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Burden of multidrug resistant respiratory pathogens in intensive care units of tertiary care hospital

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ABSTRACT

Background: Lower respiratory tract infections are one of the most common infections among the patients in Intensive Care Units (ICUs). Admission in ICUs and use of life supporting devices increase the risk of infection with multidrug resistant pathogens. **Aims and Objectives:** This study was aimed to determine the prevalence and antibiograms of the bacterial pathogens causing lower respiratory tract infections among patients of ICUs. **Materials and Methods:** A total of 184 specimens from patients admitted in ICUs with lower respiratory tract infections were included in this study. Isolation, identification and antibiotic susceptibility testing of the isolates was performed by standard microbiological techniques. Carbapenamase detection was performed by modified Hodge test method. Detection of metallo beta lactamase (MBL) was tested by imipenem and imipenem/EDTA disc. Detection of *Klebsiella pneumoniae* carbapenamase (KPC) was performed by imipenem and imipenem/phenyl boronic acid. **Results:** Out of 184 samples, 131 showed significant growth of bacterial pathogens. *Acinetobacter* species (42.6%), *Staphylococcus aureus* (16.9%) and *Pseudomonas aeruginosa* (13.9%) were the three most common isolates. Out of 22 imipenem resistant isolates of *Acinetobacter* species, 9 were KPC producer, 4 were MBL producers and 3 isolates were positive for MBL and KPC both. Among the *Acinetobacter* species, 5.1% isolates were resistant to tigecycline and colistin. One isolate of *Pseudomonas aeruginosa* was positive for MBL. **Conclusions:** High prevalence of multidrug resistant bacteria in ICUs was recorded. Gram negative bacilli were predominantly associated with LRTI among ICU patients; *Acinetobacter* species being most common isolate. Detection of carbapenamase among the *Acinetobacter* and emergence of tigecycline resistance limits the therapeutic options. Regular monitoring of such resistant isolates would be important for managing infection control in critical units.

Key words: Intensive Care Unit; Drug resistance; Carbapenamase; Metallo beta *lactamase*; Klebsiella pneumoniae *carbapenamase*; Tigecycline.

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INTRODUCTION

Respiratory tract infections are one of the most common and significant cause of mortality and morbidity across the world.[1] These infections are mostly of community origin but increasing cases of hospital acquired infections have been reported.[2] Patients with chronic and severe underlying disorders need Intensive Care Unit (ICU) admission. Prolonged hospitalization in ICU, invasive devices, decreased mobility and variety of medications increase the risk of nosocomial infections.[3] The prevalence of hospital acquired infections (HAIs), especially among the patients admitted in critical care units is higher in developing countries as compared to developed countries.[4,5] The incidence of nosocomial infections among ICU patients ranges from 2.3%-49.2% across the centers.[6] A

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multicenter prospective study from 75 countries reported 50% of the patients admitted in ICU had nosocomial infections accounting for 20-30% of all nosocomial infections.[7] Patients with underlying respiratory disorders require aid of respiratory equipments such as mechanical ventilators which increase risk the microbial entry in the lower respiratory tract.

Bacteriological profile and antimicrobial resistance profile of the isolates vary greatly from hospital to hospital within and outside country. The spread of multidrug resistant bacteria in ICU is believed to be associated with widespread use of higher generation antibiotics. Exposure to such antibiotics exerts selective pressure on bacteria and forces them to explore novel mechanisms to bypass the lethal action of drug. Methicillin resistant *Staphylococcus aureus* (MRSA), vancomycin resistant *Enterococci* (VRE), extended spectrum beta lactamase (ESBL) producing Gram negative bacteria (*E coli*, *Klebsiella* species, *Pseudomonas aeruginosa* and *Acinetobacter* species) are common pathogens reported from ICUs.[8]

Burden of antimicrobial resistance is increasing in developing countries. In Nepal, data regarding prevalence of bacterial pathogens of lower respiratory tract infections in ICU patients and their resistance pattern is poorly reported. This study was designed to determine the common pathogens prevalent in ICUs along with their antibiotic resistance pattern in a tertiary care hospital. Findings of the study would be helpful in formulating guidelines for prophylactic and empirical antibiotic therapy of patients admitted in ICUs.

