

New Delhi Metallo- β lactamase (NDM-1) in Enterobacteriaceae: Treatment options with Carbapenems Compromised

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Abstract

Background : Carbapenems are among the few useful antibiotics against multidrug resistant gram negative bacteria particularly those with extended spectrum beta lactamase. However resistance to carbapenems occurs and is mediated by mechanisms like loss of outer membrane proteins and production of beta lactamase that is capable of hydrolyzing carbapenems. An alert issued in the UK in 2009 warned of an increasing number of carbapenem resistant Enterobacteriaceae strains identified in UK hospital patients. Many of them were recently hospitalized in India and Pakistan and had new type of metallo beta lactamase designated as New Delhi Metallo-1 (NDM-1).

Objective : To assess the production of NDM-1 type Metallo beta lactamase enzyme in Enterobacteriaceae at a tertiary care centre in Mumbai.

Materials and Methods : Consecutive carbapenem resistant Enterobacteriaceae isolates were collected from August 2009 to November 2009. Susceptibility testing for carbapenems was performed by the disc diffusion method. Carbapenemase production was confirmed by Modified Hodge test. These strains were then subjected to single target PCR. A 475bp product was amplified by the NDM primers and visualized on 3% agarose gel.

Results and Conclusions : Modified Hodge test was positive for all carbapenem resistant isolates. Of 24 carbapenem resistant Enterobacteriaceae 22 were NDM producers while 2 were NDM non producers. Amongst the 22 NDM producing organisms 10 were Klebsiella spp, 9 were Escherichia coli, 2 were Enterobacter spp and 1 was Morganella morganii. This high number in a relatively short span is a worrisome trend that compromises the treatment options with the carbapenems.

Introduction

Carbapenems are the agents of last resort against many multi drug resistant, gram negative bacteria. Resistance to carbapenems is mediated by mechanisms like loss of outer membrane proteins, and production of carbapenemases that are capable of hydrolyzing the carbapenems. The growing incidence and also the diversity of carbapenemase producing strains is therefore a major concern. Pseudomonas and Acinetobacter baumannii have significant carbapenem resistance. The emergence of carbapenemases in Enterobacteriaceae provides an added risk of dissemination in the community. These enzymes confer resistance to the other beta-lactam agents as well, including extended spectrum cephalosporins. Metallo beta lactamases (MBLs) are one such type of carbapenemase, that are characterized by the ability to hydrolyze carbapenems and are inhibited by EDTA, chelators of Zn²⁺.^{2,3} Most Enterobacteriaceae carrying an MBL gene will appear sensitive, with imipenem MIC's between 1 and 2 $\mu\text{g/ml}$.^{2,4} These organisms colonise the gut. Thus, they can spread via faeco oral route to the community.

An alert issued in the UK in 2009 warned of an increasing number of carbapenem resistant Enterobacteriaceae strains identified in UK hospital patients.¹ Many of them were recently hospitalized in India and Pakistan and had a new type of

metallo beta lactamase designated as NDM-1 (New Delhi Metallo-1). These isolates were clonally diverse. Due to lack of epidemiological data within India, the exact prevalence of NDM-1 enzyme is not known. We studied carbapenem resistant Enterobacteriaceae at our tertiary centre in Mumbai to find if NDM-1 was found among them.

Materials and Methods

Setting : The study design and protocols were approved by the Scientific and Ethical Research Committee of P.D.Hinduja National Hospital and Medical Research Centre, Mumbai, India where this study was conducted.

Consecutive carbapenem resistant Enterobacteriaceae isolates were collected from August 2009 to November 2009.

Antimicrobial susceptibility testing : Organisms were tested by disc diffusion method using Mueller –Hinton agar as described by CLSI (Clinical and Laboratory Standards Institute) guidelines. All antibiotic discs were obtained from Himedia Laboratories P. LTD. E.coli ATCC 25922, Pseudomonas aeruginosa ATCC 27853 and Staphylococcus aureus ATCC 25923 strains were used for quality control.

Phenotypic detection of carbapenemase production : The modified Hodge test was performed on all isolates on Mueller Hinton agar. After overnight incubation the plates were observed for clover leaf-type indentation at the intersection of the test organism and the standard strain, within the zone of inhibition of the carbapenem susceptibility disc.

