ANTIBIOTIC SENSITIVITY PATTERN OF BACTERIAL ISOLATES FROM CLINICAL SPECIMENS OF PATIENTS ADMITTED IN INTENSIVE CARE UNIT

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ABSTRACT

Introduction

Antibiotics resistance is increasing nowadays. Factor to antibiotic resistance is its misuse. There is no national guideline for use of antibiotics.

Objective

Study was conducted to determine bacteriological profile, and the bacterial antibiotic sensitivity pattern of the pathogens isolated from patients in intensive care unit.

Methodology

Study was conducted between 1st October 2020and 30th March 2021. The samples collected from Intensive care units patients were processed in Microbiology Laboratory following Clinical and Laboratory Standard Institute guideline. Organisms were identified morphologically and biochemically and antibiotic sensitivity pattern was determined by disc diffusion methods.

Result

Out of total 397 samples, only 46 were found to be culture positive, of which 32 (69.56%) were gram negative organisms and 14 (30.43%) were gram positive organisms. Out of gram negative organisms *Klebsiella*pecies 10 (21.73%) were predominant which is more sensitive to colistin, polymyxin B and tigecycline. Among gram positive organisms *Staphycoccus* aureus 12 (85.71%) was found more sensitive to Vancomycin than others.

Conclusion

It is concluded that *Klebsiella* species and *Staphylococcus* aureus were predominant pathogens isolated from the patients. Regular surveillance of antibiotic sensitivity pattern and stewardship program will beuseful in treating patients in Intensive care unit.

KEYWORDS

Antibiotics susceptibility, bacterial pathogens, intensive care unit.

INTRODUCTION

Microbes are the organism that causes infections.[1] The major cause of mortality worldwide is infection which targeted low and lower middle countries.[2] Infection is common among hospitalized patients of intensive care unit.[3,4]

Intensive care unit (ICU) is a confined setting where antibiotics are extremely used.[5] Rather than other hospital patients, Intensive care unit patients are 5-10 times more likely of acquiring hospital infection.[6] Health care associated infection is high risk factor with critically ill patients in Intensive Care Unit which occurs due to various factors like decreased host defences, use of devices and due to cross transmission of infection among patients and staffs.[7,8] Health care associated infection and antibiotic resistance are prime treats among patients in ICU.[9] Therapeutic drugs are constantly found to be ineffective in infection, which is decreasing the success of routine treatment.[10] One study conducted in 2007 with ICU patients, from 75 different countries, reported that patients with longer ICU stay got higher rate of infection especially due to resistant *Staphylococci*, *Acinetobacter*, *Pseudomonas* species and *Candida* species.[11] ICU patients acquiring infections are mostly associated with invasive devices and ventilators.[12] The major consequences of the problem is increase in patients morbidity, mortality which is the result of the treatment failure.[13,14] Infections caused by gram negative bacteria in ICU has increased with which lack of treatment options against multi drug resistant (MDR) strains is in doubt. Infection that is caused by MDR gram negative organisms brings high morbidity and mortality. So, infection control guideline should be followed to improve patient outcome and decrease cost for treatment.[15] Antimicrobial Stewardship programs can optimize the proper use of available antimicrobial agent which can improve infections caused by organisms.

The results from this study can help in building up strategies for proper use of prophylactic and ampiric antibiotic therapy among ICU patients in developing countries like ours. The aim of the study was to reduce the advance consequence of antimicrobial use.

