Review Article

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Carbapenemase among Clinical Bacterial Isolates in Nepal

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ABSTRACT

Gram-negative bacterial isolates producing carbapenemase enzymes are great public health problem in developing countries and their control is challenging task due to the involvement of multiple factors including the practice of self-medication, use of antibiotics on animal farms, poor hospital hygiene, etc. In this review, we searched various relevant publications on carbapenemase-producing bacterial isolates in Nepal.

Various classes of carbapenemase producing bacteria have been reported in Nepal. Most frequent was the New Delhi Metallo beta lactamase with many variants. Similarly, oxacillinase and *Klebsiella pneumoniae* carbapenemase producers were also prevalent in Nepal. Likewise, other carbapenemase like Verona integron-encoded metallo-β-lactamase, imipenemase, and Dutch imipenemase were also detected. Ten variants of class B carbapenemase were detected including seven variants of New Delhi Metallo-β-lactamase and one variant each of Verona integron-encoded metallo-β-lactamase, Dutch imipenemase, and imipenemase. Similarly, 12 variants of oxacillinase were reported while no variants of Klebsiella pneumoniae carbapenemase were reported. The isolates producing carbapenemase were extremely drug-resistant as they also co-produced various other carbapenemase, beta-lactamase, 16S rRNAmethylase. Such isolates had very few treatment options as only last line drugs like colistin, fosfomycin, and tigecycline were effective against most of these isolates. Carbapenemase production by almost all gram-negative pathogens is a matter of concern because some of these enzymes are located on plasmids and pose rapid dissemination among various gram-negative pathogens. Timely surveillance for carbapenemase producers throughout the nation, their proper treatment, and proper hospital hygiene to prevent nosocomial infections by carbapenemase producers, controlled use of carbapenems, educating health care workers, students, and the general public about the adverse effects of antimicrobial resistance are imminent.

Keywords: Carbapenemase; KPC; NDM; Nepal; OXA.

INTRODUCTION

Carbapenemase are the member of ß-lactamase having the flexible ability of ß-lactam hydrolysis.[1] Carbapenemase are the global public health challenge since their discovery and are classified as class A, B, and D carbapenemase as per Ambler classification.[2] Class A carbapenemases are serine carbapenemases as they must contain serine for their hydrolytic activity. *Klebsiella pneumoniae* carbapenemase (KPC), *Serratia marcescens* enzyme (SME), Guiana extended spectrum ß-lactamase (GES), and imipenem-hydrolyzing ß-lactamase (IMI) are the major genes in this class of carbapenemases with many variants.[1] Metallo-ß-lactamases or class B carbapenemases contain metal ions at the active site of enzymes. New Delhi Metallo-ß-lactamase (NDM), Verona integron-encoded metallo-ß-lactamase (VIM), and imipenemase (IMP) are the significant genes of this carbapenemase. [2] Class D carbapenemases or oxacillin-hydrolyzing ß-lactamases (OXA) carbapenemases are common in *Acinetobacter baumannii*. OXA-23, OXA-24, OXA-51, OXA-55, OXA-58, OXA-48, OXA-50, OXA-60, and OXA-62 are the major subgroups.[1]

Carbapenem-resistant isolates also carry various other resistance determinants in many instances limiting the options for their treatment. Not only this, the mortality rate is higher in case of infections by these pathogens in comparison to carbapenem susceptible isolates.[3] Excess use of carbapenem for the treatment of ESBL

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producers is the cause of carbapenem resistance and this resistance mechanism is more severe in *Klebsiella* with more than 50 % prevalence in South East Asia.[4]

Prevalence of carbapenemase-producing gram-negative isolates is high in Nepal but there are very few studies about these pathogens. As a result various characteristics of these isolates are less known in our country. Hence, this study was carried out to sum up the information about these isolates.

METHODS

A systematic literature search was done for various carbapenemase including class B (NDM, VIM, IMP, SPM, GIM, SIM), class A (KPC, SME, IMI, GES, NMC), and class D (OXA) carbapenemase genes and enzymes in Nepal from various electronic databases (Medline via PubMed, Embase, NepJOL and other databases) published till January 2019. Original research articles, as well as review articles available in English indicating any of class A, B and D carbapenemase detection using both phenotypic and molecular methods from Nepal, were included in this study (Figure 1). Various keywords used for literature search in the abstract and title of the articles were: NDM gene/enzyme, VIM gene/enzyme, IMP gene/enzyme, SPM gene/enzyme, GIM gene/enzyme, SIM gene/enzyme, KPC gene/enzyme, SME gene/enzyme, IMI gene/enzyme, GES gene/enzyme, NMC gene/enzyme, OXA gene/enzyme, Nepal, class A carbapenemase, class B carbapenemase, class D carbapenemase, multi-drug resistant, gram-negative isolates, prevalence.

