    |
| P. aeruginosa | 12 | 57.1|     |              | 9  | 42.9   |    |
| Acinetobacterspp | 13 | 92.9|     |              | 1  | 7.1    |    |
| P. mirabilis  | 7  | 87.5|     |              | 1  | 12.5   |    |
| C. freundii   | 8  | 66.7|     |              | 4  | 33.3   |    |
| K oxytoca     | 2  | 100.0|   |              | -  | -      |    |
| P. vulgaris   | 1  | 100.0|   |              | -  | -      |    |
| S. marcescens | -  | -   |    |              | 1  | 100    |    |
| E. aerogens   | 2  | 66.7|     |              | 1  | 33.3   |    |
| Total         | 114|     |     |              | 33 |        |    |

Table 4. Multidrug-resistant Gram-negative bacteria
Among the isolated \textit{S. aureus} 100\% were susceptible to second-line antibiotics vancomycin, teicoplanin, and tigecycline (Table 3).

Among the 147 Gram-negative bacteria, 114 were multidrug-resistant, and 33 were non-multidrug resistant. The isolated MDR bacteria were \textit{E. coli} 46 (78\%), \textit{K. pneumonia} 23 (88.2\%), \textit{P. aeruginosa} 12(57.1\%), \textit{Acinetobacterspp} 13 (92.9\%), \textit{P. mirabilis} 7 (87.5\%), \textit{C. freundii} 8 (66.7\%), \textit{K. oxytoca} 2 (100\%), \textit{P. vulgaris} 1 (100\%) and \textit{E. aerogens} 3 (66.7\%) (Table 4).

A total of 41 isolates of MDR \textit{E. coli}, 34 isolates were ESBL producers. Among 20 isolates of MDR \textit{K. pneumoniae}, 10 isolates were ESBL producers. Similarly, out of 12 isolates of MDR \textit{Acinetobacterspp}, 2 isolates were ESBL producers. Also, 12 isolates were MDR \textit{P. aeruginosa}, 1 isolate was ESBL producers, a total of 8 isolates of MDR \textit{C. freundii}, 5 isolates were ESBL producers, among 3 isolates of MDR \textit{P. mirabilis}, 2isolates were ESBL producers. Besides, a total of 2 isolates of MDR \textit{K. oxytoca}, 1 isolate was ESBL producers, but no isolates of \textit{P. vulgaris} and \textit{E. aerogens} were ESBL producers (Table 5).

Table 5. ESBL producers among MDR Gram negative bacteria

| Bacterial isolates | Total | ESBL No | % |
|--------------------|-------|---------|---|
| \textit{E. coli}   | 41    | 34      | 82.9 |
| \textit{K. pneumoniae} | 20    | 10      | 50.0 |
| \textit{P. aeruginosa} | 12    | 1       | 8.3  |
| \textit{Acinetobacterspp} | 12    | 2       | 16.7 |
| \textit{C. freundii} | 8     | 5       | 62.5 |
| \textit{P. mirabilis} | 3     | 2       | 66.7 |
| \textit{K. oxytoca} | 2     | 1       | 50.0 |
| \textit{P. vulgaris} | 1     | 0       | 0.0  |
| \textit{E. aerogens} | 2     | 0       | 0.0  |

In this study, out of 339 samples collected, 193 (56.9\%) samples showed aerobic bacterial growth, and 146 (43.1\%) samples showed no growth. The overall prevalence rate of wound infections was 50\% (Shrestha et al.,2009). In a similar study conducted at TUTH, 50.7\% of total samples showed growth (Acharya et al.,2008) and 49.3\% with no growth. Similarly, a study carried out by Chitwan Medical College Teaching Hospital showed that out of 200 samples 150 (75\%) showed growth (Gautam et al., 2013) and 60\% showed the growth positive (Bhatta and Lakhey 2007).

In a present study, out of total samples collected, 187 (58.1\%) were collected from male patients, and 112 (41.9\%) were collected from female patients. Though our study showed a higher number of male cases than female cases, the growth rate didn't differ significantly between male and female populations (p>0.05). A similar study was carried out in Lahore which showed 20\% more male patients than females (Zafar et al., 2007) and a higher percentage of males (76.5\%) patients were found than females in Nigeria (Adegoke et al., 2010).

