Antibiotic resistance pattern of bacterial isolates retrieved from febrile neutropenic patients with hematological disorders

Gayatri Prajapati (gayapraja@gmail.com)
Civil Service Hospital https://orcid.org/0000-0002-6922-1288
Bishesh Sharma Poudyal
Civil Service Hospital
Krishna Kumar Maharjan
Civil Service Hospital
Sunita Prajapati
Patan Academy of Health Sciences
Janak Raj Dhungana
Tribhuvan University - Tri-Chandra Multiple Campus

Research

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Abstract

Background

Antibiotic resistance is nowadays becoming a threat in the treatment of immunosuppressed patients. The aim of this study was to find out the antibiotic resistance pattern of bacteria isolated from febrile neutropenic patients with hematological disorders so that it would help to select the empirical antibiotic for prompt effective treatment of the febrile neutropenic patients.

Methods

A cross-sectional descriptive study was conducted at a tertiary care hospital of Nepal from October 2018 to November 2019. Blood was drawn aseptically in blood culture bottles. The bacteria were identified by standard microbiological methods with observation of colony morphology, gram staining and biochemical tests of bacteria. The antibiotic susceptibility tests were done by Kirby Bauer disc diffusion method. Extended Spectrum Beta Lactamase (ESBL) and Metallo Beta Lactamase (MBL) producers, and Methicillin Resistant *Staphylococcus aureus* (MRSA) were detected by phenotypic methods.

Results

Of the total 214 blood samples, 33.9% (71) yielded the bacterial growth. Gram negative bacteria were isolated from 23.8% of total samples and Gram-positive bacteria were isolated from 9.3% of the total samples. The Gram negative bacteria isolated were *Escherichia coli* (7.9%), *Klebsiella pneumoniae* (4.7%), *Citrobacter* spp. (4.7%), *Acinetobacter* spp. (3.7%) and *Pseudomonas aeruginosa* (2.8%). The Gram-positive bacteria isolated were *Staphylococcus aureus* (5.6%), Coagulase Negative *Staphylococcus* (2.3%) and *Enterococcus* spp. (1.4%). About 66.7% of the total Gram-negative bacteria isolated and 50% of the total Gram-positive bacteria were MDR (Multidrug-resistant). About 19.6% of the total Gram-negative bacteria were ESBL producers and 19.6% of them were MBL producers. About 41.6% of *Staphylococcus aureus* isolated were MRSA (Methicillin Resistant *S. aureus*). In our institution, piperacillin-tazobactam is the preferred first choice empirical antibiotic. But 58.8% of the Gram negative organisms were found to be resistant towards piperacillin-tazobactam. Hence there is a prompt necessity to switch to another antibiotic with high sensitivity for effective treatment of the febrile neutropenic patients in our institution.

Conclusion

Antibiotic surveillance data should be evaluated periodically to select the empirical therapeutic antibiotic for effective treatment of febrile neutropenic patients.

Background

Febrile neutropenia is a common observation among patients undergoing treatment of hematological malignancies. It leads to prolonged hospital stays, increase in medical costs and increase in mortality in spite of therapeutic advances available including broad spectrum antibiotics, antifungals, antivirals and granulocyte colony stimulating factors (1).

Neutropenia is the predisposing factor for bacteremia as neutrophil is the first line of defense mechanism against bacterial infection (2). Neutropenia occurs in the hematological patients after cytotoxic chemotherapy that suppresses the hematopoietic system. Often fever is the only sign of infection in neutropenic patients as neutropenia reduces the signs and symptoms of infection (2).

Bacteremia is the important cause of fever in neutropenic patients (3). Emergence of antimicrobial resistance has become a global problem (4). Treatment of bacteremia due to so called “Superbugs”, MDR pathogens, is becoming a clinical challenge especially in neutropenic patients. Microbiological profile of bacteremia in febrile neutropenic patients is unknown on the onset of fever. The first choice empirical antibiotic for the treatment must be selected on the basis of local epidemiological bacterial isolates and resistance patterns (5).

