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RESEARCH ARTICLE

DETECTION OF BLA NDM-1 GENE ENCODING METALLO BETA LACTAMASE AMONG URINARY ISOLATES OF KLEBSIELLA SPECIES ISOLATED FROM TERTIARY CARE HOSPITAL IN KANCHEEPURAM DISTRICT

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ABSTRACT

Topic: Presence of bla NMD 1 gene in urinary isolates of klebsiella species
Aim : To detect the presence of bla NDM 1 gene for the production of MBL in urinary isolates of klebsiella species in tertiary care hospital.
Objective: A sum total of 20 will be used to detect the presence of bla NDM 1 gene for the production of MBL in urinary isolates of klebsiella species in tertiary care hospital.
Background: Recently the B BETA-lactamase NDM 1has become a source of serious concern. Initially isolated from klebsialla pneumonia and Eschirichia coli isolates recovered in Sweden from a patient who was initially admitted in India, NDM 1 producers have subsequently been identified in various other countries in UK and Pakistan and indentified in K pneumonia, Citrobacterfreundii, enterobacter cloacae.
Reason: To study the presence boa NDM 1 gene in our isolates for antibiotic resistance and for better treatment procedures.

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INTRODUCTION

Klebsiella pneumoniae is one of the most frequently encountered pathogen of Enterobacteriaceae family responsible for various nosocomial infections, especially in intensive care units (ICU) and in neonates. (Podschun and Ullmann, 1998) Carbapenems are the beta-lactam antibiotics which bind to the bacterial penicillin-binding proteins which results in the elongation and cross linking of peptidoglycan of the bacterial cell wall leads to impaired cell wall synthesis and cell death. Incidence of multi drug resistance in organisms is increasing due to spread of resistance determinant genes mediated by transposons, plasmids and gene cassettes in integrons. However, due to the presence of extended-spectrum beta-lactamase and AmpC enzymes in these Gram-negative bacilli, carbapenems have become the drug of choice to treat such infections. Carbapenems, most commonly meropenem and imipenem (IP), have been considered as most promising beta-lactams against multi drug resistant Gram-negative bacteria. However, the increased use of carbapenems has led to the emergence of resistant strains and outbreaks due to these are mostly associated with significant morbidity and mortality. Due to production of carbapenemases in clinical isolates of Enterobacteriaceae, the treatment of ICU patients is becoming difficult. Resistance to carbapenems due to carbapenemase production poses serious challenges in the treatment of such infections with resistant strains. (Livermore and Woodford, 2006) Mobile genetic elements are being associated with carbapenemases production. The genetic trait of blaNDM-1 likely facilitates the rapid dissemination of this gene within K. pneumoniae isolates. Spreading of NDM-1 producing K. pneumoniae in a clinical settings is a complex event involving several modes of spread, such as dissemination of several unrelated strains or the propagation of a single clone from patient to patient and from the environment to patients. With this background, our study was undertaken to detect the blaNDM-1 gene in clinical isolates of K. pneumonia.

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MATERIALS AND METHODS

Bacterial isolates

A total of 20 non repetitive urinary isolates of Klebsiella pneumoniae were collected from Saveetha Medical College and Hospitals, Chennai. They were processed for a battery of standard biochemical tests and confirmed. Isolates were preserved in semisolid trypticase soy broth stock and were stored at 4 ºC until further use.

Antibiotic susceptibility testing

Antibiotic sensitivity test was carried out by Kirby Bauer disk diffusion method with routinely used commercially available

antibiotics (HiMedia, Mumbai). These antibiotics include Ampicillin, Amoxycillin, Ceftazidime, Cefotaxime, Amikacin, Gentamicin, Imipenem, Ciprofloxacinas per CLSI 2015 guidelines. (Clinical and Laboratory Standards Institute, 2015)

Detection of blaNDM-1 gene in K. Pneumonia

Klebsiella pneumoniae isolates were detected for the presence of blaNDM-1 gene by PCR analysis. Detection of the gene was carried out using primer as depicted in table 2. Bacterial DNA was extracted by boiling lysis method. 1 µL of DNA extract was used as template for PCR reaction. The reaction mixture contained 1mM of Mgcl20.2mM dNTP mix and 0.8µM of blaNDM-1 gene with 1U of Taq polymerase (New England Biolabs) in a 1x PCR buffered reaction. A positive control of K. pneumoniae with blaNDM-1 gene was also included in this study. PCR amplification was carried out using thermal cycler (Eppendorf) with the following cycling condition. Initial denaturation at 96°C for 3 minutes, 30 cycles of denaturation at 95°C for 1 minute, primer annealing at 54°C for 40 seconds and primer extension at 75°C for 1 minute and final extension at 72°C for 5 minutes were used. PCR products were resolved in 1.5% agarose gel. A 100bp ladder was including in all the gel analysis. (Bora *et al*., 2013)

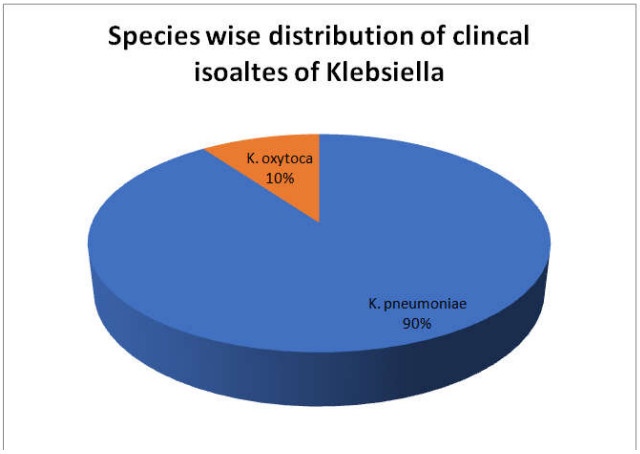
Table 1. Gene sequencing of *bla*$_{NDM-1}$gene

Primer	Primer sequence	Product size
bla$_{NDM-1}$	CACTTCCTATCTCGACATGC GGGCCGTATGAGTGATTG	621 bp

RESULTS

Sample wise distribution of clinical isolates of Klebsiella pneumoniae:

Of the 20 clinical isolates of Klebsiella pneumoniae, 12/20(60%) were from urine, 4/20(20%) from stool, 3/20(15%) and 1/20(5%) were from the wound swab and pus respectively.