Out of 193 positives samples, 146 (75.6\%) showed monomicrobial Growth, and 47 (24.4\%) showed polymicrobial growth. The single isolate was higher than multiple isolates in both pus swab and aspirated pus. Various studies carried out in wound infection showed a higher rate of monomicrobial infection than polymicrobial infection (Karki 2012; Komolafe et al., 2003; Kumari 2008; Nepal and Shrestha et al., 2009).

We identified 244 bacterial isolates obtained from the 193 positive samples, 97 (39.8\%) bacterial isolates were Gram-positive, and 147 (60.2\%) bacterial isolates were Gram-negative. Another study from Kathmandu Model hospital showed that among the total isolates, 273 (64.08\%) were Gram-positive bacteria, and 153 (35.92\%) were Gram-negative bacteria (Shrestha et al., 2009). In wound swab, \textit{E. coli} 31 (23.3\%) was most predominant, followed by 24 (18\%) \textit{CoNS} and \textit{S. aureus} 22 (16.5\%). Similarly, in pus aspirates \textit{E. coli} 28 (25.2\%) was most predominant, followed by \textit{S. aureus} 26 (23.4\%) and \textit{CoNS} 19 (17.1\%).

We found that the most common bacterial isolates were \textit{E. coli}, followed by \textit{S. aureus}. The most predominant bacteria were \textit{E. coli} supported by (Gautam et al., 2013; KC et al., 2013). The most predominance of \textit{S. aureus} and the second most predominant bacteria is \textit{E. coli} in a wound is supported by many studies (Karki 2012; Shrestha...
et al., 2009). The predominance of *E. coli* in a wound is supported by many studies (Gautam et al., 2013). Among the 244 bacterial isolates, 14 different species were isolated. (Kansakar et al., 2003) in TUTH, which reported that 82.5% of the sample cultured aerobically showed bacterial growth and 13 different bacterial species were isolated. *S. aureus* was the most frequently isolated organisms (57.7%), followed by *E. coli* (11%) and CoNS (3%) (Basnet, 2011), found that the most predominant organism was *S. aureus* (19.71%) followed by *E. coli* (15.5%). Gautam et al., (2013), found most predominant bacterial species as *S. aureus* (65.3%) followed by *K. pneumoniae* (8%), *E. coli* (7.3%), CoNS (6%), *P. aeruginosa* (5.3%), *Enterococcus* spp., (3.3%), *Enterobacter* spp., (2%), *Acinetobacter* spp. (1.3%), *P. mirabilis* (0.6%) and *P. vulgaris* (0.6%). *S. aureus* was the predominant organism followed by hemolytic *Streptococcus* (Ruth and Keith 2004). Bhatta and Lakhey (2007); Singh et al., (2006) and Shrestha et al., (2009) reported that after *S. aureus*, *E. coli* was the second predominant isolate. Mumtaz et al., (2002), samples from aerobic pyogenic isolates from wounds and abscesses, reported that *S. aureus* was the most common pathogen (49%) followed by *E. coli* (25.9%), *Klebsiella* (9.5%), *P. aeruginosa* (8.6%), *Proteus* spp. (4%) and *Acinetobacterspp* (2.7%). Another study conducted by B.D. Sharma Postgraduate Institute of Medical Sciences in Rohtak, India found that the most common wound isolate was *S. aureus* (32.3%) followed by *K. pneumoniae* (22.0%), *Pseudomonas* spp (18.7%) and *E. coli* (17.4%) (Gupta et al., 2002).

This study also showed that the most susceptible first-line antibiotic was amikacin and among the total isolated *E. coli* 83.1% were susceptible to amikacin, levofloxacin, and ofloxacin were second most effective antibiotic against the 37.3% *E. coli*. Similarly, 18.6% *E. coli* were least susceptible to amoxycillin and amoxyclyl. Among the isolated *E. coli* 91.5% were susceptible to meropenem, imipenem, piperacillin/tazobactam, and chloramphenicol and 100% of isolated *E. coli* were susceptible to third-line antibiotics colistin, polymixin-B, and tigecycline. *E. coli* was found to be sensitive to gentamycin (80%), ciprofloxacin (60%), cefotaxime (50%) and co-trimoxazole (40%). The least effective antibiotic was ampicillin followed by cephazolin (20%) and ceftriaxone (30%). A study conducted by Karkee (2008) in Bir Hospital, 78% of isolates were sensitive to gentamycin whereas 55.3% of isolates were resistant to ciprofloxacin, 65.8% were equally resistant to co-trimoxazole and amoxicillin. In a study carried out by Nwachukwa et al., (2009), 55% of *E. coli* isolates were sensitive to ciprofloxacin. The studies carried out by Bhatta and Lakhey (2007), and Singh et al., (2006) found that *E. coli* was equally susceptible to cephalaxin, co-trimoxazole, and ciprofloxacin (57%).