METHODOLOGY

This is a descriptive cross sectional study conducted at Kathmandu Medical College and Teaching Hospital from 1st October 2020 to 30th March 2021. Ethical approval was taken from Institutional review board (IRC) Reference no: 170920205. Clinical specimens collected from ICU patients were included in this study. Specimens after been collected in the Microbiology laboratory were further been processed for Grams staining and culture. After isolating the organisms from the culture they were further characterized by conventional biochemical test to identify the specific microorganism. Antibiotic susceptibility test of the bacterial isolates were done by Kirby Bauer disc diffusion method on Mueller Hinton Agar (MHA) as per Clinical Laboratory Standard Institute (CLSI) guideline. With a sterile loop four or five colonies of the isolated cultured organisms were mixed with 2ml of sterile saline and was vortexed to create a suspension. Turbidity of the solution was adjusted to 0.5 Mc Farland standard. A sterile swab stick was then dipped into the formed suspension, firmly pressed to remove excess fluid and then lawn culture was performed on Muller Hinton agar (MHA). Antibiotic discs were then applied to MHA plates and was included at 37ºC for 18-24 hours. Zone of inhibition was measured and interpreted using standard chart and organisms were reported susceptible, intermediate or resistant accordingly. Himedia, Mumbai, India and MAST diagnostics, Merseyside, England antibiotic discs were obtained.[16] Antibiotics of following concentrations used were: Amikacin (30µg), Gentamycin (10µg), Ciprofloxacin (5µg), Chloramphenicol (30µg), Co-Trimoxazole (25µg), Ceftazidime (30µg), Piperacillin-Tazobactam (100/10µg), Imipenem (10µg), Meropenem (10µg), Azithromycin (15µg), Penicillin (10µg), Oxacillin (1µg), Vancomycin (5µg), Linezolid (30µg), Tigecycline (30µg), Polymyxin B (30 units) and Colistin Sulphate (10µg) from Hi-Media laboratory. Zone diameter were interpreted according to Clinical and Laboratory standard Institute (CLSI 2017) recommendations.

Quality control for culture plates and antibiotic susceptibility were performed using Escherichia coli ATCC 25922, Staphylococcus aureus 25923, Pseudomonas aeruginosa ATCC 27853.

Multi Drug Resistance (MDR) bacterial isolates were identified according to the criteria recommended by international expert committee of the European Centre for disease Prevention and Control (ECDC) and the Centers for disease control and Prevention (CDC). The isolates resistant to at least one antimicrobial from three different group of first line drugs tests were regarded as Multidrug Resistant (MDR).[17]

Sample size (n) $=Z^2\alpha/2*(p)*(1-p)/d^2=366.23\sim367$

Where: Z = degree of confidence level=95%=1.96

p=prevalence=60.8%=0.608[18] d = allowable error=5%

RESULTS

During the study period a total of 397 samples were processed of which 46 (11.58%) were culture positive. Of total positive culture specimens higher number was isolated from pus 20 (43.47%), followed by fluid 9 (19.56%), urine 8 (17.39%), blood 5 (10.86%), catheter tip 2 (4.43%), endotracheal tip 1 (2.17%) and high vaginal swab 1 (2.17%). (Table No. 1)

Table 1: Clinical manifestations

Sample	Bacterial Isolate (%)
Pus	20 (43.47%)
Fluid	9 (19.56%)
Urine	8 (17.39%)
Blood	5 (10.86%)
Catheter tip	2 (4.34%)
Endotracheal tip	1 (2.17%)
High Vaginal Swab	1 (2.17%)
Total	**46 (100%)**

Among the bacterial isolates isolated from intensive care unit *Klebsiella* species was predominant among gram negative bacteria 10 (31.25%) and was mostly isolated from urine 5 (50.00%). Among gram positive organisms predominant was *Staphylococcus aureus* 12 (85.71%) and was mostly isolated from pus (91.66%).(Table No. 2)

Table 2: Distribution of bacterial isolates in Clinical Samples

Organisms	Pus	Fluid	Urine	Blood	Catheter tip	Endotrachial tip	High vaginal swab
S.aureus	11 (91.66%)	-	-	-	1 (8.33%)	-	-
Streptococcus Species	-	-	-	-	-	-	1 (100%)
Coagulase negative Staphylococcus		-	-	1 (100%)	-	-	-
***Klebsiella*species**	-	2 (20%)	5 (50%)	2 (20%)	1 (10%)	-	-
Pseudomonas aeruginosa	4 (50%)	2 (25%)	1 (12.50%)	-	-	1 (12.50%)	-
***Acinetobacter* species**	2 (28.57%)	4 (57.14%)	1 (14.28%)	-	-	-	-
***Enterobacter* species**	-	1 (100%)	-	-	-	-	-
***Proteus* species**	3 (100%)	-	-	-	-	-	-
***Citrobacter*species**	-	-	1 (100%)	-	-	-	-
Escherichia coli	2 (100%)	-	-	-	-	-	-

