Prevalence of Carbapenem-Resistant Gram-Negative Clinical Isolates and its Antibiotic Susceptibility Patterns

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Keywords
Carbapenem
carbapenemase
MBL
MDR
KPC

Received: 12 October 2024
Revised: 15 November 2024
Accepted: 28 November 2024

ISSN: 3059 - 9687

Abstract

Carbapenem-resistant Gram-negative pathogens are recognized as a major health concern. However little information is available in Nepal regarding carbapenem-resistant Gram-negative bacteria. Therefore, a cross-sectional study was carried out at a tertiary care hospital to examine the prevalence of carbapenemase, metallo-beta-lactamases, and AmpC-beta-lactamases producing organisms in carbapenem-resistant Gram-negative bacteria. A total of 82 Gram-negative bacteria were identified from 825 clinical specimens based on colony characters, Gram staining catalase test, oxidase test, and other biochemical tests. Antibiotic susceptibility testing was performed according to clinical standard institute (CLSI) guidelines 2014. Of 82 isolates, 24 (29.3%) were carbapenem-resistant. Of these 33.4% were *E. coli*, 25% were *Pseudomonas*, 25% were *Klebsiella pneumoniae* and 16.7% were *Acinetobacter* spp. A total of 13 (54.2%) were MBL producers. All MBL producers were multi-drug resistant (MDR). Single isolate of *K. pneumoniae* was KPC positive. Out of 24 carbapenem-resistant isolates, 20.8% isolates were positive for the AmpC beta-lactamases test. All AmpC beta-lactamases and single isolates of KPC producers were MDR. Among the total of 82 Gram-negative bacterial isolates, 48.8% were MDR organisms. Based on *in-vitro* testing polymyxin B was found effective against all isolates. Although, MBL and other beta-lactamase producers were not higher than that found in other studies from Nepal, beta-lactamases and MDR organisms should be detected in the hospital setting to prevent the spread of these organisms.

Introduction

Antibiotic resistance is an important public health problem. Antibiotic resistance is now well recognized as a major problem in the treatment of infections in hospitals and the community. The World Health Organization identified antimicrobial resistance as one of the three greatest threats to human health (Davies *et al.,* 2010). Drug-resistant bacterial infections and their morbidity and mortality are on the rise all over the world (Bisht *et al.,* 2009).

Beta-lactams are the major antibiotics used in the world today. It comprises more than 65% of the total antibiotics consumed in the world today (Elander, 2003). Major mechanisms causing resistance to the beta-lactam antibiotics are the production of beta-lactamases, reduced outer membrane permeability, and altered affinity of target Penicillin-binding proteins. The infections due to beta-lactamase are difficult to treat because of the emergence of newer beta-lactamases such as Extended Spectrum beta-

lactamases (ESBL), AmpC beta-lactamases, and Carbapenemases. The beta-lactamases inactivate beta-lactam antibiotics by cleaving the structural beta-lactam ring. Failure to detect these enzymes producing strains has contributed to their uncontrolled spread in the Health Care setup and therapeutic failure (Parker *et al.,* 1990, Washington *et al.,* 2006).

Among all of the bacterial resistance problems, Gram-negative pathogens both fermentative and non-fermentative bacteria are the leading cause of human infection. Antibiotic-resistant pathogens are common in Nepal. This is due to the misuse of antibiotics. Many patients use antibiotics without a prescription from a physician and also some physicians prescribed antibiotics without the results of antibiotics susceptibility testing. Also, people fail to finish the full course of treatment. In both circumstances, the improper dosing will fail to eliminate the disease agent and will furthermore; encourage the growth of most resistant strains. The study was mainly conducted to evaluate the burden of MDR with special reference to Gram-negative bacteria and to determine their antibiotic-resistant, MBL, AmpC beta-lactamase, and carbapenemase.

Materials and Methods

This was a cross-sectional study conducted at a tertiary care hospital in Kathmandu. Patients of all age groups and both sexes were included in the study population after getting consent from the patient. Nonrepeating samples which were sent for routine culture and antibiotic susceptibility testing were processed.