Antibiotic susceptibility testing

Increased percentage of isolates were showing resistance to cephalosporins and other group of antibiotic (80-100%). We found very less number of isolates were sensitive to imipenem (20%) which is considered to be a most potential drugs The detailed resistant pattern of Klebsiella isolates were showed in Table 1.

Table 1. Results of antibiotic susceptibility patterns of Klebsiella pneumoniae

Antibiotics	Sensitivity (%)	Intermediate (%)	Resistant (%)
Ampicillin	5	0	95
Amoxicillin	5	0	95
Ceftazidime	5	0	95
Cefotaxime	0	0	100
Amikacin	0	0	100
Gentamicin	15	5	80
Imipenem	20	0	80
Ciprofloxacin	0	0	100

Result of blaNDM-1 gene in K. Pneumonia

2/20 (10%) clinical isolates of K. pneumoniae were found to harborblaNDM-1 gene.

Representative gel picture showing positive for *bla*$_{NDM-1}$ gene

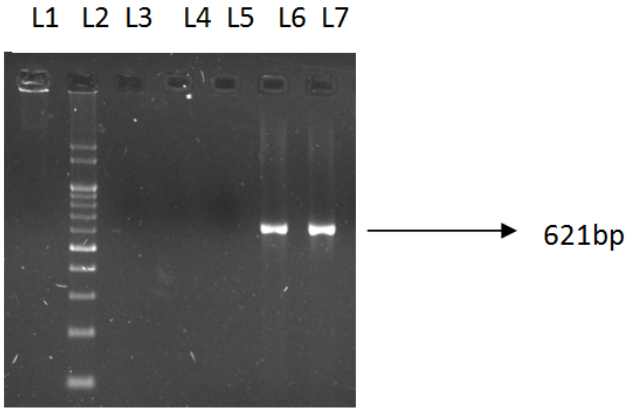


Figure 2. Representative gel picture showing positive for blaNDM-1 gene

L2-100bp ladder; L6,L7-621bp blaNDM-1 gene

DISCUSSION

The result of our study showed that, most of these isolates were resistant to multiple antibiotics tested, however only 30% of isolates were resistant to imipenem. Increased percentage of resistance was observed in cephalosporin group of antibiotics. They were subjected for the presence of blaNDM-1 gene by PCR. It showed only 2/20 (10%) strain was found to harborblaNDM-1. blaNDM-1 is a transferable class B MBL gene. Since its 1st appearance in 2008, it has been identified in different Gram-negative isolates from different parts of the world including UK, Pakistan, Australia and USA, mostly from patients who are epidemiologically linked to the Indian subcontinent. (Nordmann *et al*., 2011) Several reports from India have shown there is 5-8% prevalence of blaNDM-1, a finding that is somewhat similar to our study findings. (Deshpande *et al*., 2011) Since all the NDM-1 possessing isolates exhibited high-level of resistance to a different generation cephalosporins, it is understood that it may have other genes for multiple antibiotic resistance. Study conducted by Bora and coworkers in 2013 adopted PCR detection for some of the important types of ESBL genes as well as AmpC gene. As expected, each of the blaNDM-1 positive isolate harbored two or more additional bla genes. (Bora *et al*., 2013) Of these, blaCTX-M was the most common and found in all

isolates, whereas blaTEM was found in 78.57% (11/14) isolates. Only 21.43% (3/14) of NDM-1 producing isolates was positive for plasmid-mediated blaAmpC. However, in our study we did not detect for these genes. Earlier studies from India (Roy *et al*., 2011) and abroad, (Mulvey *et al*., 2011) also reported the co-existence of different types of ESBL genes (mostly, blaTEM-1 and blaCTX-M-15) along with AmpC genes (mostly, blaCMY) in blaNDM-1 positive E. coli isolates. The presence ESBL and AmpC genes in the blaNDM-1 positive isolates might contribute to the high level of resistance.

Conclusion

Transmission of plasmid conveying these genes to different individuals from Enterobacteriaceae will build the occurrence of multidrug resistance. Early location of these genes will help in counteractive action and satisfactory contamination control by restricting the spread of these creatures. Spreading of NDM-1 creating K. pneumoniae in a clinical settings is an unpredictable occasion including a few methods of spread, for example, scattering of a few random strains or the proliferation of a solitary clone from patient to persistent and from nature to patients. A sum of 20 clinical separates of K. pneumoniae were subjected to anti-toxin weakness design took after by the identification of blaNDM-1 quality by PCR. Our outcomes demonstrates the expanded level of imperviousness to the greater part of the routinely utilized anti-infection agents.10% of our isolates were found to posses this gene among our isolates. Early recognition of these qualities will help in anticipation and sufficient disease control by restricting the spread of these creatures.

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