bla$_{NDM-1}$ possessing *Escherichia coli* and *Klebsiella pneumoniae* isolates exhibiting multidrug-resistant and pandrug-resistant phenotypes in Northeast India

Arijit Bora[1], Giasuddin Ahmed[2], Naba Kumar Hazarika[3]

[1]Department of Applied Sciences, GUIST, Gauhati University, Assam, India, [2]Department of Biotechnology, Gauhati University, Guwahati, Assam, India. [2]Department of Microbiology, Gauhati Medical College and Hospital, Guwahati, Assam, India.

INTRODUCTION

- Increasing reports on New Delhi metallo-β-lactamase-1 (NDM-1) producing *Enterobacteriaceae*, particularly *Escherichia coli* and *Klebsiella pneumoniae* constitute a serious threat to global health [1].
- NDM-1 is a novel type of metallo-β-lactamase that hydrolyzes all the β-lactam antibiotics except aztreonam, which is usually inactivated by the coproduction of the extended-spectrum or the AmpC β-lactamases.
- The gene encoding NDM-1 is known as *bla*$_{NDM-1}$, which is located on a transmissible plasmid and its association with other resistant determinants leads to the extensive drug resistance [1].
- However, the data on the prevalence of NDM-1 producing *Enterobacteriaceae* in Indian hospitals is limited due to constrained resources. Therefore, the present study was designed evaluate the incidence of *bla*$_{NDM-1}$ gene in *E. coli* and *K. pneumoniae* isolates at a tertiary care referral hospital in Northeast India.

METHODS

- A total of 412 consecutive, non-duplicate isolates of *E. coli* (n = 221) and *K. pneumoniae* (n = 191) were recovered from various clinical samples at a tertiary care referral hospital in northeast India. The samples were obtained from both hospitalized and non-hospitalized patients between August 2011 and January 2012.
- Kirby-Bauer disc diffusion method was performed to determine the susceptibilities of different β-lactam and non-β-lactam antibiotics [2].
- All the isolates with reduced susceptibility to meropenem or ertapenem (diameter of zones of inhibition, ≤ 21mm) were screened for production of carbapenemase [2].
- Minimum inhibitory concentration (MIC) values for imipenem, meropenem, ertapenem, tigecycline and colistin were determined by using Etest strips.
- Metallo-β-lactamase (MBL) production was detected by performing combined disc test by using imipenem discs with and without ethylenediaminetetraacetic acid (EDTA), which chelates zinc required for MBL activity [3].
- *bla*$_{NDM-1}$, *bla*$_{TEM}$, *bla*$_{SHV}$, *bla*$_{CTX-M}$ and *bla*$_{AmpC}$ genes in the screened isolates were detected by employing monoplex PCRs [4,5].

RESULTS

- On the basis of their reduced susceptibility to meropenem or ertapenem, 55 (24.88%) *E. coli* and 52 (27.22%) *K. pneumoniae* were screened for detection of *bla*$_{NDM-1}$ by PCR.
- All the screened isolates were found to be positive for *bla*$_{NDM-1}$ as well as showed positive results in combined disc test for MBL production.
- Each of the *bla*$_{NDM-1}$ possessing isolates of *E. coli* and *K. pneumoniae* was also found to be positive for two or more additional *bla* genes, such as *bla*$_{TEM}$, *bla*$_{SHV}$, *bla*$_{CTX-M}$ and *bla*$_{AmpC}$.
- All the *bla*$_{NDM-1}$ possessing isolates were multidrug-resistant (MDR) as well as 56.36% *E. coli* and 63.46% of *K. pneumoniae* isolates with *bla*$_{NDM-1}$ were "pandrug- resistant".
- Each of the *bla*$_{NDM-1}$ possessing isolate of *E. coli* and *K. pneumoniae* was found to be sensitive to tigecycline and colistin.
- However, 9.1% of *E. coli* and 11.5% of *K. pneumoniae* with *bla*$_{NDM-1}$ showed reduced susceptibility to tigecycline as well as 5.4% of *E. coli* and 7.7% of *K. pneumoniae* with *bla*$_{NDM-1}$ showed reduced susceptibility to colistin.

