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Antibiotic Susceptibility Pattern of Bacterial Pathogens Isolated from Infected Lesions

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ABSTRACT

Background: Wound infection with multidrug resistant bacteria along with risk factors is a major burden and challenge to the health care persons. This study focuses on antibiotic susceptibility pattern of bacterial isolates and risk factors of patients with infected wounds.

Methods: This was a cross-sectional study conducted between November 2017 to June 2018 in Kathmandu University Hospital. Pus sample and wound swabs collected from patients during the study period were included. All microbiological processing were done following standard guidelines and patient's information was collected following ethical guidelines.

Results: Total number of patients observed for wound infection was 2,763. Pathogenic bacteria were detected in 252(9.12%) samples. 167(66.27%) were multidrug resistant. 118(46.82%) had risk factors. Among risk factors 14 had malignancy, 12 had diabetes, 32 were above age 60 without other risk factors, 45 received prior antibiotics and had critical illness and 15 were renal failure patients. Multidrug resistant bacteria was detected more among patients with risk factors 101(85.59%) in comparison to patients without risk factor 66(49.25%). When p value was calculated it was found significant. ($p=<0.05$).

Conclusions: Significant amount of multidrug resistant bacteria were found in wounds of patients with risk factors. Prevention of wound infection by taking care of postoperative wounds, controlling and treating the risk factors, avoiding misuse of antibiotics and early microbiological analysis of infected wound might help to reduce the burden in healthcare centers.

Keywords: Extended spectrum beta-lactamase; methicillin resistant staphylococcus aureus; multidrug resistant.

INTRODUCTION

Treatment of patients infected with multidrug resistant bacteria has become a major challenge to the physicians these days. *Extended spectrum beta-lactamase (ESBL)* and *Methicillin resistant Staphylococcus aureus (MRSA)* producing bacterial strains are associated with skin and soft tissue infections in hospital and community settings and presence of these organisms in the community poses a substantial concern, due to the high morbidity and mortality associated with possible consequent hospital infections.[1] In developing countries, there is irrational prescription of antibiotics.[2] In Nepal, there are number of studies done which investigate antimicrobial resistance among bacterial pathogens causing wound infection.[3,4]

METHODS

This study was a cross-sectional study carried out at Kathmandu University Hospital, Dhulikhel, Nepal. Pus sample and wound swabs from outpatients and inpatients collected between November 2017 to June 2018 from which pathogenic bacteria was isolated was included in the study. Clinical and epidemiological information was collected from the patient after taking informed consent from the patient.

Ethical clearance was taken from Institutional Review Committee of Kathmandu University Hospital before the study was conducted.

Specimen collection, culture, identification tests were done according to the guidelines given by American Society for Microbiology.[5] The antibiotic susceptibility test of the pathogens isolated from the clinical specimen against different antibiotics was done using Mueller Hinton agar by the standard disk diffusion technique

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of modified Kirby-Bauer method as recommended by Clinical and Laboratory Standards Institute (CLSI).[6]

The test for the production of ESBL was performed by using ceftriaxone (CRO) (30 µg), ceftazidime (CAZ) (30 µg) and cefotaxime (CTX) (30 µg) If the zone of inhibition (ZOI) was ≤ 25mm for CRO, ≤22mm for CAZ and/or ≤27mm for CTX, the isolate was considered a potential ESBL- producer as recommended by CLSI.[6]

Combination disk method was used for the phenotypic confirmation of ESBL-producing strains in which CTX and CAZ (30 µg), alone and in combination with clavulanic acid (CA) (10 µg) was used (Becton Dickinson, USA). An increased ZOI of ≥ 5 mm for either antimicrobial agent tested in combination with CA versus its zone when tested alone confirmed ESBL.[6]

Detection of MRSA was done by cefoxitin disc diffusion test in which direct colony suspension in saline was prepared and matched with the turbidity standard equivalent to 0.5 MacFarland standard. A plate of Mueller Hinton Agar was inoculated and cefoxitin disc 30 µg was applied to the plate. The plate was incubated at 37°c for 24 hours. The results were interpreted according to clinical and laboratory standards institute guidelines for cefoxitin susceptibility testing: a zone size ≤19mm was considered resistant and ≥ 20mm was considered susceptible.[6]

Data were analyzed by (SPSS) version 11.5 software and P value less than 0.05 was considered significant.