Emergence and spread of antimicrobial resistance is growing rapidly in South Asian countries (6). Nepal is one of those countries with high burden of antibiotic resistance (7). There is lack of data regarding antimicrobial surveillance from Nepal. To our best knowledge, this is probably the first report on microbial surveillance in febrile neutropenia patients with hematological malignancies from Nepal.

Methods

This cross-sectional descriptive study was conducted in patients admitted in the hematology ward at a tertiary care hospital in Kathmandu, Nepal from October 2018 to November 2019. Ethical approval was obtained from the Institutional Review Committee of the hospital. Oral informed consent was obtained from all the enrolled patients.

Patient's enrollments

The patients admitted in hematology ward with hematological disorders were selected for the study. Only the patients with febrile neutropenic conditions were enrolled in the study. In this study, febrile condition was defined as rise in temperature of the body more than 38^0C (100.4^0F) which lasted for more than one hour (5). Neutropenia was defined as absolute neutrophil count $< 0.5 \times 10^9$/L (500 cells/mm^3) (5).

Blood cultures and bacteremia

Blood was drawn aseptically in blood culture bottles from BD BACTEC™ (Becton, Dickinson and company). The bottles were incubated in BD BACTEC™ Fx model instrument. A patient was considered as blood culture positive if > 1 bottle of the same patient grew same organism. Since only aerobic vials were used, isolation of anaerobic bacteria was excluded from this study.

Microbiological procedures

The positive blood culture vials were then sub cultured onto 5% sheep blood agar and MacConkey agar media plates. The media plates were then incubated at 37^0C for 24 hours. The organism was identified by gram staining, by its morphological characteristics and using biochemical tests. The antibiotic susceptibility tests were done by following the disk diffusion method (modified Kirby-Bauer method) on Mueller Hinton agar (Hi-Media, India) following standard procedures recommended by the Clinical and Laboratory Standards Institute (CLSI), Wayne, USA (8).

The antibiotics used for Gram negative bacteria were amikacin, amoxicillin, cefixime, ceftriaxone, cefepime, ciprofloxacin, cotrimoxazole, piperacillin-tazobactam, imipenem, meropenem, tigecycline and polymixin B. In case of Gram-positive organisms, the antibiotics used were amikacin, amoxicillin, cloxacillin, cephalexin, ceftriaxone, ciprofloxacin, erythromycin, clindamycin, cotrimoxazole and vancomycin. The drugs were chosen so as to classify the organisms as MDR, XDR (extensively drug-resistant) and PDR (pandrug -resistant) as per the international expert proposal for interim standard definitions for acquired resistance by the European Centre for Disease Prevention and Control & Centre for Disease Control and Prevention (4).

MDR is defined as non-susceptibility to at least one agent in three or more antimicrobial categories. XDR is defined as non-susceptibility to at least one agent in all but two or fewer antimicrobial categories (i.e. bacterial isolates remain susceptible to only one or two categories). PDR is defined as non-susceptibility to all agents in all antimicrobial categories (i.e. no agents tested as susceptible for that organism) (4).

ESBL and MBL enzymes were detected by double disc combined synergy method. For ESBL detection, cefotaxime and cefotaxime + clavulanic discs were used (8). For MBL detection, imipenem and imipenem + EDTA discs were used (9). Cefoxitin discs were used for the detection of MRSA (8).

Statistics

The demographic data of patients (age, sex and disease type) and the results of microbiological investigations were entered in a computer program. SPSS 20.0 (SPSS Inc., IBM Co., Chicago, IL, USA) software was used to enter and analyze the data. Descriptive analysis was done by calculating frequency and percentages.

Result

Patient demographics

From October 2018 to November 2019, 214 febrile neutropenic episodes from 91 patients were observed and enrolled in this study. A bacterium isolated from a single patient with same antibiotic sensitivity pattern was considered as single pathogen. The age of patient ranged from 4 to 77 years. Of the total patients, 74.7% of patients were male and 25.3% were females (Table 1).

