Prevalence of carbapenem resistant bacterial strains isolated from different clinical samples: study from a tertiary care hospital in Kathmandu, Nepal.

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Prevalence of carbapenem resistant bacterial strains isolated from different clinical samples: study from a tertiary care hospital in Kathmandu, Nepal.

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ABSTRACT

Background
Carbapenems are considered as drugs of choice for the treatment of the infections caused by drug resistant bacteria. However, in the recent years the prevalence of carbapenem resistant gram negative bacteria has increased significantly. The main objective of this study was to determine the prevalence of carbapenemase producing gram negative bacteria among all the clinical isolates.

Material and methods
A total of 3246 non-repeated, different clinical specimens from patients attending Kathmandu Model Hospital, from July 2013 to January 2014 were cultured and the gram negative bacterial isolates obtained were subjected to identification with the help of colony morphology, Gram’s stain and conventional biochemical tests. Kirby-Bauer disk diffusion technique was used to perform antimicrobial susceptibility testing. Phenotypic confirmation of carbapenemase and AmpC beta-lactamase production was done by combined disc method.

Results
890 samples showed the growth of bacterial pathogens. Out of total 769 gram negative bacteria, 57 were found to be carbapenem resistant. Of which, highest number (47) of the isolates were found to be metallo-β-lactamase (MBL) producers. Six bacterial isolates produced both (Klebsiella pneumoniae carbapenemase) KPC and MBL, whereas only one isolate was found to be positive for both MBL and AmpC. Three bacterial strains showed carbapenem resistance due to over production of AmpC β-lactamase.

Conclusion
Among carbapenem resistant gram negative bacteria, MBL was present as the major enzyme responsible for resisting carbapenem antibiotics.

Keywords
Multidrug resistance, carbapenemase, metallo-β-lactamase, Klebsiella pneumoniae carbapenemase, AmpC-β-lactamase, Nepal
**Introduction:**

The gram-negative bacteria are the leading causes of serious infections in human. *Escherichia coli* and *Klebsiella* spp. (among the members of Enterobacteriaceae family), and *Pseudomonas* spp. and *Acinetobacter* spp. (among the non-fermenting gram negative bacilli) are the major bacterial pathogens causing infections in human [1].

Antibiotics play important role in the treatment and control of bacterial infections. Due to high efficacy and good safety profile of β-lactam antibiotics, their global uses account for greater than 60% of all antibiotics used. Further, β-lactam antibiotics can easily be subjected to chemical manipulation to extend or restore their activities [2, 3]. Among the β-lactam antibiotics, carbapenems are the antibiotics with very broad spectrum of activity [4]. They are stable to most of the β-lactamases, including extended spectrum beta lactamase (ESBL) and AmpC β-lactamase [5]. So, the carbapenem antibiotics are considered as the drugs of choice for the treatment of the infections caused by multidrug resistant (MDR) bacteria [6].

Antibiotic resistance is not the recent problem. It has been known since the discovery of antibiotics [7]. But recently, drug resistance among the bacteria is present as more serious public health problem. The irrational uses of the antibiotics have driven the evolution of antibiotic resistance, leading to increased morbidity and mortality along with increased health care cost and longer period of hospitalization [8, 9].

In terms of the β-lactam antibiotics, bacteria are mainly resistant by producing enzymes, such as extended spectrum beta lactamase (ESBL), AmpC-beta-lactamase and carbapenem hydrolyzing enzymes (carbapenemases) [10]. Carbapenemase is considered as a versatile group of β-lactam with very broad spectrum of activities. Many of the carbapenemases are able to hydrolyze the wide spectrum of β-lactam antibiotics, which include penicillins, cephalosporins, carbapenems, monobactams and can also show resistance to commercially available β-lactamase inhibitors such as clavulanic acid, sulbactam, and tazobactam [3, 11]. So, carbapenamase producing bacteria resist majority of the antibiotics, leaving only limited treatment options for infections caused by them [12].