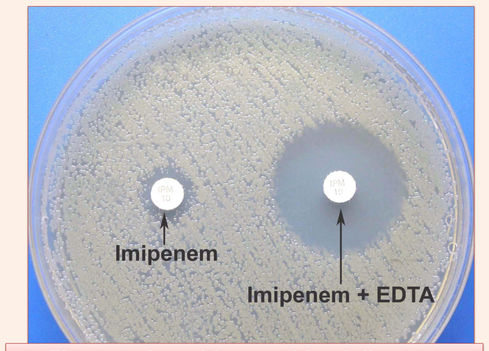

Figure 1: A positive combined disc test

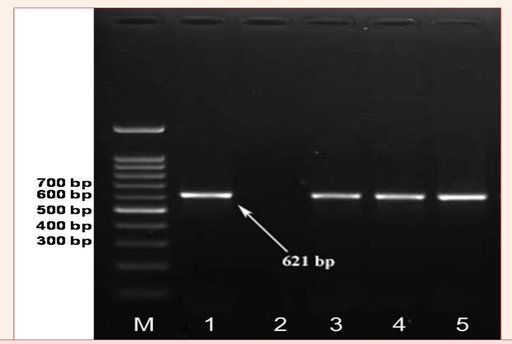

Figure 2: PCR amplified product for *bla*$_{NDM-1}$ gene

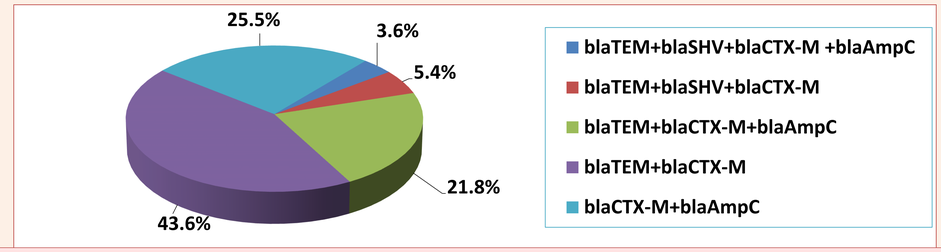

Figure 3: Coexistence of *bla*$_{TEM}$, *bla*$_{SHV}$, *bla*$_{CTX-M}$ and *bla*$_{AmpC}$ in *bla*$_{NDM-1}$ positive isolates of *E. coli*

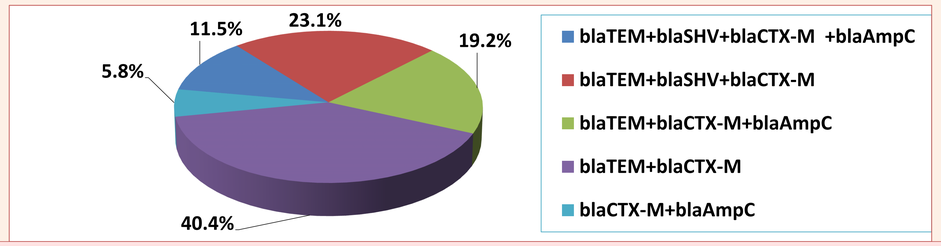

Figure 4: Coexistence of *bla*$_{TEM}$, *bla*$_{SHV}$, *bla*$_{CTX-M}$ and *bla*$_{AmpC}$ in *bla*$_{NDM-1}$ positive isolates of *K. pneumoniae*

bla$_{NDM-1}$ possessing isolate	MIC range (µg/ml)				
	Imipenem	Meropenem	Ertapenem	Tigecycline	Colistin
E. coli	2.0–8.0	3.0–16	8.0–>32	0.125–0.75	0.125–0.5
K. pneumoniae	2.0– >32	2.0– >32	6.0– >32	0.38–2.0	0.125–0.5

Table 1: MIC ranges found for *bla*$_{NDM-1}$ possessing isolates of *E. coli* and *K. pneumoniae*

CONCLUSIONS

- The observed high level resistance to the different β-lactam antibiotics, including aztreonam might be contributed by the coexistence of additional *bla* genes in the *bla*$_{NDM-1}$ possessing isolates.
- Our findings showed that all the *bla*$_{NDM-1}$ possessing isolates were MDR as well as a considerable number of *E. coli* and *K. pneumoniae* isolates possessing *bla*$_{NDM-1}$ were exhibiting pandrug-resistant phenotypes.
- In addition, few of the *bla*$_{NDM-1}$ positive isolates showed reduced susceptibility to tigecycline and colistin, which extremely limits the therapeutic options for infections cause by NDM-1-positive isolates.
- Perceptive of the antibiotic resistance genes in important bacterial pathogens from a geographical area is of paramount importance for surveillance and control of antibiotic resistance.

BIBLIOGRAPHY

1. Berrazeg M, Diene S, Medjahed L, Parola P, Drissi M, Raoult D and Rolain J. New Delhi Metallo-beta-lactamase around the world: an eReview using Google Maps. EuroSurveill. 2014;19(20).
2. Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing, 21st informational supplement (M100-S21). Wayne (PA): the Institute; 2011.
3. Franklin C, Liolios L, Peleg AY. Phenotypic detection of carbapenem susceptible metallo beta-lactamase-producing gram-negative bacilli in the clinical laboratory. J Clin Microbiol. 2006;44(9):3139-44.
4. Nordmann P, Poirel L, Carrer A, Toleman MA, Walsh TR. How to detect NDM-1 producers. J Clin Microbiol 2011;49:718-21.
5. Shahid M. Citrobacter spp. simultaneously harboring *bla*$_{CTX-M}$, *bla*$_{TEM}$, *bla*$_{SHV}$ *bla*$_{ampC}$, and insertion sequences IS26 and orf513: an evolutionary phenomenon of recent concern for antibiotic resistance. J Clin Microbiol. 2010;48(5):1833-8.

Acknowledgement: The first author acknowledges UGC, India for providing UGC-RFSMS fellowship and DST, Govt. of India for providing visiting fellowship for N.E. Research Student.