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Carbapenem Resistance in Non-Fermentative Gram-Negative Bacilli Isolated from Intensive Care Unit Patients of a Referral Hospital

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ABSTRACT

Background: Non-fermentative Gram-negative bacilli or non-fermenters are opportunistic pathogens associated with serious infections in intensive care unit patients. Although carbapenems were considered as a backbone of treatment for life-threatening infections, these bacteria are increasingly acquiring resistance to carbapenems. Carbapenem-resistant *Acinetobacter baumannii* and *Pseudomonas aeruginosa* are prioritized as critical pathogens by the World Health Organization. The objective of the study was to document the status of carbapenem-resistant and carbapenemase-producing non-fermenters isolated from intensive care unit patients.

Methods: This study was conducted at Tribhuvan University Teaching Hospital, Kathmandu, Nepal. The clinical specimens collected from intensive care unit patients were processed for isolation and identification of non-fermenters and antibiotic susceptibility profile of bacterial isolates was determined. The multidrug-resistant isolates were identified and carbapenemase enzyme was detected in the carbapenem-resistant isolates.

Results: A total of 157 non-fermenters were isolated from 1063 samples which included *Acinetobacter* species (n=85), *Pseudomonas aeruginosa* (n=55), *Burkholderia cepacia* complex (n=15), and *Stenotrophomonas maltophilia* (n=2). Carbapenem resistance was reported in 85.9%, 72.7%, and 33.3% of *Acinetobacter* species, *Pseudomonas aeruginosa,* and *Burkholderia cepacia* complex, respectively. Among total non-fermenters, 91.1% isolates were multidrug-resistant and 60.8% carbapenem-resistant isolates were carbapenemase producers. The carbapenem-resistant isolates demonstrated an extremely high degree of resistance than carbapenem-susceptible isolates towards other antimicrobial classes.

Conclusions: This study reported high rates of carbapenem-resistant, carbapenemase-producing, and multidrug-resistant non-fermenters isolates. Therefore, preventing the spread of these superbugs among the critically ill patients in intensive care units should be a major initiative in hospitals.

Keywords: Carbapenem-resistant; carbapenemase; intensive care unit; non-fermentative Gram-negative bacilli

INTRODUCTION

Carbapenems are considered as mainstay of treatment for life-threatening infections but carbapenem resistance in Gram-negative bacteria is increasing at an alarming rate.[1,2] The non-fermenters are opportunistic pathogens and are progressively reported as a cause of serious infections in intensive care unit (ICU) patients.[3] Among different mechanisms of carbapenem resistance in Gram-negative bacteria, carbapenemase production is the most worrying mechanism due to their easily transmissible property to other bacterial pathogens.[2,4]

Carbapenem-resistant non-fermenters are emerging causes of healthcare-associated infections that pose a huge threat to clinical settings and are hard to treat due to their high degree of antibiotic resistance and are related to significant mortality.[5] The World Health Organization (WHO) published a list of antibiotic-resistant bacteria including carbapenem-resistant *Acinetobacter baumannii* and *Pseudomonas aeruginosa* within the critical priority level.[6]

Therefore, this study was designed to document the status of carbapenem-resistant and carbapenemase-

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producing non-fermenters isolated from ICU patients.

METHODS

The study had a cross-sectional design and was conducted at the clinical microbiology department of Tribhuvan University Teaching Hospital, Kathmandu, Nepal from January 2017 to December 2017. Ethical approval for the study was obtained from Nepal Health Research Council with reference number 1559. The non-replicative clinical specimens like sputum, tracheal aspirate, bronchoalveolar lavage fluid, blood, urine, pus, wound swabs, catheter tips, and body fluids collected for culture and sensitivity tests from the patients of any age, suspected infection and admitted to different ICUs were included in the study. We had not accepted repeated specimens from the same patient within 48 hours of the prior one which nullifies the chances of strain biasedness from the same patient. The specimens from the upper respiratory tract and gastrointestinal tract were excluded because of the presence of non-fermentative Gram-negative bacteria as commensals on these sites.