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Fig. 1 : Photograph shows the results of Modified Hodge test (All the four isolates seen in the figure are positive for the Modified Hodge test, showing the cloverleaf type indentation)

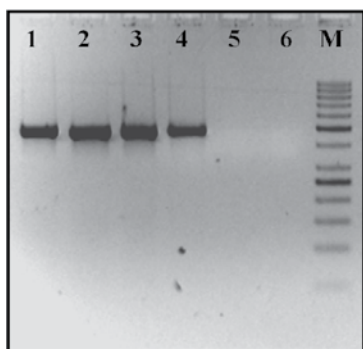


Fig. 2 : Gel picture of NDM PCR results [1,2,3,4 – Represent NDM positives (Group A); 5, 6 – Represent NDM negatives (Group B); M- 50 bp DNA ladder]

Molecular analysis technique : DNA was extracted from all the strains by heat boil method and this DNA was subjected to single target PCR. Amplified products (475 bp) were visualized under UV light on 3% Agarose Gel Electrophoresis.

Results

A total of 24 carbapenem resistant gram negative organisms were collected in a period of 3 months. They were found to be carbapenem resistant by disc diffusion method. All these were positive for carbapenemase production by the Modified Hodge test. Clover leaf type indentation was observed in all these tests (Fig1). NDM PCR was positive for 22 of 24 isolates (Fig 2). There were two isolates that tested positive by Modified Hodge test and were negative by NDM PCR. Both of them were *Klebsiella pneumoniae*.

Amongst the 22 NDM producing organisms 10 were *Klebsiella* spp while 9 were *Escherichia coli* (Table 1). Two strains of *Enterobacter* spp and one of *Morganella morganii* were NDM positive. 14 of the samples (NDM producers and non producers) were obtained from the ICU (Table 2). Of these, 11 samples were urine samples, 4 were sputum, 3 were blood and one each from tracheal secretion, stool, bronchoalveolar lavage (BAL), swab, endotracheal secretion and pus.

Discussion

Resistance to carbapenems is of great concern as carbapenems are considered to be antibiotics of last resort to combat infections by multidrug resistant bacteria, especially in ICUs and high risk wards. While carbapenem resistance in *Pseudomonas* and *Acinetobacter* spp is well known, resistance among *Enterobacteriaceae* is increasing. Carbapenem resistance in *Enterobacteriaceae* has increased from 0% in 2006 to 8% in Jan – Aug 2009 (Fig 3) in ICU blood cultures. This is worrisome as

Table 2 : Strain and Sample Distribution for Carbapenem Resistant *Enterobacteriaceae*

Sr. No.	Organism	Samples	Location
1	<i>Klebsiella pneumoniae</i>	Swab	ICU
2	<i>Klebsiella pneumoniae</i>	Blood	ICU
3	<i>Klebsiella pneumoniae</i>	Sputum	ICU
4	<i>Klebsiella pneumoniae</i>	Urine	ICU
5	<i>Klebsiella pneumoniae</i>	Urine	PICU
6	<i>Klebsiella pneumoniae</i>	Urine	ICU
7	<i>Klebsiella pneumoniae</i>	Pus	SSS
8	<i>Enterobacter cloacae</i>	Tracheal	ICU
9	<i>Escherichia coli</i>	Blood	ICU
10	<i>Morganella morganii</i>	Urine	Wards
11	<i>Klebsiella ozonae</i>	Stool	Wards
12	<i>Escherichia coli</i>	Urine	Wards
13	<i>Escherichia coli</i>	Blood	ICU
14	<i>Escherichia coli</i>	Urine	Wards
15	<i>Escherichia coli</i>	Urine	Wards
16	<i>Klebsiella pneumoniae</i>	Urine	ICU
17	<i>Escherichia coli</i>	Urine	Wards
18	<i>Klebsiella pneumoniae</i>	ET secretion	PICU
19	<i>Klebsiella pneumoniae</i>	Sputum	ICU
20	<i>Escherichia coli</i>	Sputum	Wards
21	<i>Escherichia coli</i>	Urine	Wards
22	<i>Escherichia coli</i>	Urine	Wards
23	<i>Klebsiella pneumoniae</i>	BAL*	ICU
24	<i>Enterobacter aerogenes</i>	Sputum	ICU