MATERIALS AND METHODS

This cross sectional hospital based study was carried out at ICUs of Manipal Teaching Hospital, a 750 bedded tertiary care center of Western Nepal. Approval from the Institutional Ethical Committee (IRC) of Manipal College of Medical Sciences (MCOMS), Pokhara, Nepal, was obtained before the commencement of the study. The study was conducted over a period of five months (July 2018 to November 2018) in adult Intensive Care Unit (ICU), Pediatric ICU, Neonatal ICU, Neuro ICU and Critical Care Unit.

Isolation and identification

Lower respiratory tract specimens (sputum, suction tip, endotracheal specimen, and bronchoalveolar lavage) received from ICUs formed the material of this study. All the specimens were subjected to Gram stain and culture. Specimens were inoculated immediately on 5% Sheep Blood agar and Chocolate agar (Hi media, Mumbai, India) plates. Inoculated plates were incubated at 37^{0}C in a candle jar with increased CO2 partial pressure. The isolates were identified by standard microbiological techniques such as colony morphology, Gram stain, catalase test, coagulase test, oxidase, sugar fermentation and IMViC test.[9]

Antibiotic susceptibility test

Antibiotic susceptibility testing of the isolates was performed on Mueller Hinton agar (HI media, Mumbai, India) by Kirby Bauer disc diffusion method.[10] Methicillin resistance amongst *Staphylococcus aureus* was screened by cefoxitin (30μg) disc diffusion method as described earlier.[10] Isolates showing resistance to at least one agent in three or more antimicrobial categories were labeled as multidrug resistant.[11]

Detection of carbapenamase

Screening of carbapenamase production was performed by imipenem disc (10μg) by Kirby Bauer disc diffusion method. Carbapenamase detection was phenotypically confirmed by Modified Hodge test method.[12,13] Indicator organism (*E coli,* ATCC 25922) suspension of 0.5 McFarland was prepared and further diluted to 1:10. Carpet culture of diluted indicator organism was performed on MHA. Imipenem disc (10μg) was placed on the center of plate. Test organism was streaked from the edge of the disc to the edge of the plate in a straight line. After 24 hours of incubation, plate was examined for clover leaf-type indentation at the intersection of the test organism and *E coli*, within the zone of inhibition of the carbapenem susceptibility disc.

Metallo Beta Lactamases (MBLs) detection

For confirmation of MBL production, 0.5 McFarland bacterial suspensions were inoculated on MHA plates. Imipenem (10μg) disc alone and imipenem with 10μl of 100mM EDTA were placed and plates were incubated at 37^{0}C for 18-24 hrs. Increase in the zone diameter by >5mm with zone around combined imipenem-EDTA disc as compared to imipenem alone were considered as MBL producer.[14]

Detection of Klebsiella pneumoniae carbapenamase (KPC)

For confirmation of KPC production, bacterial suspensions matching the turbidity with 0.5 McFarland were inoculated on MHA plates. Imipenem (10μg) disc alone and imipenem with phenyl boronic acid (PBA) were placed and plates were incubated at 37^{0}C for 18-24 hrs. Increase in the zone diameter by >5mm with zone around combined imipenem-EDTA disc as compared to imipenem alone were considered as KPC producer.[15]

RESULTS

A total of 184 specimens (102 male and 82 female patients) from lower respiratory tract infections were studied.