The calculated minimum sample size was 514. As convenient sampling was done, the sample size was doubled to 1028. However, 1063 samples had been taken so as to increase the validity of the result. The collected samples were cultured on suitable media in the bacteriology laboratory for the isolation of bacterial isolates. The isolated bacteria were further subjected for identification of non-fermentative Gram-negative bacilli (NFGNB) by employing different microbiological techniques which involved the morphological appearance of the colonies, Gram's staining, and different biochemical reactions by standard methods recommended by the American Society for Microbiology (ASM).[7]

The susceptibility of NFGNB isolates against different antibacterial agents was determined and interpreted by the Kirby-Bauer disk diffusion method and minimum inhibitory concentration method according to the guidelines provided by the Clinical and Laboratory Standards Institute (CLSI), M100S Document, 26th Edition (2016). The bacterial isolates were tested against a specified concentration of recommended antibiotics as applicable. *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 were used as the control organisms for the validity of the antibiotic sensitivity test.[8]

The multidrug resistant (MDR) isolates were identified by guidelines recommended by the European Centre for Disease Prevention and Control (ECDC). The isolates non-susceptible to at least one antimicrobial agent in three or more antimicrobial classes were identified as MDR phenotypes.[9]

The isolates resistant to either meropenem and/or imipenem were considered as carbapenem-resistant, and those isolates were further subjected to the detection of carbapenemase enzyme by Modified Hodge test (MHT). In this test, a suspension of *Escherichia coli* ATCC 25922 compared to 0.5 McFarland standard was prepared in 5 ml of normal saline. A one : ten (1:10) dilution of the suspension was streaked as a lawn culture on to a Mueller Hinton agar plate. A meropenem disk of 10 µg in concentration was placed at the center of the lawn culture. The test organism was streaked in a straight line from the edge of the meropenem disk to the edge of the plate. The plate was incubated overnight at 35±2°C in ambient air for 16-24 hours. After incubation, a positive MHT test showed a clover leaf-like indentation of *Escherichia coli* 25922 growing along with the test organism growth streak within the disk diffusion zone. A negative MHT test showed no growth of the *Escherichia coli* 25922 along with the test organism growth streak within the disk diffusion.[10]

All the data were analyzed using the SPSS version 16.0 and interpreted according to frequency distribution and percentage.

RESULTS

Among the total 1063 specimens collected from ICU patients, 387 (36.4%) specimens showed bacterial growth from which a total of 157 (14.8% of the total sample) non-replicate isolates of NFGNB were recovered. The total NFGNB isolates comprised of four genera of NFGNB which included *Acinetobacter* species (n=85, 54.1%), *Pseudomonas aeruginosa* (n=55, 35.0%), *Burkholderia cepacia* complex (n=15, 9.6%) and *Stenotrophomonas maltophilia* (n=2, 1.3%). The highest number of *Acinetobacter* species and *Pseudomonas aeruginosa* were isolated from lower respiratory tract specimens while about half of the *Burkholderia cepacia* complex was recovered from blood samples (Table 1).

Among total NFGNB isolates, 96 (61.6%) were isolated from male patients and 61 (38.9%) were from female patients with a male to female ratio of 1.57. The highest number of isolates were from patients with age group ≤15 years (n=49, 31.2%) and the least number from 16-32 years age group (n=24, 15.3%) (Table 2).

The proportion of carbapenem resistance was 85.9% in *Acinetobacter* species, 72.7% in *Pseudomonas aeruginosa*, 33.3% in *Burkholderia cepacia* complex,

and 100% in *Stenotrophomonas maltophilia*. Among the total carbapenem-resistant isolates, 60.8% were carbapenemase producers with a higher rate of carbapenemase enzyme detected in *Pseudomonas aeruginosa* (67.5%). Among total isolates, 91.1% were MDR isolates where 95.3% *Acinetobacter* species, 85.5% *Pseudomonas aeruginosa*, 86.6% *Burkholderia cepacia* complex, and 100% *Stenotrophomonas maltophilia* were MDR (Table 3).

The antibiotic susceptibility profile of carbapenem-resistant (CR-R) and carbapenem-susceptible (CR-S) NFGNB are shown in Table 4.

Table 1. Distribution of total sample and growth pattern of NFGNB isolates in ICU patients.