A total of 82 Gram-negative bacteria were identified from 825 clinical specimens based on colony characters, Gram staining, catalase test, oxidase test, and other biochemical tests. Antibiotic susceptibility testing was performed according to clinical standard institute (CLSI) guidelines 2014. Confirmation of MBL, AmpC, and KPC are done according to clinical standard institute (CLSI) guidelines 2014.

Results

A total of 825 different clinical specimens were received for routine culture and susceptibility testing during the study period, of which urine (54.3%), blood (26.1%), and sputum (11.2%) were the common samples submitted for culture. Of the total clinical specimens, 120 (14.5%) specimens showed significant growth from which 124 bacterial isolates were obtained. Gram-negative bacteria were predominant constituting 82 (66.2%). Gram-positive bacteria constituted 42 (33.8%) of total isolates. Among Gram-negative isolates, *Escherichia coli* (50%) was most frequently isolated, followed by *Pseudomonas* spp (15.8%) and *Klebsiella pneumoniae* (10.9%).

Table 1. Distribution of gram-negative bacteria among different clinical samples.

Clinical sample	**Number of sample (%) (n=825)**	***E. coli***	***Pseudomonas***	***K. pneumoniae***	**Others***	**Total**
Urine	448 (54.3)	29	8	6	6	**49**
Blood	215 (26.1)	4	0	1	2	**7**
Sputum	92 (11.2)	2	1	1	8	**12**
Tip	27 (3.3)	4	2	0	1	**7**
Pus	19 (2.3)	1	2	0	2	**5**
Body fluid	17(2)	1	0	1	0	**2**
Others	7 (0.8)	0	0	0	0	
Total	**825**	**41**	**13**	**9**	**19**	**82**

*Others bacterial isolates include; *Citrobacter* spp = 5, *Acinetobacter* spp=5, *Proteus mirabilis*= 3, *P. vulgaris*= 2, *Moraxella* spp =3, *Salmonella* spp=1

Multidrug resistant Gram-negative bacteria

Out of 82 Gram-negative bacterial isolates, 40 (48.8%) were MDR organisms. All the bacterial isolates showed a high degree of resistance except *Proteus* spp., *Moraxella* spp, and *Salmonella* spp.

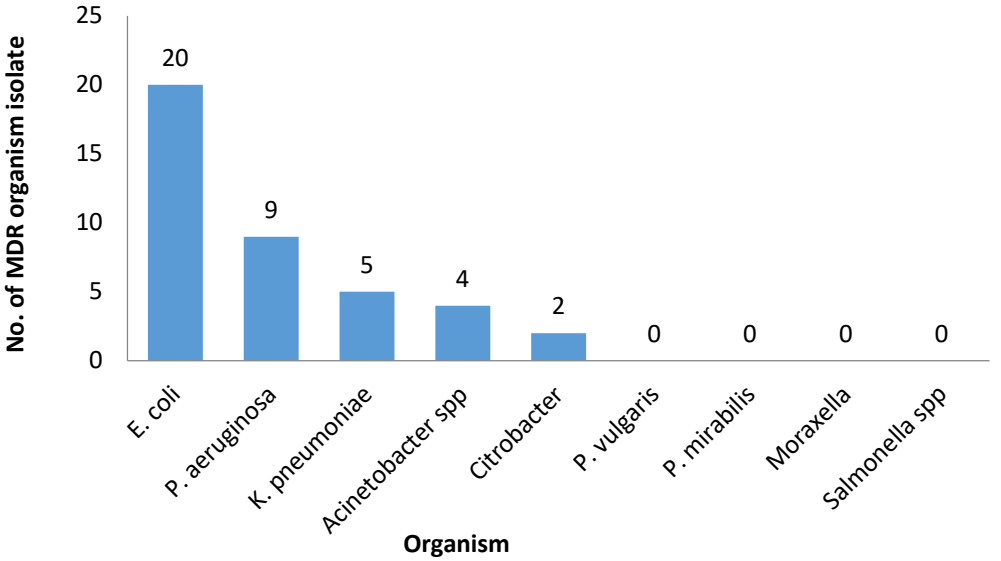

Fig. 1. Multidrug resistant Gram-negative bacteria.