Out of 184 samples, 131 showed significant growth of bacterial pathogens. A total of 136 bacterial strains were isolated. *Acinetobacter* species (42.6%), *Staphylococcus aureus* (16.9%) and *Pseudomonas aeruginosa* (13.9%) were the three most common pathogens isolated. Details of bacterial pathogens isolated are depicted in Table 1. Specimens from the patients admitted in Neuro ICU yielded the largest number of *Acinetobacter* species (30/58), *Pseudomonas aeruginosa* (11/19) and *S. aureus* (15/24). Patients admitted in NICU and PICU yielded least number of the bacterial pathogens. Majority of the isolates of *S. aureus* 78.2% (18/23), *Acinetobacter* species 77.5% (45/58) and *Pseudomonas aeruginosa* 63.1% (12/19) were multidrug resistant. Culture of endotracheal tube yielded largest number of MDR *Acinetobacter* species. Among the MDR *Acinetobacter* species, 5.1% isolates were resistant to colistin and tigecycline. Out of 13 *Klebsiella pneumoniae* isolates, 5 (38.4%) were MDR. Detail of antibiotic resistance pattern of common bacterial pathogens is depicted in Table 2. Among *S. aureus* isolates, 78.2% (18/23) were MRSA. Detail of antibiotic resistance pattern of *S. aureus* and MRSA isolates is shown in Table 3.

Out of 22 imipenem resistant isolates of *Acinetobacter* species, 9 were KPC producer, 4 were MBL producers and 3 isolates were positive for MBL and KPC both. One isolate of *Pseudomonas aeruginosa* was positive for MBL. Majority (10/16) of carbapenamase producing *Acinetobacter* species were isolated from the endotracheal tube.

Table 1: Bacteria isolated from lower respiratory tract specimen

S No	Organism	Number	Percentage
1	*Acinetobacter* species	58	42.6
2	*Pseudomonas aeruginosa*	19	13.9
3	*Staphylococcus aureus*	23	16.9
4	*Klebsiella pneumoniae*	13	9.5
5	*Escherichia coli*	08	5.8
6	*Citrobacter* species	07	5.1
7	*Enterobacter* species	04	2.9
8	*Haemophilus influenzae*	02	1.4
9	*Streptococcus pneumoniae*	02	1.4
	Total	136	

DISCUSSION

Advancement in the medical sciences resulted in interventions and increased use of life supporting medical devices. Intensive care units admission support life during critical situations but increases the risk of serious nosocomial infections like pneumonia, blood stream infections, urinary tract infections etc. Intensive care units are considered as important reservoir of microbial pathogens. Patients admitted in ICUs have increased risk of nosocomial infections due to underlying chronic illnesses and weakened immune responses. Lower respiratory tract infections are the most common infections among the

Table 2: Antibiotic resistance pattern of gram negative bacteria

S No	Antibiotic	*Acinetobacter* species n=58 (%)	*P. aeruginosa* n=19 (%)	*K. pneumoniae* n=13 (%)	*E coli* n=08 (%)	*Citrobacter species* n=07 (%)	*Enterobacter species* n=04 (%)
1	Amikacin	43 (74.1)	10 (52.6)	04 (30.7)	02 (25)	02 (28.5)	01 (25)
2	Ciprofloxacin	48 (82.7)	13 (68.4)	07 (53.8)	03 (37.5)	03 (42.8)	02 (50)
3	Ceftriaxone	45 (77.5)	13 (68.4)	06 (46.1)	03 (37.5)	04 (57.1)	02 (50)
4	Cifipime	44 (75.8)	13 (68.4)	07 (53.8)	04 (50)	03 (42.8)	02 (50)
5	Cefaperazone sulbactum	44 (75.8)	12 (63.1)	--	--	--	--
6	Colistin	03 (5.1)	0	--	--	--	--
7	Cefotaxime	45 (77.5)	13 (68.4)	07 (53.8)	03 (37.5)	03 (42.8)	02 (50)
8	Imipenem	22 (37.9)	02 (10.5)	00	00	00	00
9	Piperacillin tazobactum	34 (58.6)	08 (42.1)	--	--	--	--
10	Tigecycline	03 (5.1)	0	--	--	--	--
11	Gentamicin	38 (65.5)	10 (52.6)	05 (38.4)	03 (37.5)	02 (28.5)	01 (25)