Specimen type (number of samples)	Bacterial growth	Number of NFGNB isolates (%)				
		Acinetobacter species	*Pseudomonas aeruginosa*	*B. cepacia* complex	*S. maltophilia*	Total
LRTS* (n=321)	223	60 (70.6)	40 (72.8)	3 (20.0)	1 (50.0)	104 (66.2)
Body fluids (n=137)	27	6 (7.1)	7 (12.7)	3 (20.0)	1 (50.0)	17 (10.8)
Blood (n=326)	40	7 (8.2)	1 (1.8)	7 (46.7)	0 (0)	15 (9.6)
Pus/swabs (n=63)	43	6 (7.1)	5 (9.1)	0 (0)	0 (0)	11 (7.0)
Urine (n=190)	47	3 (3.5)	1 (1.8)	2 (13.3)	0 (0)	6 (3.8)
Catheter tips (n=26)	7	3 (3.5)	1 (1.8)	0 (0)	0 (0)	4 (2.6)
Total (N=1063)	387	85 (54.1)	55 (35.0)	15 (9.6)	2 (1.3)	157 (14.8)

**LRTS: Lower respiratory tract specimens include sputum, endotracheal aspirate, and bronchoalveolar lavage fluid.*

Table 2. Distribution of NFGNB isolates according to the demographic features of patients.

Age Group (Years)	Number (%)		
	Female	Male	Total
≤15	27 (17.2)	22 (14.0)	49 (31.2)
16-32	5 (3.2)	19 (12.1)	24 (15.3)
33-48	8 (5.1)	17 (10.8)	25 (15.9)
49-64	13 (8.3)	17 (10.8)	30 (19.1)
≥65	8 (5.1)	21 (13.4)	29 (18.5)
Total (%)	61 (38.9)	96 (61.1)	157 (100)

Table 3. Percentage of carbapenem-resistant, carbapenemase-producing, and multidrug-resistant NFGNB isolates.

NFGNB isolates	Number (%)			
	Carbapenem-resistant		Carbapenemase producers	Multidrug-resistant
	Meropenem	Imipenem		
Acinetobacter species (n=85)	73 (85.9)*	71 (83.5)	43 (58.9)	81 (95.3)
Pseudomonas aeruginosa (n=55)	40 (72.7)*	38 (69.1)	27 (67.5)	47 (85.5)
Burkholderia cepacia complex (n=15)	5 (33.3)*	5 (33.3)	3 (60.0)	13 (86.7)
Stenotrophomonas maltophilia (n=2)	2 (100)*	2 (100)	0 (0)	2 (100)
Total (N=157)		120 (76.4)	73 (60.8)	143 (91.1)

**These values were considered as the rate of carbapenem-resistant.*

Table 4. Antibiotic resistance rate of carbapenem-resistant and carbapenem-susceptible NFGNB.

Antibiotic molecules	*Acinetobacter species*			*Pseudomonas aeruginosa*			*Burkholderia cepacia complex*		
	CR-R (n=73)	CR-S (n=12)	p-value	CR-R (n=40)	CR-S (n=15)	p-value	CR-R (n=5)	CR-S (n=10)	p-value
Piperacillin	73 (100%)	9 (75.0%)	<0.001	38 (95.0%)	8 (53.3%)	<0.001	NT	NT	
Piperacillin-tazobactam	72 (98.6%)	6 (50.0%)	<0.001	36 (90.0%)	3 (20.0%)	<0.001	NT	NT	

Ampicillin-sulbactam	70 (95.9%)	4 (33.3%)	<0.001	NT	NT		NT	NT	
Ceftazidime	73 (100%)	8 (66.7%)	<0.001	39 (97.5%)	11 (73.3%)	0.005	5 (100%)	9 (90.0%)	0.500
Cefotaxime	73 (100%)	10 (83.3%)	<0.001	NT	NT		NT	NT	
Cefepime	73 (100%)	8 (66.7%)	<0.001	38 (95.0%)	11 (73.3%)	0.021	NT	NT	
Gentamicin	71 (97.3%)	5 (41.7%)	<0.001	29 (72.5%)	5 (33.3%)	0.007	NT	NT	
Amikacin	66 (90.4%)	4 (33.3%)	<0.001	21 (52.5%)	1 (6.7%)	0.002	NT	NT	
Ciprofloxacin	71 (97.3%)	9 (75.0%)	0.002	39 (97.5%)	14 (93.3%)	0.471	NT	NT	
Levofloxacin	67 (91.8%)	6 (50.0%)	<0.001	36 (90.0%)	8 (53.3%)	0.002	2 (40.0%)	10(100%)	0.003
Cotrimoxazole	72 (98.6%)	8 (66.7%)	<0.001	NT	NT		0 (0)	0 (0)	
Doxycycline	51 (69.9%)	1 (8.3%)	<0.001	NT	NT		2 (40.0%)	0 (0)	0.032
Polymyxin B	0 (0)	0 (0)		0 (0)	0 (0)		NT	NT	
Chloramphenicol	NT	NT		NT	NT		4 (80.0%)	6 (60.0)	0.475

CR-R: Carbapenem-resistant; CR-S: Carbapenem-susceptible; NT: Antibiotics not tested/not recommended by CLSI.