Among gram negative organisms *Klebsiella*species is predominant in intensive care unit which is more sensitive to antibiotics like Colistin, Polymyxin B and Tigecycline. Out of gram positive organisms the most predominant organism was *Staphylococcus aureus* which is more sensitive to Vancomycin. (Table No. 3)

Table 3: Antibiotic sensitivity pattern of patients given in percentage in Intensive care unit

Antibiotics	*Klebsiella*sps (%)	*Pseudomonas aeruginosa* (%)	*Acinetobacter*sps (%)	*Citrobacter*sps (%)	*Entero-bacter*sps (%)	*Proteus* sps (%)	*E.coli* (%)	*S.aureus* (%)	*CONS* (%)	*Strep.*sps (%)
Amikacin	70.00	50.00	42.84	100.00	100.00	66.66	50.00	33.33	100.00	100.00
Cefexime	60.00	25.00	28.57	00.00	00.00	00.00	00.00	33.33	00.00	00.00
Ceftriaxone	40.00	50.00	28.57	00.00	00.00	33.33	00.00	58.33	00.00	00.00
Cotrimoxazole	30.00	12.50	42.85	00.00	00.00	66.66	00.00	41.66	00.00	00.00
Ciprofloxacin	4 0.00	25.00	57.14	00.00	00.00	66.66	50.00	41.66	00.00	00.00
Chloramphenicol	30.00	37.50	28.57	00.00	00.00	66.66	50.00	33.33	100.00	00.00
Imipenem	70.00	37.50	28.57	100.00	100.00	100.00	100.00	-	-	-
Meropenem	80.00	37.50	28.57	100.00	100.00	100.00	100.00	-	-	-
Piperacillin/ Tazobactam	90.00	25.00	57.14	100.00	100.00	100.00	100.00	-	-	-
Azithromycin	30.00	00.00	00.00	00.00	00.00	33.33	50.00	33.33	100.00	100.00
Ceftazidime	50.00	37.50	42.85	00.00	00.00	33.33	100.00	-	-	-
Penicillin	-	-	-	-	-	-	-	33.33	100.00	100.00
Colistin	100.00	100.00	100.00	100.00	100.00	100.00	100.00	-	-	-
Polymyxin B	100.00	100.00	100.00	100.00	100.00	100.00	100.00	-	-	-
Tigecycline	90.00	-	100.00	100.00	100.00	100.00	100.00	-	-	-
Gentamicin	-	-	-	-	-	-	-	41.66	100.00	100.00
Oxacillin	-	-	-	-	-	-	-	41.66	100.00	100.00
Vancomycin	-	-	-	-	-	-	-	100.00	-	100.00
Linezolid	-	-	-	-	-	-	-	91.66	100.00	-

DISCUSSION

Antimicrobial resistance in the hospital infection is increasing day by day mostly when the infection is caused by multidrug resistance organisms. Intensive care unit is led by a team which involves intensivist, clinical microbiologist associated with medical and surgical specialities.In Intensive care unit coordination between the intensivist and clinical microbiologist is required to get a better outcome.[19] Intensive care unit is common for secondary infection as compared to primary infection. In this study we have taken total 397 samples of which only 46 (11.58%) were reported as culture positive. This study showed that most of the isolates were from Pus 20 (43.47%) samples, followed by Fluid 9 (19.56%), Urine 8 (17.39%), Blood 5 (10.86%), Catheter tip 2 (4.34%), Endotracheal tube (ET) 1 (2.17%) and High Vaginal Swab 1 (2.17%). This study is slightly different with the study conducted by other authors[20] which shows highest percentage of growth in Blood 48 (37.79%), followed by ET 40 (31.49), Urine 39 (30.70%), Sputum 17 (13.38%), Pus 11 (8.66%), Catheter 4 (3.14%), Ear Swab 2 (1.57%) and Stool 1 (0.78%).In this study, we isolated gram negative bacteria 32 (69.56%) as compaired to gram positive cocci 14 (30.43%). In this study, we isolated more gram negative bacteria 32 (69.56%) compaired to gram positive cocci 14 (30.43%). In the Canadian National ICU cases the result observed was *Pseudomonas aeruginosa*,