Table 3: Antibiotic resistance pattern of *Staphylococcus aureus* (MRSA and MSSA) isolates

Antibiotic	*S. aureus* isolates (n=23) Frequency (%)	MRSA isolates (n=18) Frequency (%)	MSSA isolates (n=05) Frequency (%)
Penicillin	23 (100)	18 (100)	05 (100)
Erythromycin	20 (86.9)	18 (100)	02 (40)
Ciprofloxacin	19 (82.6)	18 (100)	01 (20)
Gentamicin	02 (8.7)	02 (11.1)	00
Clindamycin	10 (43.4)	08 (44.4)	02 (40)
Ceftriaxone	18 (78.2)	17 (94.4)	01 (20)
Cefoxitin	18 (78.2)	18 (100)	00
Co-trimoxazole	17 (73.9)	16 (88.8)	01 (20)
Amikacin	02 (8.7)	02 (11.1)	00

MRSA: Methicillin resistant *Staphylococcus aureus*, MSSA: Methicillin sensitive *Staphylococcus aureus*

patients admitted in ICU.[16,17] This study was aimed to determine the prevalence and antibiograms of bacterial pathogens causing LRTI among the patients of ICUs.

Ability of pathogen to resist commonly used antibiotics and disinfectants increase their survival in hospital environment. Gram negative bacteria are most adept in acquiring resistance to antibiotics. In this study also, Gram negative bacterial isolates were significantly higher (81.7%) as compared Gram positive bacteria (18.4%). Findings of our study are similar to other studies from neighboring countries.[18,19] In our study, *Acinetobacter* species, *S. aureus*, *Pseudomonas aeruginosa* and *Klebsiella pneumonia*e were the common bacterial pathogens. Findings of our study are similar to studies from Nepal and India.[18,20] *Pseudomonas aeruginosa*, *Klebsiella* species, *E coli*, *S. aureus* and *Enterococcus* species are the most frequent pathogens reported from ICUs of different hospitals of Asian countries.[16,18,19]

In this study, *Acinetobacter* was the most common pathogen. Parajuli et al, from Kathmandu, Nepal, reported *Acinetobacter* as the most common pathogens from ICU patients followed by *Klebsiella, E coli* and *Pseudomonas species*.[21] Study from Bangladesh reported *Klebsiella pneumoniae* as most common bacterial pathogens from lower respiratory tract.[19] The spectrum of pathogens and drug resistance pattern vary from hospital to hospital, within and outside country along with type of illness among the patients.[22] The prevalence of pathogens in various units of the hospital varies greatly depending upon infection control policies.

Increasing antimicrobial resistance in healthcare setting is global burden, particularly in developing countries. Higher drug resistance among hospital strains compared to community strains has been reported. Isolation of multidrug resistant bacteria like *S. aureus*, *Acinetobacter* species, *Pseudomonas aeruginosa* and *Klebsiella pneumonia*e from patients of ICU is worrisome. The most common isolate of our study *Acinetobacter,* showed higher resistance to most of the antibiotics tested including imipenem. Emergence of imipenem resistant isolates has been reported.[21,23] Resistance to imipenem is alarming, a threat to patients admitted in ICU and challenge for the infection control team. Majority of multidrug resistant strains of *Acinetobacter* species were susceptible to colistin and tigecycline. High resistance to common antibiotics limits the treatment options with financial burden and long term hospitalization among the patients. *Pseudomonas aeruginosa* isolates revealed comparatively lower percentage of resistance towards commonly used antibiotics. Resistance to imipenem was significantly lower among *Pseudomonas aeruginosa* and none of the isolates were resistant to colistin and tigecycline. Among other Gram negative bacteria, low resistance for amikacin and gentamicin but higher resistance to ciprofloxacin, ceftazidime, ceftriaxone and cefipime was observed. The high level of drug resistance has been attributed to production of enzymes, decreased uptake of drugs and efflux pumps.[24] High resistance to cephalosporins and quinolone could be due to extensive use of these drugs in past few years.