DISCUSSION

After the introduction of the carbapenem group of antibiotics in the 1980s, they remain the backbone of antimicrobial therapy for the treatment of serious infections. The resistance to carbapenems was seen by the early 1990s and the incidence of infections caused by carbapenem-resistant Gram-negative bacilli in ICUs has also increased which constitutes a global problem.[1,11] The concern species of NFGNB are *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, *Stenotrophomonas maltophilia,* and members of *Burkholderia cepacia* complex. These are generally saprophytic bacteria but cause opportunistic infections like ventilator-associated pneumonia (VAP), surgical site infections (SSI), urinary tract infections (UTI), and bacteremia in critically ill, hospitalized, and immunocompromised patients.[4]

Carbapenem resistance in NFGNB can be mediated by different mechanisms like over-expression of efflux systems, decreased permeability due to porin mutations, and alterations in penicillin-binding proteins (PBPs) but the production of carbapenemase enzyme is the most worrying mechanism due to their easily transmissible property to other bacterial pathogens, increasing prevalence and their association with resistance to other antimicrobial categories leading to multidrug resistance phenotypes.[2] The carbapenemase enzymes belong to Ambler class A (*Klebsiella pneumoniae* carbapenemase), class B (metallo-ß-lactamases), and class D (mainly oxacillinase) ß-lactamases.[12] The documentation and regular monitoring of carbapenem-resistant and carbapenemase-producing NFGNB are important as they can cause infection outbreaks in ICU settings.

Analysis of the case records of ICU patients revealed that the highest number of NFGNB isolates (n=104, 66.2%) were detected from lower respiratory tract specimens followed by body fluids (n=17, 10.8%), blood (n=15, 9.6%), and least number from catheter tips (n=4, 2.6%). Nautiyal et al[13] from India also reported the majority of NFGNB isolates from respiratory tract specimens infections followed by pus samples. *Acinetobacter* species was the most common NFGNB isolates (54.1%) detected in our study followed by *Pseudomonas aeruginosa* (35.0%), *Burkholderia cepacia* complex (9.6%) and the least number was *Stenotrophomonas maltophilia* (1.3%). Parajuli et al[3] also reported *Acinetobacter* species as major NFGNB isolates from ICU patients followed by *Pseudomonas aeruginosa*. In our study, both *Acinetobacter* species (70.6%) and *Pseudomonas aeruginosa* (72.8%) were the common isolates from lower respiratory tract specimens while most of the *Burkholderia cepacia* complex (46.7%) were isolated from blood samples. Samawi et al[14] from Qatar also reported a majority of *Acinetobacter* species from respiratory tract infection, Parajuli et al[3] reported most of the *Pseudomonas aeruginosa* from VAP and Dizbay et al[15]and multidrug resistance makes them a serious threat in hospital settings. The aim of this study was to evaluate the epidemiology of *B. cepacia* infections in our hospital. Methodology: The incidence, clinical characteristics, antimicrobial susceptibility, and outcomes of nosocomial *B. cepacia* infections during a five-year period were retrospectively analysed according to the infection control committee records. Results: A total of 39 cases with nosocomial *B. cepacia* infection were included in the study. *B. cepacia* was identified from 0.7% of the nosocomial isolates, its incidence was 0.26 per 1,000 admissions with 53.8% crude mortality rate. The most frequent nosocomial B. cepacia infection was pneumonia (58.9% from Turkey reported major *Burkholderia cepacia* complex from bloodstream infection.