Table 1
Patient demographics

Characteristics	Value
Age in years	
Range	4–77
Median	27
Sex, no. of males / no. of females	68/23
Disease	
Acute lymphoblastic leukemia	43
Acute myeloblastic leukemia	38
Myelodysplastic Syndrome	4
Multiple myeloma	3
Lymphoma	3
Total	91

Bacterial isolates

Of the total 214 blood samples, 33.9% (71) yielded the bacterial growth. Gram negative bacteria were isolated from 23.8% of total samples and Gram-positive bacteria were isolated from 9.3% of the total samples. Among Gram negative isolates, *E. coli* was the predominant pathogen and *S. aureus* was the predominant pathogen among Gram positive isolates (Table 2).

Table 2
Distribution of Gram negative and Gram positive bacteria in blood culture

Bacteria	Frequency	Percentage
Gram negative bacteria		
Escherichia coli	17	7.9
Klebsiella pneumoniae	10	4.7
Citrobacter spp.	10	4.7
Acinetobacter spp.	8	3.7
Pseudomonas aeruginosa	6	2.8
Gram positive bacteria		
Staphylococcus aureus	12	5.6
CONS	5	2.3
Enterococcus spp.	3	1.4
Total	71	33.9

All the Gram-negative isolates were susceptible to polymixin B and majority of the isolates were susceptible to tigecycline. Besides tigecycline and polymixin B, amikacin was the drug towards which Gram-negative bacteria showed the lowest resistance (35.2%) (Table 3). Imipenem and meropenem are considered as a drug of choice for MDR bacteria but a marked resistance was observed towards them in this study (41.1% and 37.2% respectively). All the Gram-negative bacteria were sensitive towards polymixin B (Table 3).

Table 3
Antibiotic resistance pattern of Gram-negative bacteria

Antibiotics	% resistance of the bacterial isolates					
	E.coli (n = 17)	*K. pneumoniae* (n = 10)	*Citrobacter* spp.(n = 10)	*Acinetobacter* spp.(n = 8)	*P. aeruginosa* (n = 6)	Total (n = 51)
Amikacin	35.2	90.0	30.0	0	0	35.2
Amoxicillin	94.1	100	60.0	-	-	86.5
Cefixime	88.2	90.0	80.0	75.0	-	84.5
Ceftriaxone	82.4	90.0	60.0	75.0	50.0	74.5
Cefepime	76.4	90.0	50.0	62.5	33.3	66.6
Ciprofloxacin	94.1	90.0	40.0	62.5	0	62.7
Cotrimoxazole	82.4	90.0	50.0	75.0	-	75.5
Piperacillin-tazobactam	76.4	90.0	40.0	25.0	33.3	58.8
Imipenem	58.8	80.0	20.0	0	16.6	41.1
Meropenem	52.9	80.0	20.0	0	0	37.2
Tigecycline	17.6	50.0	10.0	-	-	24.3
Polymixin B	0	0	0	0	0	0

About 19.6% of total Gram-negative bacteria isolated were found to be ESBL producers and 19.6% of them were found to be MBL producers (Table 4). One isolate of *K. pneumoniae* was found to be both ESBL and MBL producer (Table 4). A total of 34 isolates of Gram-negative bacteria were found to be MDR which was 66.7% of total Gram-negative bacteria isolated (Table 5). About 33.3% of the Gram-negative bacteria were found to be XDR and none of the isolates was found to be PDR. Most of *K. pneumoniae* (90%) were MDR and about 88.2% of *E. coli* were MDR (Table 5).