Although in the recent years, increased rates of carbapenem resistance have been reported from all around the world, in Nepal, limited data are available among the clinical isolates. In this study, we determined the prevalence of carbapenemase and AmpC beta-lactamase producing gram-negative bacteria in causing human infections.

**Material and methods**

**Study design**

A total of 3246 non-repeated, different clinical specimens (urine, sputum, blood, sterile body fluids, pus/pus swab, catheter tip) from patients attending Kathmandu Model Hospital, from July 2013 to January 2014 were used in our study. The samples were collected in sterile, screw capped and leak proof plastic containers.

**Isolation and identification of the bacterial isolates**

The clinical samples were cultured following standard microbiological techniques [13] and the gram negative bacterial isolates obtained were subjected to identification with the help of colony morphology, Gram’s stain and conventional biochemical tests [14].

**Performance of antimicrobial susceptibility testing**

Kirby-Bauer disk diffusion technique was used to perform antimicrobial susceptibility testing [15]. The bacterial isolates showing resistance toward three or more different classes of antibiotics were reported as multidrug resistant (MDR) [9].

**Phenotypic confirmation of carbapenemase and AmpC beta-lactamase production**

The gram-negative bacterial strains, which were found to be resistant to carbapenem antibiotic were subjected to phenotypic confirmation of carbapenemase and AmpC beta-lactamase production by combined disc method. Confirmation of metallo-beta-lactamase production was performed by using imipenem and imipenem+ ethylenediaminetetraacetic acid (EDTA) discs [16]. Similarly, for the detection of *Klebsiella pneumoniae* carbapenemase production, imipenem and imipenem+ phenyl boronic acid discs were used [17]. Further, for confirmation of AmpC beta-lactamase production cefoxitin and cefoxitin+phenyl boronic acid discs were used [16].

**Quality control**

For quality control of biochemical tests purity plate was used. Similarly, for the standardization of the antimicrobial susceptibility testing and phenotypic confirmation of carbapenemase production, control strains *Escherichia coli* (ATCC 25922) and *Staphylococcus aureus* (ATCC 25923) were used.

**Data analysis**

SPSS version 16.0 was used for statistical analysis. Chi-square test was applied and p<0.05 was taken as statistically significant. The present study was approved by the institutional review board of Kathmandu Model Hospital, Kathmandu, Nepal.

**Results**

Out of total 3246 clinical specimens, 890 (27.41%) samples showed growth of bacterial pathogens and total of 769 gram negative bacterial isolates were isolated from them.

**Multidrug resistance among different gram negative bacteria:**

In our study, among 769 gram negative bacteria isolated from different samples, 42.91% were found to be multidrug resistant.
Among the different bacteria, 268/623 of the Escherichia coli, 21/60 of the Klebsiella pneumoniae and 15/25 of Acinetobacter baumannii were MDR (table 1).

Table - 1 Multidrug resistance among different gram negative bacteria

<table>
<thead>
<tr>
<th>Name of bacteria</th>
<th>Total isolates</th>
<th>Total MDR strains</th>
</tr>
</thead>
<tbody>
<tr>
<td>Escherichia coli</td>
<td>623</td>
<td>268</td>
</tr>
<tr>
<td>Klebsiella pneumoniae</td>
<td>60</td>
<td>21</td>
</tr>
<tr>
<td>Klebsiella oxytoca</td>
<td>5</td>
<td>4</td>
</tr>
<tr>
<td>Acinetobacter baumannii</td>
<td>25</td>
<td>15</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>17</td>
<td>7</td>
</tr>
<tr>
<td>Proteus mirabilis</td>
<td>7</td>
<td>1</td>
</tr>
<tr>
<td>Proteus vulgaris</td>
<td>5</td>
<td>3</td>
</tr>
<tr>
<td>Citrobacter freundii</td>
<td>15</td>
<td>8</td>
</tr>
<tr>
<td>Citrobacter koseri</td>
<td>6</td>
<td>2</td>
</tr>
<tr>
<td>Enterobacter cloacae</td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td>Salmonella Typhi</td>
<td>1</td>
<td>0</td>
</tr>
</tbody>
</table>