Metallo beta-lactamases producing Gram-negative bacteria

Of 82 total Gram-negative isolates, 24 (29.3%) were carbapenem-resistant isolates. Among 24 carbapenem-resistant isolates, 13 (54.2%) were MBL producers. The Highest MBL producer's isolates were among *P. aeruginosa* (66.7%).

Table 2. Metallo beta-lactamases producing Gram negative bacteria.

Organisms	**Total no. of isolates**	**Carbapenem resistant isolates (%)**	**% among total**	**MBL-positive (%)**	**% among total**
E. coli	41	8 (19.5)	33.4	4 (50)	16.7
P. aeruginosa	13	6(46.2)	25	4 (66.7)	16.7
K. pneumoniae	9	6 (66.7)	25	3 (50)	12.5
Acinetobacter spp	5	4 (80)	16.7	2 (50)	8.4
Citrobacter	5	0	0	0	0
P. vulgaris	2	0	0	0	0
P. mirabilis	3	0	0	0	0
Moraxella	3	0	0	0	0
Salmonella spp	1	0	0	0	0
Total	**82**	**24 (29.3)**		**13 (54.2)**	54.2%

Klebsiella pneumoniae carbapenemase (KPC) producing Gram-negative bacteria

Out of 24 carbapenem-resistant isolates tested for KPC, only one isolate of *K. pneumoniae* was positive. All others were negative for KPC production. Of a total 6 carbapenem resistant, *K. pneumoniae* isolates 1 (16.7%) isolate was a KPC producer. This isolate was negative for the MBL test. KPC producers' isolate was MDR and sensitive to polymyxin B.

AmpC beta-lactamases producing bacterial isolates

Out of 24 carbapenem-resistant isolates tested for AmpC beta-lactamases, 5 (20.8%) isolates were positive for the AmpC beta-lactamases test (Figure 1). AmpC beta-lactamase producers were *E. coli* (1 isolate), *K. pneumoniae* (1 isolate), *P. vulgaris* (1 isolate), *Citrobacter* spp (1 isolate), and *Acinetobacter* spp. (1 isolate). These isolates were negative for MBL detection and KPC detection test.

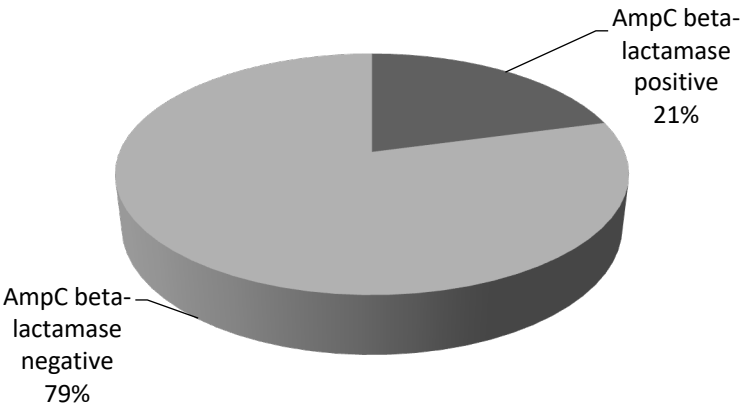

Fig. 2. AmpC beta-lactamase positive and negative isolates.

Discussion

In the present study, a total of 124 bacterial species were isolated from 825 various clinical specimens. Of 124 total isolates, Gram-negative bacteria were predominant constituting 82 (66.2%). Gram-positive bacteria constituted 42 (33.2%) of total isolates. The study was in consonant with the study of Ali *et al.* (2016). Among Gram-negative isolates, *E. coli* (50%) was most prevalent followed by *Pseudomonas* (15.8%), *K. pneumoniae* (11%), and single isolates of *Salmonella* spp were obtained. Out of total Gram-negative bacteria, a majority (59.7%) of isolates were obtained from urine samples; *E. coli* (70.7%), *Pseudomonas* (61.5%), *K.pneumoniae* (66.7%), and others (31.6%). This may be due to the larger number of urine samples included in our study as the hospital has got dialysis facility from which a large number of urine samples were obtained *E. coli* and *K. pneumoniae* were the most commonly isolated organisms from a urine sample. This finding was supported by other results of other studies from Nepal showing the urine as the major source of *E. coli* (Dhakal *et al.,* 2012; Raut *et al.,* 2015) and *K. pneumoniae* (Nepal *et al.,* 2017). Further, *E. coli* and *K. pneumoniae* are common causes of urinary tract infections.