Carbapenem-resistant *Pseudomonas aeruginosa* (CRPA) and *Acinetobacter baumannii* (CRAB) remain important causes of hospital-acquired infections (HAIs) and are prioritized by the WHO as critical pathogens requiring urgent discovery, research, and development of new antibiotics.[16] In this study, we have well documented the status of carbapenem resistance in NFGNB isolated from ICU patients. In our study, 85.9% of *Acinetobacter species* and 72.7% of *Pseudomonas aeruginosa* were found resistant to carbapenems and 33.3% *Burkholderia cepacia* complex were carbapenem-resistant. Both the isolates of *Stenotrophomonas maltophilia* were found resistant to meropenem as this bacteria is intrinsically resistant to carbapenems. Parajuli et al[3] from Nepal (in 2014) and Xia et al[17] China. Ventilator-associated pneumonia (VAP from China (in 2011) have also documented about 86% carbapenem-resistant *Acinetobacter* species while Mishra et al[18] and Shrestha et al[19] from Nepal reported a relatively lower rate of 50.0% (in 2008) and 69.3% (in 2015) carbapenem-resistant *Acinetobacter* species, respectively. Among *Pseudomonas aeruginosa*, Parajuli et al[3] from Nepal documented 62.5% (in 2014), Agarwal et al[1] from India reported 56% (in 2017) while Mishra et al[18] reported only 17.6% (in 2008) carbapenem-resistant isolates. Similarly, Parajuli et al[3] from Nepal and Gautam et al[20] from India have reported 20% and 72.2% carbapenem-resistant *Burkholderia cepacia* complex, respectively. The above data indicates that the rate of carbapenem resistance in NFGNB has been increased dramatically within the last decade. Carbapenemase production by MHT has been seen in 58.9%, 67.5%, and 60.0% of carbapenem-resistant *Acinetobacter* species, *Pseudomonas aeruginosa*, and *Burkholderia cepacia* complex, respectively. The bacterial isolates harboring carbapenemase enzymes result in limited therapeutic options as they are often resistant to multiple classes of antibiotic classes.[12]

The carbapenem-resistant isolates demonstrated a much higher degree of antibiotic resistance than carbapenem-susceptible isolates towards other antimicrobial classes. The carbapenem-resistant *Acinetobacter* species showed a very high resistance rate towards penicillins, cephalosporins, fluoroquinolones, and aminoglycosides (>90%) and were moderately resistant to doxycycline (69.9%). Our resistance rate result of *Acinetobacter* species is higher than the previous results from the same hospital.[3,21] Similarly, more isolates of carbapenem-resistant *Pseudomonas aeruginosa* showed resistance towards penicillins, cephalosporins, and fluoroquinolones (>90%) and 72.5% resistance to gentamicin. Mishra et al[18] from Nepal and Xie et al[17]China. Ventilator-associated pneumonia (VAP from China reported a lower resistance rate in *P. aeruginosa* than our report against beta-lactam antibiotics. All the carbapenem-resistant isolates of *Burkholderia cepacia* complex were resistant to ceftazidime, 80.0% resistant to chloramphenicol, and 40% resistant to levofloxacin and doxycycline. Parajuli et al[3] also reported 100% *Burkholderia cepacia* complex resistant to ceftazidime but Gautam et al[20] from India reported only 10.8% isolates resistant to ceftazidime. In this study, both *Stenotrophomonas maltophilia* isolates were resistant to ceftazidime and chloramphenicol which is higher than the report of Sattler et al.[22] Polymyxin B showed excellent effect (100% sensitivity) against both carbapenem-resistant and carbapenem-susceptible *Acinetobacter* species and *Pseudomonas aeruginosa* isolates while cotrimoxazole was found to be effective against *Burkholderia cepacia* complex and *Stenotrophomonas maltophilia*.

We have reported a high MDR rate of 91.1% in NFGNB isolates comprising 95.3% MDR *Acinetobacter* species which is extremely high. Shrestha et al[23] and Mishra et al[21] also reported about 96% and 95% MDR *Acinetobacter* species respectively. We found 85.5% MDR *Pseudomonas aeruginosa* isolates which is higher than the reported of Mishra et al[18] from Nepal in 2008 (65.9% MDR) and Hassuna et al[24] from Egypt in 2015 (56% MDR). This high prevalence of MDR NFGNB is due to the high chance of dissemination of resistance genes among different bacterial isolates and the rate of MDR is in an upward trend globally especially in developing countries resulting in a threatening situation.

Due to the lack of sophisticated instruments for performing molecular typing methods in our setting and also due to budget constraints to get those tests done in foreign labs, we could not evaluate the molecular characterization of resistant phenotypes.

CONCLUSIONS

Our study highlights a rise in the rate of infections caused by carbapenem-resistant, carbapenemase-producing, and multidrug-resistant NFGNB in ICU patients and also reducing the availability of effective antimicrobial agents. Therefore, preventing the spread of these superbugs among critically ill patients in the ICUs should be a major initiative in hospitals.

CONFLICT OF INTEREST: None

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