MDR-Multidrug resistant

Carbapenemase production among different gram negative bacteria:
Out of total 769 gram negative bacteria, 57 were found to be carbapenem resistant. The most common carbapenamase producing bacteria isolated were Acinetobacter baumannii, Proteus vulgaris, Klebsiella spp., Pseudomonas aeruginosa (table 2).

Table - 2 Carbapenemase production among different microorganisms

<table>
<thead>
<tr>
<th>Organisms</th>
<th>Total. No.</th>
<th>MBL +</th>
<th>MBL + KPC</th>
<th>MBL + AmpC</th>
<th>AmpC</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Escherichia coli</td>
<td>623</td>
<td>23</td>
<td>3.69</td>
<td>1.01</td>
<td>0.16</td>
<td>0.16</td>
</tr>
<tr>
<td>Klebsiella pneumoniae</td>
<td>60</td>
<td>8</td>
<td>13.33</td>
<td>1.64</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>Klebsiella oxytoca</td>
<td>5</td>
<td>1</td>
<td>20</td>
<td>0.40</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Acinetobacter baumannii</td>
<td>25</td>
<td>9</td>
<td>36</td>
<td>1.40</td>
<td>3</td>
<td>1.40</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>17</td>
<td>3</td>
<td>17.64</td>
<td>0.40</td>
<td>3</td>
<td>17.64</td>
</tr>
<tr>
<td>Proteus vulgaris</td>
<td>5</td>
<td>1</td>
<td>20</td>
<td>0.40</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Citrobacter freundii</td>
<td>16</td>
<td>2</td>
<td>12.5</td>
<td>0.40</td>
<td>2</td>
<td>12.5</td>
</tr>
</tbody>
</table>

MBL-Metallo-β-lactamase, KPC- Klebsiella pneumoniae carbapenemase, AmpC- AmpC β-lactamase

Discussion
In our study, among 769 gram negative bacteria isolated from different samples, 42.91% were found to be multidrug resistant. Similar findings were also reported by Awasthi et al. (42.86%) [9], Pokhrel et al. (47%) [18] and Mishra et al. (53.7%) [19]. In developing countries like Nepal, due to easy availability of the antibiotics there is increased haphazard use of antibiotics and self medication is a common practice [20]. Use of expired antibiotics, counterfeit drugs and inadequate infection control measures in hospitals can further promote the development of antibiotic resistance among bacteria [20].

In present study, the prevalence of carbapenem resistance among the bacteria belonging to enterobacteriaceae ranged from 4.49% to 20%. In a similar study done by Datta et al. from India, the prevalence of carbapenem resistant bacteria belonging to enterobacteriaceae family was 7.87% and the rate of MBL type carbapenemase production was 5.75% [12]. Accordingly, in a study by Wattal et al. the prevalence of carbapenem resistance in E. coli and Klebsiella spp. isolated from ICUs and wards of a tertiary care hospital in Delhi, ranged from 13% to 51% [21]. In a study conducted in Nepal, 18.98% of the E. coli and 21.08% of the Klebsiella pneumoniae were found to be carbapenemase producers [22]. Similarly, Gupta et al. reported the prevalence of carbapenem resistance among enterobacteriaceae to be varying from 17% to 22% [23]. Quite high rates of AmpC β-lactamase (37.2%) and MBL (36.7%) production (among gram negative bacteria) in comparison to our study were reported by Khanal et al. [24] but as in our study highest rate of MBL production was reported to be shown by Acinetobacter spp. [24].