MDR Gram-negative bacteria have been frequently reported from different parts of the world as the emergence of treatment problems.

In this study, 48.8% of Gram-negative isolates were MDR. Yadav *et al.* (2015) have observed 96.84 % MDR Enterobacteriaceae. A higher rate of MDR was also reported by Sharma *et al.* (2013) (90.8%) whereas Khanal *et al.* (2013) reported that 50% of Gram-negative isolates were MDR, similar to our findings. In the present study, 69.3% of *Pseudomonas* isolates and 80% of *Acinetobacter* isolates were MDR. *Pseudomonas* and *Acinetobacter* are intrinsically resistant to many commonly used antibiotics and a high rate of MDR among these isolates was observed worldwide. Diverse resistance mechanisms are implicated in MDR isolates. Gram-negative bacteria are involved in various infection types and the increasing prevalence of MDR Gram-negative bacteria makes therapeutic options limited.

In this study, among 24 carbapenem-resistant isolates, 54.2% were MBL producers. The Highest MBL producer's isolates were among *P. aeruginosa*; 66.7% of carbapenem-resistant isolates were MBL producers. In accordance with our findings, Acharya *et al.* (2017) reported that 68.6% of imipenem-resistant isolates were MBL producers. In a tertiary level hospital in Egypt, 68.7% of carbapenem-resistant *P. aeruginosa* were MBL producers which is comparable to the result of the present study (Zafer *et al.,* 2015). Figueiredo-Mendes *et al.* (2005) described higher rates of MBLs production (77.8%) among *P. aeruginosa* strains previously resistant to carbapenems in hospitals in São Paulo, Brazil. However, another study from Nepal reported MBL-producing *P. aeruginosa* in 33.3% of imipenem-resistant isolates (Khanal *et al.,* 2013), according to which MBL may not play a major role in imipenem resistance. These great differences in MBL prevalence between different countries are probably due to different antibiotic therapy policies.

In this study, 50% of each isolate of *E. coli* and *K. pneumoniae* resistant to carbapenem were MBL producers, indicating carbapenem resistance in MBL negative isolates is probably due to other mechanisms. However, in another study from Nepal, Bora *et al.* (2014) reported all carbapenem-resistant *E. coli* and *K. pneumoniae* isolates were MBL producers. In the present study, the prevalence of MBL in carbapenem-resistant *Acinetobacter* was relatively low (50%) than that reported in India, where 80.3% of imipenem-resistant *Acinetobacter* isolates were MBL producers (Kaur *et al.,* 2014).

It has been observed that MBL encoding genes are usually associated with many other non-Beta-lactam resistance determinants, which give rise to “MDR and pan-drug resistant” isolates. We also observed 100% MBL producers as “MDR”.

Although the prevalence of KPC-producing Enterobacteriaceae is increasing worldwide, in this study, single isolates of carbapenem-resistant isolates of *K. pneumoniae* were KPC producers. In some parts of the world, up to 60% of carbapenem-resistant isolates were KPC producers (Mathers *et al.,* 2015).

AmpC enzymes possess a low potential for carbapenem hydrolysis and their overproduction may contribute to carbapenem resistance combined with diminished outer-membrane permeability and/or efflux pump overexpression (Meletis *et al.,* 2016). In this study out of 24 carbapenem-resistant isolates tested for AmpC beta-lactamases, 5 (20.8%) isolates were positive for AmpC beta-lactamases.

Conclusion

Despite the increasing prevalence of carbapenem-resistant Gram-negative bacteria globally, pooled data suggest the prevalence of such pathogens within the reported range in Nepalese hospitals. The full in vitro susceptibility of isolates to polymyxin B shows the effectiveness of such a drug in the curing of infections caused by them. Data also suggest that the dissemination of carbapenem-resistant bacteria may worsen the therapeutic options in Nepalese hospitals.

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