The most effective antibiotic in first-line antibiotics was chloramphenicol (93.6%) followed by gentamycin and doxycycline (89.4%). The least susceptible to amoxycillin and amoxyclyl (6.2%). Among the isolated *S. aureus* 100% were susceptible to second-line antibiotics vancomycin, teicoplanin, and tigecycline. Gautam et al., (2013) have found that *S. aureus* was highly sensitive to amikacin (83.6%) followed by ceftriaxone (67.3%), ciprofloxacin (65.3%), cefotaxime (55%), gentamycin (53.06%). It was highly resistant to ampicillin (67.3%), and co-trimoxazole (65.3%) Andragachew et al., (2006) has reported ampicillin (55%) and co-trimoxazole (65%) as a highly resistant drug against *S. aureus*.

Similarly, out of 147 Gram-negative bacteria, 114 were multidrug-resistant, and 33 were non-multidrug-resistant. The isolated MDR bacteria were *E. coli* 46 (78%), *K. pneumoniae* 23 (88.2%), *P. aeruginosa* 12 (57.1%), *Acinetobacter* spp 13 (92.9%), *P. mirabilis* 7 (87.5%), *C. freundii* 8 (66.7%), *K. oxytoca* 2 (100%), *P. vulgaris* 1 (100%) and *E. aerogens* 3 (66.7%). A similar study found in (Edward et al., 2013), *A. baumannii* isolates recovered from patients with burns greater than 30% of total body surface were more likely to be MDR (61%) with no significant difference for *P. aeruginosa* and *K. pneumoniae*. Another study found that total *P. aeruginosa* isolates, 62 were found to multidrug resistance, of which 2 were resistant to three antimicrobial classes. (Yakha et al., 2014). In a similar study, overall multi-drug resistant isolates were 66.7% (Raza et al., 2013).

From the total 48 (23.4%) *S. aureus* isolated
from wound samples, 16 (33.3%) were MRSA. The overall prevalence of MRSA was 68% (Khanal et al., 2010). Out of 36 S. aureus, 15 isolates were MRSA (Raza et al., 2013).

Out of 41 isolates of MDR E. coli, 34 isolates were ESBL producers. Likewise, among 20 isolates of MDR K. pneumoniae, 10 isolates were ESBL producers. Similarly, a total of 12 isolates of MDR Acinetobacter spp, 2 isolates were ESBL producers. Also, 12 isolates MDR P. aeruginosa, 1 isolate was ESBL producers, a total of 8 isolates of MDR C. freundii and 5 isolates were ESBL producers. Besides, a total of 3 isolates of MDR P. mirabilis, 2 isolates were ESBL producers, among 2 isolates of MDR K. oxytoca, 1 isolate was ESBL producers but no isolates of P. vulgaris and E. aerogens were ESBL producers. In the tertiary care hospital of eastern Nepal, A total of 300 Gram-negative bacilli isolated from the pus samples were identified phenotypically, and antimicrobial activity was determined. MDR was found in 92.6% of ESBL producers (Shrestha et al., 2011).

Another study in pus and wound swabs from Saudi Arabia, E. coli (21) and K. pneumoniae (11) were found to be ESBL producers. The highest numbers of ESBL producing E. coli were detected by cefpodoxime, followed by aztreonam, ceftazidime, cefotaxime, and ceftriaxone. For ESBL producing K. pneumoniae, it was cefpodoxime followed by cefotaxime, ceftazidime, aztreonam, and ceftriaxone (Al-Zahrani et al., 2005).

In a study from Uganda, the ESBL producing Gram-negative bacteria in wound swab was 100%, and in pus swab was 47.4% (Kateregg et al., 2015). In Saudi Arabia, among the K. pneumoniae isolated from pus sample, 50% were found to be ESBL producers. This study was done by the double-disk synergy test method (Rahim et al., 2014).