Research articles containing following information in text and/ or abstract were selected in the study; i) reported various carbapenemase producing gram-negative bacterial pathogens that were isolated in Nepal, ii) used standard phenotypic and molecular techniques for the detection of various carbapenemase producers, iii) included various properties of carbapenemase producers like duration of study, isolate producing carbapenemase, variants of the carbapenemases, site of study, prevalence and antibiotic susceptibility profile. Similarly, the exclusion criteria were; i) not used the standard phenotypic (carbapenemase inhibition methods using EDTA, boronic acid derivatives, and dipicolinic acid) and molecular method (PCR and/ gene sequencing) for carbapenemase detection, ii) not included various characteristics of carbapenemase producers, iii) duplicate articles of one study, iv) meta-analysis, v) articles on languages other than English, and vi) articles containing abstract only.

DATA EXTRACTION

Variables extracted from the selected studies were: carbapenemase positive isolate, source specimen, study period, co-existence of other resistant determinants, variants of class A, B and D carbapenemase, the prevalence of carbapenemase producers, study site and antibiotic susceptibility profile. Selected articles were independently reviewed by four reviewers fairly and consensus was made to solve any inconsistencies among reviewers.

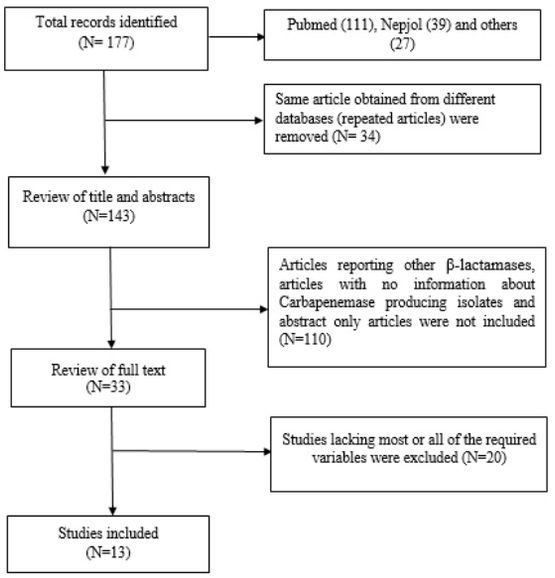

Figure1. selection process of the included studies.

RESULTS

Various carbapenemase producing Gram-negative pathogens have been reported in Nepal including class A, B, and D carbapenemase using both molecular and phenotypic methods. Major carbapenemase reported were NDM, OXA, KPC, VIM, and IMP. Significant carbapenemase producers reported as per the articles reviewed were *E. coli, Klebsiella* spp, *Acinetobacter baumannii, Pseudomonas aeruginosa,* and *Providencia rettegeri*. Almost all carbapenemase bearing isolates were positive for many other resistance determinants and all were multidrug resistant in nature.

CLASS A CARBAPENEMASE

Only *Klebsiella pneumoniae* carbapenemase (KPC) had been reported in Nepal among *E. coli, K. pneumoniae,* and *A. baumannii* isolates with the prevalence range of 0.8 to 8 percentages. All studies detected this resistance mechanism using a phenotypic method using imipenem and imipenem/phenylboronic acid. Most of these isolates were also co-producing metallo beta-lactamase enzyme increasing their drug resistance pattern. Other subclasses of this carbapenemase like SME, IMI, GES, NMC, etc. have not been detected yet and there is imminent need of screening for these carbapenemases.

Table 1. Characteristics of Class A Carbapenemase producing Gram-negative isolates.

Subclass	Isolates	Study period	Prevalence among total GNB studied (%)	Other resistance genes/factors	Study site*	Ref.
KPC	*Enterobacteriaceae*	2013-14	1.3 (4/310)	MBL	KCH	5
KPC	*E. coli, K. pneumoniae, A. baumannii*	2013-14	0.8 (6/769)	MBL	KMH	6
KPC	*Klebsiella Acinetobacter*	2014-15	4.2 (7/165)	NA	TUTH	7
KPC	*Acinetobacter*	2018	8 (9/111)	MBL	MTH	8
SME, IMI, GES, NMC	NR	NR	NR	NR	NR	NR

*KCH-Kanti Children Hospital, KMH- Kathmandu Model Hospital, TUTH-Tribhuvan University Teaching Hospital, MTH-Manipal Teaching Hospital, MBL- Metallo beta-lactamase enzyme, NA-Not available, NR-Not reported

CLASS B CARBAPENEMASE

Four subclasses of this carbapenemase have been reported in Nepal including NDM, VIM, IPM, and DIM. NDM was the most common carbapenemase enzyme reported with seven variants. While only two variant of VIM have been reported. The highest prevalence of VIM, NDM, and IMP were 18.3%, 6.5%, and 6.1% respectively. Many human pathogens including *E. coli, Klebsiella, Acinetobacter, Pseudomonas, Enterobacter, Citrobacter, Proteus,* and *Providencia* spp. were positive for various class B carbapenemase genes. These isolates were highly drug-resistant as most of these isolates were resistant to imipenem, meropenem, aztreonam, ceftazidime, amikacin, arbekacin, ciprofloxacin while most of them were sensitive only to colistin.[9,11] Subclasses like SPM, GIM, SIM were not prevalent among gram-negative pathogens of Nepal.