RESULTS

Total number of patients included in the study period was 2,763. Out of this 1,550(56.10%) were postoperative pus sample/wound swabs and 1213(43.90%) were non-post-operative pus sample/wound swabs. Out of this growth of pathogenic bacteria was detected in 252(9.12%) samples. 146 patients from whom pathogenic bacteria was isolated were male patients and 106 were female patients. 233 patients were inpatients and 19 were out patients. All isolates of both *Staphylococcus aureus* and *Staphylococcus epidermidis* isolates were sensitive to aminoglycosides, chloramphenicol, clindamycin, vancomycin and linezolid but only 10(19.23%) *S. aureus* and 12(28.57%) *S. epidermidis* were sensitive to erythromycin, ciprofloxacin and cotrimoxazole. 39(75%) of *S. aureus* and 28(66.67%) of *S. epidermidis* were sensitive to tetracyline. 42(80.77%) *S. aureus* and 30(71.43%) *S. epidermidis* were Methicillin resistant as shown in table 1. Out of 6 *Enterococcus faecalis* isolates 5 were multidrug resistant and out of 2, *Streptococcus pyogenes* isolates 1 was multidrug resistant. Both isolates of *S. pyogenes* were isolated from posttraumatic infected wound. All strains of enterococci and streptococci isolated were sensitive to vancomycin and linezolid as observed in table 1.

Among *E. coli*, 68(66.67%) strains were *ESBL* and 2(1.96%) strains were resistant to carbapenem too. Among *Acinetobacter* isolates, 6(40%) were multidrug resistant and out of this 1(6.67%) was resistant to carbapenem. Among *Klebsiella pneumoniae*, 8(80%) isolates were *ESBL*.

All 3(100%) *Enterobacter* isolates and both 2(100%) *Klebsiella oxytoca* isolates were non-multidrug resistant. Single isolates each of *Proteus mirabilis* and *Proteus vulgaris* were all multidrug resistant (*ESBL*).

Single isolate of *Serratia marcescens* was non-multidrug resistant as observed in table 2.

Table 1. Antibiotic sensitivity profile of the Gram positive bacterial isolates.

Antibiotics	*S. aureus* N=52	*S. epidermidis* N=42	*E. faecalis* N=6	*S. pyogenes* N=2
Penicillin	10(19.23%)	12(28.57%)	1(16.67%)	1(50%)
Amoxicillin-clavulanic acid	10(19.23%)	12(28.57%)	NT	NT
Erythromycin	10(19.23%)	12(28.57%)	1(16.67%)	2(100%)
Cloxacillin	10(19.23%)	12(28.57%)	NT	NT
Gentamicin	52(100%)	42(100%)	1(16.67%)	NT
Ciprofloxacin	10(19.23%)	12(28.57%)	1(16.67%)	1(50%)
Tetracycline	39(75%)	28(66.67%)	3(50%)	2(100%)
Cotrimoxazole	10(19.23%)	12(28.57%)	1(16.67%)	1(50%)
Chloramphenicol	52(100%)	42(100%)	3(50%)	2(100%)
Amikacin	52(100%)	42(100%)	1(16.67%)	NT
Vancomycin	52(100%)	42(100%)	6(100%)	2(100%)
Linezolid	52(100%)	42(100%)	6(100%)	2(100%)

Table 2. Antibiotic sensitivity profile of the Gram negative bacterial isolates.

Antibiotics	*E. coli* N=102	*Acinetobacter species* N=15	*P. aeruginosa* N=15	*K. pneumoniae* N=10	*Enterobacter species* N=3	*K. oxytoca* N=2	*S. marcescens* N=1	*P. mirabilis* N=1	*P. vulgaris* N=1
Amoxicillin	32(31.37%)	9(60%)	NT	2(20%)	3(100%)	2(100%)	1(100%)	0(0%)	0(0%)
Amoxicillin-clavulanic acid	32(31.37%)	9(60%)	NT	2(20%)	3(100%)	2(100%)	1(100%)	0(0%)	0(0%)
Cefazolin	32(31.37%)	9(60%)	NT	2(20%)	3(100%)	2(100%)	1(100%)	0(0%)	0(0%)
Cefuroxime	32(31.37%)	9(60%)	NT	2(20%)	3(100%)	2(100%)	1(100%)	0(0%)	0(0%)
Ceftriaxone	32(31.37%)	9(60%)	12(80%)	2(20%)	3(100%)	2(100%)	1(100%)	0(0%)	0(0%)
Gentamicin	32(31.37%)	9(60%)	12(80%)	2(20%)	2(66.67%)	2(100%)	1(100%)	0(0%)	0(0%)
Tobramycin	32(31.37%)	9(60%)	12(80%)	2(20%)	2(66.67%)	2(100%)	1(100%)	0(0%)	0(0%)
Amikacin	32(31.37%)	9(60%)	6(50%)	2(20%)	2(66.67%)	2(100%)	1(100%)	0(0%)	0(0%)
Ciprofloxacin	32(31.37%)	9(60%)	12(80%)	2(20%)	2(66.67%)	2(100%)	1(100%)	0(0%)	0(0%)
Cotrimoxazole	32(31.37%)	9(60%)	NT	2(20%)	3(100%)	0(0%)	0(0%)	0(0%)	0(0%)
Cefepime	32(31.37%)	9(60%)	12(100%)	2(20%)	3(100%)	2(100%)	1(100%)	0(0%)	0(0%)
Ceftazidime-clavulanic acid	32(31.37%)	9(60%)	NT	2(20%)	3(100%)	2(100%)	1(100%)	0(0%)	0(0%)
Piperacillin	NT	NT	12(80%)	NT	NT	NT	NT	NT	NT
Piperacillin-tazobactum	NT	NT	12(80%)	NT	NT	NT	NT	NT	NT
Ticarcillin - clavulanic acid	NT	NT	12(80%)	NT	NT	NT	NT	NT	NT
Meropenem	100(98.04%)	14(93.33%)	12(100%)	10 (100%)	3(100%)	2(100%)	1(100%)	1 (100%)	1(100%)
Colistin	102(100%)	15(100%)	12(100%)	10 (100%)	3(100%)	2(100%)	1(100%)	1 (100%)	1(100%)
Polymixin-B	NT	15(100%)	12(100%)	NT	NT	NT	NT	NT	NT