Among Enterobacteriaceae isolates, 25% of isolates of E. coli were ESBL producers, 40% of K. pneumoniaeis isolates were ESBL producer and 33.3% of C. freundii were ESBL producer. But no species of Proteus were ESBL producers. Baral (2008) showed the presence of 28.12% ESBL producers out of 96 MDR isolates, Bomjan (2005) found the presence of 28.3% ESB producers among various clinical isolates and Sharma (2004) found 8% K. pneumoniae, 12.5% E. coli, 12.5% C. freundii, 25% A. calcoaceticus and 5% P. aeruginosa as ESBL-producing strains. Poudyal (2010) reported 62.72% of ESBL producers, of which 86.96% were E. coli. Of all the organisms studied till date, the most potent ESBL producers belong to the family Enterobacteriaceae (E. coli, K. pneumoniae, E.aerogenes, P. mirabilis, etc. (Bradford 2001; Senekal 2010).

Wound infections have a problem in the field of medicine for a long time. Advances in control of infections have not completely eradicated this problem because of the emergence of drug resistance (Thomas 1991). As compared to the previous study done, antibiotic resistance pattern is increasing. Many factors may have contributed to such a level of resistance, including the misuse of antibiotics by health professionals and unskilled practitioners (Karki 2012). In Nepal, it is a common practice that antibiotics can be purchased without a prescription, which leads to the misuse of antibiotics, thus contributing to the emergence and spread of antimicrobial resistance. MRSA is proving to be the scourge of modern-day surgery and can colonize the skin and body like other strains; they appear to be increasing in frequency and are displacing resistance to a broader range of antibiotics including vancomycin. Hence, they must be considered a severe problem.

**Conclusion**

Gram-negative bacteria were found to be more predominant compared to Gram-positive bacteria in wound infections. E. coli was one of the major pathogens responsible for causing wound infections followed by S. aureus. Besides these, other organisms most frequently encountered in this study were P. aeruginosa, Acinetobacter spp, Enterobacter spp, C. freundii, K. pneumoniae, CoNS, P. vulgaris, P. mirabilis, S. viridans, E. faecalis, S. marcescens, and K. oxytoca.

Gentamycin and chloramphenicol were the most effective for both Gram-positive and Gram-negative bacteria.
negative organisms. Most of the organisms were resistant to amoxycillin, ceftazidime, and co-trimoxazole. Among total isolates of *S. aureus*, 33.3% of isolates were found to be resistant to cefoxitin which indicated the increasing rate of MRSA in wound infection. Among total Gram-negative bacteria, 82.9% *E. coli* were ESBL producers followed by *P. mirabilis* 66.7%. Increasing the resistance pattern of antibiotics is being a threat to human life which is progressing at an alarming rate.

**Acknowledgements**

We acknowledge the Master Thesis support grant provided by the University Grants Commission (UGC). We are very thankful to Kathmandu Model Hospital, Kathmandu and Central Department of Microbiology, Tribhuvan University, Kritipur, Nepal for making facilities available.

**References**

Adegoke, A.A. and A.O. Komolafe. 2008. Nasal colonization of school children in Ile-Ife by multiple antibiotic-resistant *Staphylococcus aureus*. *Int J Biotech All Sci* 3: 3876-3881.

Al-kasaby, N. and V. Sachdeve. 2015. Antibiotic resistance patterns of bacterial isolates in the adult intensive care unit at Nizwa Hospital, Oman. *British microbiology journal* 10: 1-10.

Banjara, M.R., A.P. Sharma, A.B. Joshi, N.R. Tuladhar, P. Ghimire and D.R. Bhatta. 2003. Surgical wound infection in Tribhuvan University Teaching Hospital. *Journal of Nepal Health Research Council* 1: 41-45.

Bhatta, C.P. and M. Lakhey. 2007. The distribution of pathogens causing wound infection and their antibiotic susceptibility pattern. *J NEP Health Res Coun* 5: 22-25.

Chan, W.S., B.S. Tang, M.V. Boost, C. Chow, and P.H. Leucing. 2014. Detection of methicillin-resistant *Staphylococcus aureus* using a gold nanoparticle-based colourimetric polymerase chain reaction assay. *Biosensors and bioelectronics* 53: 105-111.

Cheesbrough M (2006). District Laboratory Practice in Tropical Countries. 2nd edn. Cambridge. Newyork Melbourne, Madrid, Cape Town, Singapore, Sao Poulo: Cambridge University Press.

Church, D., S. Elsayed, O. Reid, B. Winston and R. Lindsay. 2006. Burn wound infection. *Clin. Microbiol. Rev.* 19: 403-434. CLSI. 2013. Performance standards for antimicrobial susceptibility testing. Twenty-Third Informational Supplement. 33:1-199.