Table 2.Characteristics of Class B Carbapenemase producing Gram-negative isolates.

Sub class	Variants	isolates	Study period	Prevalence (%)	Source specimen	Other resistance genes	Study site*	Ref.
NDM	NA	*E. coli, Klebsiella, Acinetobacter, Pseudomonas, Enterobacter, Citrobacter, Proteus, Providencia*	2015-16	6.5	Urine, pus, sputum blood	NA	MTH	9
NDM	NDM-1	*P. rettgeri*	2012	NA	Pus Sputum	*bla* $_{OXA-10,}$ *aadA1*, *bla* $_{VEB-1}$, *bla* $_{TEM-1}$, *bla* $_{ADC-67}$, *armA*	TUTH	10
NDM and VIM	NDM-1 VIM-2	*P. aeruginosa*	2012-13	NA		RmtB4, PDC-35	TUTH	11
NDM IPM, VIM	NDM-1	*E. coli*	2012-13	2.15, 2.15, 3.22	NA	bla_{TEM}, bla_{SHV}, bla_{CTX-M}	KMC	12
NDM	NDM-1, NDM-3, NDM-4, NDM-7, NDM-8, NDM-12, and NDM-13	*E.coli Klebsiella Acinetobacter*	2012-15	NA	Urine, pus, sputum blood	bla OXA-23, blaOXA-69, aacC1, aadA1, aadA5armA, blaOXA-32, blaOXA-420, blaPSE-1, aacA2blaOXA-104, blaPER-7, RmtC, blaOXA-94, blaCTX-M-15, blaOXA-181, blaTEM-166, rmtB, aadA2, RmtF, aphA6, OXA-72, SHV-158, TEM-1, SHV-28, SHV-11, SHV-1,	TUTH PH	13
DIM	DIM-1	*P. aeruginosa*	2012-13	NA	NA	PDC-32, RmtF2	TUTH	11
VIM	VIM-2	*P. aeruginosa*	2015-16	18.3	Pus, Urine, Sputum		ANIAS	14
IMP	IMP-1	*P. aeruginosa*	2015-16	6.1	Pus Urine Sputum		ANIAS	14
SPM, GIM,SIM	NR	NR	NR	NR	NR		NR	NR

CLASS D CARBAPENEMASE

Twelve variants of OXA genes have been reported among *E. coli, Providencia rettegeri, A. baumannii,* and *K. pneumoniae*. These isolates also possessed various other resistance factors like beta-lactamase, carbapenemase and methyl transferase. *P. rettegeri*, an OXA-48 positive isolate from Tribhuvan University Teaching Hospital was resistant to all drugs tested (pan-drug resistant).

Table 3. Characteristics of Class D Carbapenemase producing Gram-negative isolates.

Subclass	Variants	Isolates	Study period	Prevalence	Source specimen	Other resistance genes	Study site*	Ref.
OXA	OXA-181	*E.coli*	2013-14	0.4	NA	NDM-5,CTX-M-15	TUTH	15
OXA	OXA-23 OXA-58	*A. baumannii*	2013-14	NA	NA	NDM-1, *armA*	TUTH	16
OXA	OXA-48	*Providencia rettegeri*	2015	NA	Urine	NA	TUTH	17
OXA	OXA-23, OXA-51 OXA-10 OXA-72 OXA-69 OXA-32 OXA-420 OXA-104 OXA-94	*Providencia rettegeri, Acinetobacter K. pneumoniae*	2012-15	NA	Pus, sputum, urine	aacC1, aadA1, aadA5,armA, blaPSE-1, aacA2, blaPER-7, RmtC, blaCTX-M-15, blaTEM-166, rmtB, aadA2, RmtF,aphA6, SHV-158, TEM-1, SHV-28, SHV-11, SHV-1, NDM-1, NDM-3, NDM-4, NDM-7, NDM-8, NDM-12, and NDM-13	TUTH	13

DISCUSSION

Based on the articles reviewed, all three types of carbapenemase-producing pathogens have been detected in Nepal since the beginning of this decade. Carbapenemase positive isolates were reported from various specimens including urine, pus, sputum, and blood. Almost all significant gram-negative pathogens were capable of producing these resistance factors. Most common isolates producing carbapenemase were *E. coli, K. pneumoniae, A. baumannii, P. aeruginosa, P. rettegeri* with a prevalence range of 0.4 to 18.3%. Most of the carbapenem producing isolates also coproduced a wide variety of other drug-resistance genes including metallo beta-lactamases, extended spectrum beta-lactamase, acetyltransferase, methyltransferase, aminoglycoside phosphotransferase, streptomycin adenyltransferase, beta-lactamase, etc. Due to the coproduction of these resistance determinants almost all carbapenemase bearing isolates were highly drug-resistant.