Table 4
Differentiation of the Gram-negative bacteria on the basis of ESBL and MBL producers

Bacteria	ESBL producer no. (%)	MBL producer no. (%)	Both ESBL and MBL producer no. (%)
E. coli (n = 17)	5 (29.4)	1 (5.8)	o
K. pneumoniae(n = 10)	1 (10)	6 (60)	1 (10)
Citrobacter spp.(n = 10)	1 (10)	1 (10)	0
Acinetobacter spp.(n = 8)	3 (37.5)	1 (12.5)	0
P. aeruginosa(n = 6)	0	1 (16.6)	0
Total (N = 51)	10 (19.6)	10 (19.6)	1 (1.96)

Table 5
Multi-drug resistant Gram-negative bacteria

Bacteria	Non-MDR no.(%)	MDR no. (%)	XDR no. (%)	PDR no. (%)
E. coli (n = 17)	2(11.7)	15 (88.2)	7 (41.1)	0
K. pneumoniae(n = 10)	1(10)	9(90)	8 (80)	0
Citrobacter spp.(n = 10)	5(50)	5 (50)	2 (20)	0
Acinetobacter spp.(n = 8)	5(62.5)	3 (37.5)	0	0
P. aeruginosa(n = 6)	4(66.7)	2 (33.3)	0	0
Total (N = 51)	17(33.3)	34 (66.7)	17 (33.3)	0

Gram-positive bacteria in the study were found to be less resistant to antibiotics as compared to Gram-negative bacteria. All *Enterococcus* spp. bacteria were found to be sensitive to all antibiotics those were used. None of the Gram-positive bacteria were resistant to vancomycin. As the Gram-negative isolates, majority of the Gram-positive bacteria were sensitive to amikacin (Table 6). But marked resistance was found towards other antibiotics used (Table 6).

Table 6
Antibiotic resistance pattern of Gram-positive bacteria

Antibiotics	% resistance of the bacterial isolates			
	S. aureus (n = 12)	CONS (n = 5)	Enterococcus spp. (n = 3)	Total (N = 20)
Amikacin	8.3	20.0	0	10.0
Amoxicillin	91.6	60.0	0	70.0
Cloxacillin	50.0	60.0	0	45.0
Cephalexin	50.0	40.0	0	40.0
Ceftriaxone	41.6	40.0	0	35.0
Ciprofloxacin	83.3	60.0	0	65.0
Erythromycin	91.6	100	0	80.0
Clindamycin	41.6	20.0	0	30.0
Cotrimoxazole	58.3	80.0	0	55.0
Vancomycin	0	0	0	0

About 41.6% of *S. aureus* were found to be MRSA (Table 7). None of *Enterococcus* spp. was found to be MDR but majority of *S. aureus* and CONS were found to be MDR with few XDR as well. None of the Gram-positive bacteria were found to be PDR (Table 8).

Table 7
Methicillin and vancomycin resistance of the Gram-positive bacteria

Bacteria	MRSA no. (%)	Non-MRSA no. (%)	Vancomycin resistant
S. aureus(n = 12)	5 (41.6)	7(58.3)	0
CONS(n = 5)	-	-	0
Enterococcus spp.(n = 3)	-	-	0
N = 20	-	-	0

Table 8
Multi-drug resistant Gram-positive Bacteria

Bacteria	Non-MDR no. (%)	MDR no. (%)	XDR no. (%)	PDR no.(%)
S. aureus(n = 12)	5(41.7)	7 (58.3)	1 (8.3)	0
CONS *(*n = 5)	2(40)	3(60)	1 (20)	0
Enterococcus spp.(n = 3)	3(100)	0	0	0
N = 20	10(50)	10(50)	2(10)	0

Discussion

Increasing antimicrobial resistance is the emerging global issue. This study has pointed towards increasing antibiotic resistance among the bacteria isolated from febrile neutropenic patients. Most of the bacteria isolated in the current study were found to be MDR. About 66.7% of total gram-negative bacteria and 50% of the total gram-positive bacteria were found to be MDR. The incidence in this study is higher than the study done in India by Babu K G et al in 2014 (1) where 35.2% of gram-negative bacteria and 12.8% of gram-positive bacteria were MDR. Our observation regarding MDR in this study is lower than that of a study done in patients admitted in critical care unit of a tertiary care hospital in Nepal by Parajuli et al in 2015 where 95.8% of the bacterial isolates were MDR and 43.3% of them were XDR (10). Our study shows that the prevalence of MDR is alarmingly high.