Collier, M., 2002. Wound bed management, key principles for practice. *Prof. Nurs* 18: 221-225.

Esebelahie, N.O., F.O. Esebelahieand R. Omorogie. 2013. Aerobic bacterial isolates from wound infection. *Afr. J. Clin. Exper. Microbiol.* 14: 155-159.

Gautam, R., A. Acharya, H.P. Nepal and S. Shrestha. 2013. Antibiotic susceptibility pattern of bacterial isolates from wound infection in Chitwan medical college teaching hospital, Chitwan Nepal. *International journal of biomedical and advance research* 4: 249-252.

Gupta, N., V. Gautam, S. Saini, L. Singh, and D.R. Arora. 2002. Prevalence of multidrug organism in wound infection. *J. Infect. Dis. Antimicrobial. Agent.* 19: 111-117.

Karki, S., 2012. *Antibiotic susceptibility pattern of bacterial isolates from wound infection in patients visiting Kanti Children's Hospital*. M.Sc. Dissertation submitted to the Central Department of Microbiology, Tribhuvan University.

Kateregga, J.N., R. Kantume, A. Atuhaire, M.N. Lubowaand J. Ndukui.2015. Phenotypic expression and prevalence of ESBL- producing Enterobacteriaceae in samples collected from patients in various wards of Mugalo Hospital Uganda. *BMC pharmacology and toxicology* 16:14.

KC, R., A. Shrestha and V.K. Sharma. 2013. Bacteriological study of wound infection and antibiotic susceptibility pattern of the isolates. *Nepal journal of science and technology* 14: 143-150.

Khanal, L.K. and B.K. Jha. 2010. Prevalence of methicillin-resistant *Staphylococcus aureus* (MRSA) among skin infection cases at a hospital in Chitwan, Nepal. *Nepal med coll J* 12: 224-228.

Komolafe, O., J. James, L. Dalongolera and M. Koka. 2003. Bacteriology of burns at the Queen Elizabeth Central Hospital, Blantyre, Malawi. *Burns* 29: 235-238

Kumari, K. 2008. *A pattern of bacterial isolates and antibiogram from open wound infection among the indoor patients of Bir Hospital*. M.Sc. Dissertation submitted to the Central Department of Microbiology, Tribhuvan University.

Mera, M.A, Abdissa and T. Sewunet. 2014. Antimicrobial susceptibility pattern of bacterial isolates from wound infection and their sensitivity to alternative topical agents at Jimma University specialized hospital, south-west Ethiopia. *Annals of clinical microbiology and antimicrobials* 13: 14.

Rakhem, K.A.A. and A.M.A. Mohamed. 2014.
Prevalence of extended-spectrum beta-lactamase- K. pneumonia in clinical isolates. Jundispur J Microbial \textbf{7}: e17114

Rani, R.V. and J. Nithyalakshmi. 2014. A comparative study of diabetic and non-diabetic wound infections with special reference to MRSA and ESBL. \textit{International current Microbiol applied sciences} \textbf{3}: 546-554.

Raza, M.S., A. Chander, A. Ranabhat. 2013. Antimicrobial susceptibility patterns of the bacterial isolates in post-operative wound infections in a tertiary care Hospital, Kathmandu, Nepal. \textit{Open journal of medical microbiology} \textbf{3}:159-163.

Shrestha, B. and R.B. Basnet. 2009. Wound infection and antibiotic sensitivity pattern of bacterial isolates. \textit{PMJN9}: 1-6.

Singh, A.P. Vaidya, N.R. Tuladharand S. Maharjan. 2006. Drug sensitivity pattern of microorganisms in infected wounds at TUTH. \textit{Journal of the Institute of medicine} \textbf{28}: 55-56.

Upadhyay, A.K., R. Maharjan and B. Shakya. 2012. Multidrug resistance bacteria in different clinical samples in National Medical College and Teaching Hospital, Birgunj, Nepal. \textit{RJPBC S} \textbf{3}: 797-807.

Yakha, J.K., A.R.Sharma, N. Dahal, B. Lekhak and M.R. Banjara. 2015. Antibiotic susceptibility pattern of bacterial isolates, causing wound infection among the patients visiting B&B Hospital. \textit{Nepal journal of science and technology} \textbf{15}: 91-96.

Zafar, A., N. Anwar and H. Ejaz. 2008. Bacteriology of infected wounds- a study conducted at children hospital Lahore. \textit{E/Biomedica} \textbf{23}: 8.