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

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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 providea 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. peumoniae, 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. aureus*is 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 Conkeyagar 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

Table 1. The pattern of bacterial isolates in wound samples

Types of organism	Sample				Total	
	Wound swab		Pus aspirates		Number	%
	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	-	-	-	-

Table 4. Multidrug-resistant Gram-negative bacteria

Bacteria	MDR Bacteria			
	MDR		No MDR	
	NO	%	No	%
E. coli	46	78.0	13	22.0
K. pneumoniae	23	88.2	3	11.5
P. aeruginosa	12	57.1	9	42.9
*Acinetobacter*spp	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	

disks test (CDT). ESBLs set consisting of Set 1: ceftazidime (30 µg) and ceftazidime (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 ceftazidime and cefotaxime discs were compared to that of the ceftazidime 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*spp14 (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 amoxycillin 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 amoxycillin and amoxyclav

(6.2%). Among the isolated *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 *E. coli* 46 (78%), *K. pneumonia* 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%) (Table 4).

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

Table 5. ESBL producers among MDR Gram negative bacteria

Bacterial isolates	Total	ESBL	
		No	%
E. coli	41	34	82.9
K. pneumoniae	20	10	50.0
P. aeruginosa	12	1	8.3
*Acinetobacter*spp	12	2	16.7
C. freundii	8	5	62.5
P. mirabilis	3	2	66.7
K. oxytoca	2	1	50.0
P. vulgaris	1	0	0.0
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, *E. coli* 31 (23.3%) was most predominant, followed by 24 (18%) *CoNS* and *S. aureus* 22 (16.5%). Similarly, in pus aspirates *E. coli* 28 (25.2%) was most predominant, followed by *S. aureus* 26 (23.4%) and *CoNS* 19 (17.1%).

We found that the most common bacterial isolates were *E. coli,* followed by *S. aureus*. The most predominant bacteria were *E. coli* supported by (Gautam *et al*., 2013; KC *et al*., 2013). The most predominance of *S. aureus* and the second most predominant bacteria is *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%), *Enterobacte*r 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 s*pp. (4%) and *Acinetobacter*spp (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 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. *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 cephalexin, 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 amoxyclav (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. pneumoniae*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 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.

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