Table 3. Risk factors and premorbid illnesses in the patients.

Risk factors	Multidrug resistant bacteria isolated	Non-multidrug resistant isolated	Total
Patients with malignancy and chemotherapy	13 (92.86%)	1(7.14%)	14(100%)
Diabetic patients with chronic ulcer	11(91.67%)	1(8.33%)	12(100%)
Age above 60 years without other risk factors	27(84.37%)	5(15.63%)	32(100%)
Prior antibiotic therapy and critically ill patients without other risk factors	37(82.22%)	8(17.78%)	45(100%)
Renal failure patients	13(86.67%)	2(13.33%)	15(100%)
Total patients with risk factors	101(85.6%)	17(14.40%)	118(100%)
Patients without any risk factors	66(49.25%)	68(50.75%)	134(100%)

Among *Pseudomonas aeruginosa* isolates, 3(20%) were multidrug resistant as shown in table 2. All multidrug resistant isolates were from burn wound infection.

Regarding risk factor, out of 252 patients, 118(46.82%) had risk factors and premorbid illnesses and 134(53.18%) had no risk factors or premorbid illnesses. Among risk factors and premorbid illnesses, 14 had malignancy, 12 had diabetes, 32 were above 60 year of age without other risk factors, 45 received prior antibiotic therapy and had critical illness and 15 were renal failure patients as shown in table 3.

Multidrug resistant bacteria was isolated more 101(85.59%) from patients with risk factors and premorbid illnesses in comparison to 66(49.25%) multidrug resistant bacteria from patients without risk factors and premorbid illnesses as shown in table 3. When p value was calculated using patients with and without risk factors as groups and isolation of multidrug resistant bacteria or non-multidrug resistant bacteria

as outcome, it was found to be statistically significant (p=<0.0001) (table 3).

DISCUSSION

This study revealed the antibiotic susceptibility pattern of various bacterial isolates from infected lesion and determined multidrug resistance. It also detected some of the risk factors among patients which might have made the patient susceptible to wound infection.

The total number of patients who was observed for wound infection during the study period was 2,763. Out of this growth of pathogenic bacteria was detected i.e. wound infection developed in 252(9.12%) of the patients which is little more than what was observed by Sule et al in which 130(7.78%) developed wound infection out of 1670 patients who were observed for wound infection for the same study duration of one year.[7] This may indicate that our healthcare setting has to focus more on prevention of wound infection in future.

Overall, out of 252 bacterial pathogens 167(66.27%) were multidrug resistant in our study which correlates with the finding or Bhatt et al in which almost same (65.38%) of the total isolates were multidrug resistant.[8] Both studies indicate that wound infection by multidrug resistant pathogen is a major burden and requires infection prevention measures.