Among Gram positive bacteria, *S. aureus* has been reported as most common cause of nosocomial infections among ICU patients.[25] Majority of the *S. aureus* isolates (78.2%) in our study were MRSA which is higher than reported from India.[16] Bhandari et al, from Nepal, reported 43.7% MRSA among ICU patients.[25] High percentage of MRSA limits the therapeutic choices and forces the clinician to resort to more toxic drugs like vancomycin.

Carbapenams have been the therapeutic choice against multidrug resistant bacteria especially in ICU patients. Resistance to carbapenams among non-fermentative Gram negative bacteria is an emerging challenge. Emergence of carbapenamase has been reported across world. *Acinetobacter* species are notorious for survival in the hospital environment, rapid spread and acquisition of drug resistance. The worldwide emergence of carbapenam resistant *Acinetobacter* isolates is grave therapeutic challenge.[26] In our study, carbapenam resistance among *Acinetobacter* species was higher 27.6% (16/58) as compared to other pathogens. Among the carbapenamases, majority of the strains (9/16) were KPC producers. Studies from other hospitals have reported MBL as the most common carbapenamase among the non-fermentative Gram negative bacteria.[27,28] The prevalence of carbapenamase has been reported from 4% to 74%.[29-32] Recent studies from other hospitals of Nepal reported the prevalence of carbapenam resistance from 4.5%- 86.4%.[21,27] This reflects that the prevalence varies from hospital to hospital within a country. Colistin and tigecycline are effective against carbapenamase producing strains of *Acinetobacter* species with low level of resistance. Previous studies have reported 57%-77% cure rates with colistin among the critically ill patients having infections of MDR *Acinetobacter* species.[33] Emergence of resistance to colistin and tigecycline limits the therapeutic choice for the treatment of infections caused by carbapenam resistant isolates. In our study, 5.1% isolates of *Acinetobacter* species were resistant to colistin and tigecycline. Similar studies from Nepal reported 100% susceptibility of *Acinetobacter* isolates to colistin and tigecycline.[21,28] Another study from Nepal reported 2% colistin resistant *Acinetobacter* isolates with high resistance to tigecycline (37.3%).[34] Similar findings have been reported from tertiary care hospitals of India.[35,36] High resistance to cefaperazone sulbactum was observed among the *Acinetobacter* and *Pseudomonas* species. Majority of carbapenamase producing organisms were isolated

from the patients with age >60 years, indicating that old age group ICU admission has greater risk of nosocomial infections. One isolate of *Acinetobacter* species from NICU was MBL producer. Isolation of such pathogen from neonatal unit is of great concern.

The top five isolates in this study were *Acinetobacter* species, *S. aureus*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *E coli*. These ESKAPE group of pathogens described by Infectious Diseases Society of America, are likely to escape the effect of many antibiotics.[37] Many studies from ICUs have reported isolation of similar organisms.[16,20] Infections caused by ESKAPE group organisms especially among ICU patients are difficult to treat and have high morbidity and mortality.[38]

CONCLUSIONS

Prevalence of multidrug resistant Gram negative bacteria causing LRTI in ICUs was high. Gram negative bacteria were predominantly associated with LRTI among ICU patients. *Acinetobacter* species are the most common pathogen isolated. Detection of KPC and MBL among the isolates of *Acinetobacter* along with emergence of colistin and tigecycline resistance limits the therapeutic options. It is necessary to have policies regarding restrictive use of antibiotics like carbapenams and colistin. Regular monitoring of such resistant isolates would be important for infection control in critical units.

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