The carbapenemase enzymes are both the chromosomal and plasmid encoded. Among the three carbapenemases, molecular class A and D include serine at their active sites whereas molecular class B contains zinc at its active site [11, 25, 26]. In molecular class-A carbapenemases, KPC is thought to be most significant in terms of clinical point of view. It is mostly confined to the Klebsiella pneumoniae. However, recently it has also been reported in other members of the enterobacteriaceae family, Pseudomonas spp. and Acinetobacter spp. [27, 28]. In our study also, KPC was found to be produced by other members of the enterobacteriaceae family and Acinetobacter spp. The bacteria producing KPC can exhibit resistance to all β-lactam agents including penicillins, cephalosporins, aztreonam, cephapymcins, carbapenems along with β-lactam/β-lactamase inhibitor combinations [27, 28]. Molecular class B carbapenemases, also called metallo-β-lactamases (MBLs) are the enzymes those require a metal ion (Zn2+) for hydrolysis of β-lactam antibiotics [6, 27, 29]. Among the MBLs, VIM and IMP are most important and mostly found in Pseudomonas spp. They are also found to be disseminated among the members of enterobacteriaceae family and Acinetobacter spp. Most MBL hydrolyze all β-lactam antibiotics except azethronam [5]. Further, they are frequently associated with integrons which contain determinants for multiple antibiotic resistance. Integrons can easily transfer from one bacterium to another but to express MBL genes the bacteria should get antibiotic pressure (mainly due to carbapenem therapy). Similar to the species-specific MBLs, the MBLs encoded by plasmids mostly appear in organisms those produce at least one more β-lactamase with same hydrolysis profile [7]. Molecular class D carbapenemases include OXA (oxacillin-hydrolyzing) enzymes. Because of slow hydrolysis rate, OXA
require additional resistance mechanism such as efflux pump and porins etc. to exhibit full resistance to carbapenem antibiotics [30]. Similar to ESBL-producing strains with decreased permeability, OXA-48 producing strains can show low-level of carbapenem resistance. There are no any phenotypic tests capable of detecting OXA-48 [31]. So we could not differentiate the strains expressing OXA-48 type carbapenemases from the strains showing resistance to carbapenems due to the production of the ESBL associated with decreased permeability.

Although carbapenem antibiotics are effective against AmpC beta-lactamase producing bacteria, over production of AmpC results in development of resistance toward carbapenem antibiotics. This is due to alteration or complete resistance of outer membrane porin, which has been mainly reported in E. coli and Klebsiella pneumoniae [10].

As the molecular methods for detection of carbapenemase production are used mainly in research settings, the simpler and cheap methods as we have used will be appropriate for the routine detection of the resistant pathogens. It will help in improving patient’s outcome and facilitating effective infection control thus reducing the escalation of the drug resistance [32].

**Conclusion**

The constantly increasing carbapenem resistant gram negative bacteria, seem to create the major problem (for public health) as carbapenem resistant gram negative bacteria resist majority of commonly used antibiotics there by greatly limiting the treatment options. Among carbapenem resistant gram negative bacteria, MBL was found as major enzyme responsible for resisting carbapenem antibiotics.

**Limitations of the study:**

This research was conducted in a resource limited low income country where the availability of the molecular technology is not easy. So, inability to use the molecular methods for the confirmation of our findings is the main drawback of our study.

**Competing interests**

The authors declare that there is no conflict of interest regarding the publication of this paper.

**Authors’ contribution**

NDP designed and conceived the study, performed the laboratory work, analyzed the data and prepared the final manuscript. SK and SN designed and conceived the study, performed the laboratory work and analyzed the data. SK helped in designing of the study and analysis of the data. SB and BS monitored the study. Final manuscript was approved by all authors.

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**Abbreviations**

- American type culture collection (ATCC), Ethylenediaminetetraacetic acid (EDTA), Extended spectrum beta-lactamase (ESBL), Klebsiella pneumoniae carbapenemase (KPC), Metallo-ß-lactamase (MBL), Multidrug resistant (MDR)

**References**