In this study, 19.6% of the total gram-negative bacteria were found to be ESBL producers and 19.6% of them were MBL producers. About 41.6% of *S. aureus* were MRSA. This result is concordant with the report by Babu KG et al (1) where 36.8% of gram-negative bacteria were ESBL producers and 16.8% were MBL producers. Our findings were lower than that of Parajuli et al where 43.7% of the isolated gram-negative isolates were ESBL producers and 50.2% of them were MBL producers (10). Regarding MRSA, our results are similar to the study done by Shrestha B. et al done in nosocomial isolates of *S. aureus* (11). In our study, the rate of ESBL producers and MBL producers was found to be lesser than that of MDR. It points towards additional mechanisms of antibiotic resistance acquired by MDR bacteria in addition to ESBL and MBL production such as resistance due to decreased antibiotic penetration and efflux, changes in target sites or resistance due to global cell adaptations (12).

Gram-negative bacteria were more predominant than gram positive bacteria in our study. Similar findings were reported by Babu K G et al from India (1). However, in studies conducted in developed countries such as Japan and America, they have found Gram-positive bacteria to be more predominant than Gram-negative bacteria (3). *E. coli* was the predominant Gram-negative bacteria followed by *K. pneumoniae* and *S. aureus* was the predominant Gram-positive bacteria isolated in the current study. These findings are concordant with other reports conducted elsewhere in febrile neutropenic patients (3, 13–18).

Though marked resistance towards the antibiotics was found in our study, the resistance percent towards the antibiotics is lesser than in the study done by Parajuli et al in Nepal in critical unit patients where

amikacin resistance ranged from 45–100%, piperacillin-tazobactam resistance ranged 48–92%, imipenem resistance 19.3–86% and meropenem resistance ranged 19–84% (10).

Piperacillin-tazobactam is the preferred initial antibiotic for febrile neutropenic patients in our institution. However, this study has shown marked resistance against piperacillin-tazobactam. Hence there is a prompt necessity to switch to another antibiotic with high sensitivity for effective treatment of febrile neutropenic patients.

Conclusion

Rapidly growing antibiotic resistance is a great concern in the modern medicine. This global problem is threatening the efficacy of the treatment of febrile neutropenic patients. Every institution must have periodic surveillance of the microbial data of bacteremia in febrile neutropenic patients in order to select the most sensitive antibiotic at that particular time for appropriate treatment of febrile neutropenic conditions.

Abbreviations

ESBL
Extended Spectrum Beta Lactamase
MBL
Metallo Beta Lactamase
MRSA
Methicillin Resistant *Staphylococcus aureus*
CLSI
Clinical and Laboratory Standards Institute
MDR
Multidrug-resistant
XDR
Extensively drug-resistant
PDR
Pandrug-resistant
EDTA
Ethylenediaminetetraacetic acid
E. coli
Escherichia coli
K. pneumoniae
Klebsiella pneumoniae
S. aureus
Staphylococcus aureus
CONS

Coagulase Negative *Staphylococcus*

Declarations

Consent for publication

Not applicable.

Availability of data and materials

The datasets (tables) supporting the conclusion of this article are included within the article. The raw data of the study are available from the corresponding author on reasonable request.

Ethical approval

Ethical approval was obtained from the Institutional Review Committee of Civil Service Hospital, Nepal. Blood culture and antibiotic sensitivity is considered as routine test for febrile neutropenic patients. Hence written consent was not needed from the patients. Oral informed consent was obtained from all enrolled patients.

Competing interests

All authors declare that they have no competing interests.

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None.

Authors' contributions

GP and JD designed the study. BP and KM identified the febrile neutropenic patients and collected patient information. GP and SP did all laboratory works and analyzed the data. GP drafted the manuscript. BP and KM revised the manuscript. All authors read and approved the final manuscript.