Among various subclasses of class A carbapenemase, only the KPC has been reported. KPC was not limited only in *K. pneumoniae* but also reported in *E. coli* and *A. baumannii*. Other studies also reported KPC from different pathogens including *Citrobacter, Salmonella, Enterobacter, E. coli, Proteus, Serratia, Pseudomonas, Acinetobacter,* and *K. oxytoca*,[18] *Morganella morganii*[19], *Providencia stuartii*.[20] and *Enterococcus spp*. and *Shigella spp*.[21] The data also showed that the prevalence of KPC among gram-negative pathogens is increasing continuously as the incidence was 0.8% in 2013 while it was 8% in 2018. This increase of KPC producers is a matter of concern as there is no regular screening of such pathogens in clinical settings of Nepal and may cause epidemic outbreaks if not monitored properly. These isolates also co-produced metallo beta-lactamase enzymes leading to increased drug resistance. Co-production of other drug resistance genes is common among these isolates.[18] Likewise, KPC producers were found to co-harbor $bla_{CTX\text{-}M\text{-}15}$, $bla_{TEM\text{-}1}$ and $bla_{SHV\text{-}11}$[22], NDM-1[23], *mcr-1*[24,25], and VIM[26,27]. Most studies of these isolates were focused only at Kathmandu valley and very less in other parts of the nation hence there is imminent need of their study throughout the country. Similarly, study of subclasses like SME, IMI, GES, NMC is not done in Nepal and this may lead to unnoticed dissemination of pathogens producing such genes.

NDM, VIM, IPM, and DIM were the reported subclasses of Class B Carbapenemase in Nepal. Among them, NDM was the most predominant one with respect to both cases reported and the number of variants. Almost all major gram-negative pathogens were positive for NDM gene which may facilitate its spread to other human pathogens as most of the isolates bear NDM gene in plasmids. Wide dissemination of NDM gene among

variety of human pathogens via plasmids is also reported earlier.[28,29] VIM, IPM, and DIM genes were also detected in Nepal but less frequently in comparison of NDM. Most of the class B carbapenemase producers also produced a wide variety of other resistance determinants conferring resistance to nearly all commonly used antibiotics. Many other antibiotic resistant factors co-exist with class B carbapenemase bearing isolates to make them resistant to wider range of antibiotics [30-37] and some of these isolates were also pan-drug resistant.[30] Only very few treatment options were there for these isolates. Extreme drug resistance among these isolates with very limited treatment options has been reported already.[38-40] Tigecycline and colistin,[41] fosfomycin[42] are the available treatment options for class B and other carbapenemases. However, there was the report of colistin and fosfomycin resistance among NDM bearing *P. rettgeri* isolated in Kathmandu in 2012.[10] Extreme drug resistance of these isolates is a serious problem for their proper treatment. In addition to this these isolates have been associated with nosocomial outbreaks further complexing their proper management.[43-46]

Many variants of oxacillinases have been reported after 2013. This carbapenemase is not limited only in *Acinetobacter* as there are the reports of oxacillinase detection among *Providencia rettegeri, K. pneumoniae,* and *E.coli*. This fact indicates that these pathogens are widespread among many members of Enterobacteriaceae in our nation. These isolates are widespread in *P. aeruginosa* and *A. baumannii* while less frequent in members of Enterobacteriaceae.[47] They have also been reported from *Acinetobacter*, *P. aeruginosa*, *Shewanella*, *Ralstonia pickettii, Pandoraea pnomenusa* and members of Enterobacteriaceae.[48] OXA positive isolates were also found to be carrying many other genes conferring resistance to other drugs including NDM, CTX-M-15, armA and others. Various carbapenemase genes frequently associated with OXA genes are NDM [49], NDM-1 and KPC.[50] Pan-drug resistance was also reported on one isolate carrying OXA-48 indicating no treatment option for such isolates. According to the study of Tavares et al the majority of OXA positive isolates were extremely drug-resistant indicating their highly elevated drug resistance.[51]

CONCLUSIONS

All three classes of carbapenemase have been detected in Nepal and those isolates were highly drug-resistant. Timely surveillance of these pathogens in clinical settings, detail molecular analysis and proper use of antibiotics is urgent.

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