Staphylococcus aureus, *Haemophilus influenza*, *Enterococcus* species and *Klebsiella pneumonia*.[21] Most of the Asian countries, the pathogens that are mostly isolated from ICU patients were *Pseudomonas aeruginosa*, *Klebsiella* species, *Escherichia coli*, *Enterococcus* species and *Staphylococcus aureus*[22] which is similar to our study. Among all admitted cases who were culture positive gram negative organisms most of the infection was caused by *Klebsiella*species 10 (31.25%), followed by *Pseudomonas aeruginosa* 8 (25%), *Acinetobacter* species 7 (21.87%), *Proteus* 3 (9.37%), *Escherichia coli* 2 (6.25%), *Enterobacter* species 1 (3.12%) and *Citrobacter* species 1 (3.12%). This study is nearly similar to the study which show predominance of *Acinetobacter* species 52 (41%) followed by *Klebsiella pneumonia* 36 (28%) and *Pseudomonas aeruginosa* 27 (21%) among ICU cases.[23] This study revealed result as the study investigated in ICU of Indonesia which shows predominancy of *Pseudomonas aeruginosa*, *Klebsiella*species and *Escherichia coli*.[24] We found Enterobacteriaceae more sensitive to piperacillin/ tazobactam and *Pseudomonas aeruginosa* more resistant to ceftazidime and more sensitive to imipenem. Similar type of study was conduced with the result showing Enterobactericeae showing 26.1% resistance to piperacillin/tazobactam and 64.1% of *Pseudomonas aeruginosa* resistant to ceftazidime and 42.0% to imipenem.[25] Our study shows that gram negative bacteria were resistant to commonly used antibiotics and were more sensitive to colistin, polymyxin B and tigecycline which is similar with other studies.[26]In this study Gram positive organism like *Staphylococcus aureus* is more sensitive to Vancomycin that is similar to other studies done[27] which shows predominance of Staphylococcus aureus in hospital setting along with its sensitivity toward Vancomycin.

Our result have got clinical importance in the treatment and management among intensive care unit patients. The study was conducted among ICU patients. For better clinical step empirical antibiotics with antibiotic stewardship program, which can prevent multidrug resistance and drug resistant organism. We have also focused on using sterile techniques while inserting devices, use of sterile gloves and gowns in ICU and most importantly hand hygiene to prevent nosocomial infections and better patient response. We have analyzed the spectrum of pathogens and the antibiotic treatment.

CONCLUSION

*Klebsiella*species, *Pseudomonas aeruginosa* and *Acinetobacter* species are the gram negative multiresistant organisms isolated. Most of the strains of these organism were resistant to cefriaxone, cotrimoxazole, ciprofloxacin, chloramphenicol, ceftazidime and were more sensitive to colistin and polymyxin B. Among gram positive organisms *Staphylococcus aureus* was the pathogen prevalent in ICU and the strains were found to be more sensitive to vancomycin. Appropriate antibiotic utilization in ICU is required for which stewardship program is useful in ICU.

LIMITATIONS OF STUDY

Antibiotic sensitivity test, its use in the hospital and hospital infection control vary from hospital to hospital. The limitation of this study is that we would have conducted study in large size but we conducted this study in only one hospital site and could not compare it with other hospital setting.

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CONFLICT OF INTEREST

There was no conflict of interest during the study.

FINANCIAL DISCLOSURE

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