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References

1. Babu KG, Lokanatha D, Lakshmaiah K, Babu MS, Jacob LA, Bhat GR, et al. Bloodstream infections in febrile neutropenic patients at a tertiary cancer institute in South India: A timeline of clinical and microbial trends through the years. Indian journal of medical paediatric oncology: official journal of Indian Society of Medical Paediatric Oncology. 2016;37(3):174.
2. Crawford J, Dale DC, Lyman GH. Chemotherapy-induced neutropenia: risks, consequences, and new directions for its management. Cancer. 2004;100(2):228–37.
3. Collin BA, Leather HL, Wingard JR, Ramphal R. Evolution, incidence, and susceptibility of bacterial bloodstream isolates from 519 bone marrow transplant patients. Clin Infect Dis. 2001;33(7):947–53.
4. Magiorakos AP, Srinivasan A, Carey R, Carmeli Y, Falagas M, Giske C, et al. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. Clin Microbiol Infect. 2012;18(3):268–81.
5. De Naurois J, Novitzky-Basso I, Gill M, Marti FM, Cullen M, Roila F, et al. Management of febrile neutropenia: ESMO clinical practice guidelines. Ann Oncol. 2010;21(suppl_5):v252–6.
6. Chereau F, Opatowski L, Tourdjman M, Vong S. Risk assessment for antibiotic resistance in South East Asia. BMJ. 2017;358:j3393.
7. Acharya KP, Wilson RT. Antimicrobial resistance in Nepal: a review. Frontiers in medicine. 2019;6:105.
8. CLSI.Performance Standards for Antimicrobial Disk. SusceptibiltyTests;Approved Standard-Eleventh Edition.CLSI document M02-A11.Wayne,PA. Clinical and Laboratory Standards Institute2012.
9. Yong D, Lee K, Yum JH, Shin HB, Rossolini GM, Chong Y. Imipenem-EDTA disk method for differentiation of metallo-beta-lactamase-producing clinical isolates of Pseudomonas spp. and Acinetobacter spp. J Clin Microbiol. 2002;40(10):3798–801.
10. Parajuli NP, Acharya SP, Mishra SK, Parajuli K, Rijal BP, Pokhrel BM. High burden of antimicrobial resistance among gram negative bacteria causing healthcare associated infections in a critical care unit of Nepal. Antimicrobial Resistance Infection Control. 2017;6(1):67.
11. Shrestha B, Pokhrel BM, Mohapatra TM. Phenotypic characterization of nosocomial isolates of Staphylococcus aureus with reference to MRSA. The Journal of Infection in Developing Countries. 2009;3(07):554–60.
12. Munita JM, Arias CA. Mechanisms of antibiotic resistance. Microbiology spectrum. 2016;4(2).
13. Basak S, Singh P, Rajurkar M. Multidrug resistant and extensively drug resistant bacteria: A study. Journal of pathogens. 2016;2016.
14. Ben-Chetrit E, Eldaim MA, Bar-Meir M, Dodin M, Katz DE. Associated factors and clinical outcomes of bloodstream infection due to extended-spectrum β-lactamase-producing Escherichia coli and Klebsiella pneumoniae during febrile neutropenia. Int J Antimicrob Agents. 2019;53(4):423–8.
15. Irfan S, Idrees F, Mehraj V, Habib F, Adil S, Hasan R. Emergence of Carbapenem resistant Gram negative and vancomycin resistant Gram positive organisms in bacteremic isolates of febrile neutropenic patients: a descriptive study. BMC Infect Dis. 2008;8(1):80.

16. Gustinetti G, Mikulska M. Bloodstream infections in neutropenic cancer patients: a practical update. Virulence. 2016;7(3):280–97.
17. Gudiol C, Carratalà J. Antibiotic resistance in cancer patients. Expert Rev Anti Infect Ther. 2014;12(8):1003–16.
18. Kanamaru A, Tatsumi Y. Microbiological data for patients with febrile neutropenia. Clin Infect Dis. 2004;39(Supplement_1):7–10.