Regarding distribution of bacterial pathogens in the samples, *Escherichia coli* was the most predominant bacterium 102(40.48%) isolated followed by *S. aureus* 52(20.63%). This finding seems similar to the findings of Mohammad et al. according to type of organism but when the predominance was considered it was opposite, where *S. aureus* was found in 37.5% of wounds with positive growth and 25% wounds had *E. coli*.[9]

70(68.63%) out of 102 *E. coli* isolates were multidrug resistant. Out of 70 multidrug resistant *E. coli*, 68(97.14%) strains were *Extended Spectrum Beta-Lactamase producer(ESBL)* and 2(2.86%) strains were resistant to carbapenem too. 8(80%) out of 10, *K. pneumoniae* isolates were *ESBL*. All 3 *Enterobacter* isolates and both *K. oxytoca* isolates were non-multidrug resistant. Single isolates each of *P. mirabilis* and *P. vulgaris* were all multidrug resistant (*ESBL*). Single isolate of *S. marcescens* was non-multidrug resistant. *E. coli and K. pneumoniae*, especially multidrug resistant *E. coli* infecting postoperative wound may be related to poor hospital hygiene, nosocomial infection and also because of acquirement of normal endogenous microbial fecal flora of the patients themselves.[10,11]

All isolates of both *S. aureus* and *S. epidermidis* isolates were sensitive to aminoglycosides, chloramphenicol, clindamycin, vancomycin, teicoplainin and linezolid but only 10(19.23%) *S. aureus* and 12(28.57%) *S. epidermidis* were sensitive to erythromycin, ciprofloxacin and cotrimoxazole. In study conducted by Mohammad et al, all *S. aureus* strain were sensitive to aminoglycosides and vancomycin but in that study, 3(75%) of *S. epidermidis* were resistant to aminoglycosides.[9] This implies that there is risk for our healthcare center also to have aminoglycoside resistant strains of *S. epidermidis* from infected wound in future. In our study 39(75%) of *S. aureus* and 28(66.67%) of *S. epidermidis* were sensitive to tetracycline which is different from the finding of *Sule et al*. in which only 16(50%) *S. aureus* and only 5(27.80%) *S. epidermidis* were sensitive to tetracycline.[7] In our findings, 42(80.77%) *S. aureus* and 30(71.43%) *S. epidermidis* were methicillin resistant but in contrast to this, 15(41.66%) *S. aureus* and 3(75%) *S. epidermidis* were methicillin resistant in study conducted by Mohammad SR et al.[9] These findings clearly indicates that our health care center needs to focus more on prevention of emergence of methicillin resistance among *S. aureus*. Our study had much higher number of cotrimoxazole resistant *S. aureus*, 42(80.77%) compared to study conducted by Mohammad et al in which only 12(33.33%) was resistant to cotrimoxazole.[9] But in that study, 4(100%) *S. epidermidis* were resistant to cotrimoxazole compared to our study, in which only 30(71.43%) were resistant. [9]

Among total of six *E. faecalis* isolates, five were multidrug resistant and among *S. pyogenes* isolates, one out of two was multidrug resistant. All strains of enterococci and streptococci isolated were sensitive to vancomycin and linezolid. Although the number of enterococcus and streptococcus isolates were less, multidrug resistance among the isolates was significant. Soft tissue and wound infection due to enterococcus species among hospitalized trauma patients in developing country has been reported and published.[12]

Out of fifteen, *Acinetobacter* isolates, six were multidrug resistant and out of this one was resistant to carbapenem also and this strain was isolated from diabetic foot ulcer. In a study conducted by Priyadarshini Shanmugam et al. too, single *Acinetobacter* resistant to carbapenem was detected in diabetic foot ulcer.[13] Out of fifteen, *P. aeruginosa* isolates, three were multidrug resistant and all of these isolates were found in infected burn wounds. *P. aeruginosa* from the patient's endogenous gastrointestinal flora or an environmental source is the most common cause of burn wound infections in many centers.[14]

Regarding risk factor, out of 252 patients, 118(46.82%)

had risk factors and premorbid illnesses and 134(53.18%) had no risk factors or premorbid illnesses. Among risk factors and premorbid illnesses, 14 had malignancy, 12 had diabetes, 32 were above 60 year of age without other risk factors, 45 received prior antibiotic therapy and had critical illness and 15 were renal failure patients. Multidrug resistant bacteria was isolated more 101(85.59%) from patients with risk factors and premorbid illnesses in comparison to 66(49.25%) multidrug resistant bacteria from patients without risk factors and premorbid illnesses. When p value was calculated it was found significant. (p=<0.05). Several studies show that multidrug resistant bacteria are isolated significantly from patients with cancer, diabetes, critically ill patients and renal failure patients.[15-18]

Some of the limitations of this study might be the study duration was short and patients were much less to determine all possible pathogens which cause wound infection. Beside this only aerobic and facultative anaerobic bacteria were investigated and no anaerobic bacteria or fungal pathogens were determined. All possible risk factors were not determined.

CONCLUSIONS

Prevention of wound infection by taking proper care of postoperative wounds, controlling and treating the risk factors, avoiding misuse of antibiotic specially prior antibiotic therapy and following antibiotic policy, early microbiological analysis of wound swab or pus sample and infection prevention might help to reduce the burden of wound infection in healthcare centers and community.

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