*BAL- Bronchoalveolar lavage

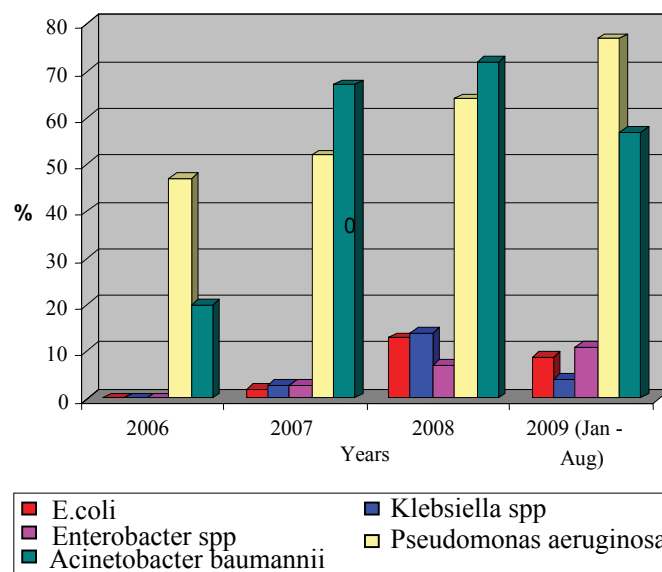


Fig. 3 : Percentage of carbapenem resistance amongst ICU blood cultures from 2006-2009

the latter are gut commensals and may spread to the community.

A novel New Delhi-1 (NDM-1) enzyme was reported in a National Alert in UK. This NDM beta lactamase is one type of metallo beta lactamase which is found only in members of *Enterobacteriaceae* and differs from other beta lactamases as Extended Spectrum Beta Lactamases (ESBLs) and Amp C by being resistant to the carbapenems. KPC is also a beta lactamase that is a serine beta lactamase which is resistant to

carbapenems. NDM-1 was strongly linked to India and Pakistan and many of the UK cases had recent medical exposure in the Indian subcontinent. NDM-1 enzyme has been accumulating swiftly probably due to efficient plasmid transfer. Recognition of patients at risk and prevention of transmission is the critical need of the hour. Till June 2009 Antibiotic Resistance Monitoring and Reference Laboratory (ARMRL) had received 17 further producers. Of the total 21 UK producers comprised *K. pneumoniae* (14), *E. coli* (4), *Enterobacter* spp., (1) and *Citrobacter freundii* (2), from 18 patients and 16 hospitals scattered across England, with one in Scotland. NDM-1 has become the most frequent carbapenemase in isolates referred to ARMRL, and the most widely scattered.¹

We sought to identify NDM-1 positive strains among the carbapenem resistant Enterobacteriaceae isolates at our tertiary care centre. In a short span of 3 months, we identified 22 such organisms. The physicians at our institute follow the hospital antibiotic policy and do not indiscriminately use carbapenems. However being a tertiary centre we receive transfer in cases / referrals from other hospitals. The majority of these organisms were from urine samples. NDM-1 was observed in *Klebsiella* spp, *E. coli*, and also in *Enterobacter cloacae* and *Morganella morganii*. This pattern is similar to that reported by the ARMRL. Only two *Klebsiella pneumoniae* carbapenem resistant strains were not producers of NDM-1 enzyme. The mechanism of resistance of these two isolates could be due to production of other Metallo beta lactamases or KPC enzymes.

Although NDM-1 organisms have been reported in UK among patients recently hospitalized in India or Pakistan, to our knowledge, this is the first report of NDM-1 producing enzyme in India.

Carbapenemases in Enterobacteriaceae may not be detected as their MICs can sometimes be below the current breakpoints. Clinical failure may result with use of carbapenem by unwary clinicians. Therefore direct detection of NDM-1 with PCR may be needed. NDMs are detected by a simple PCR that costs approximately Rs 200. Organisms with this resistance determinant will be difficult to treat as therapeutic options are limited. Colistin and perhaps Tigecycline are the only available agents for treatment and both have limitations.

In the UK report, the NDM-1 producing isolates were clonally diverse, indicating parallel evolution of resistance, doubtlessly under antibiotic pressure. This calls for curtailing indiscriminate antibiotic use. The concept of antimicrobial stewardship should be extended even to the community. Antimicrobials should be chosen carefully in every clinical situation. It is for this very reason that our group had studied and reported the outcome of treating infections due to ESBL (Extended Spectrum Beta Lactamases) producing organisms with non carbapenem antimicrobials.⁵ It

was concluded in this observational study that it is possible to successfully treat at least the less severe infections with ESBL producing organisms with non carbapenem drugs. This, we hoped, would help preserve the efficacy of carbapenems against increasingly resistant organisms. The identification of NDM-1 in 22 of 24 isolates is a worrisome development indeed. NDM-1 being present among Enterobacteriaceae has the potential for further dissemination in the community. Such dissemination may endanger patients undergoing major treatment at centres in India and this may have adverse implications for medical tourism. Besides stringent infection control in hospitals, good sanitation in the community is also needed to contain the spread of such clones.

Abbreviations

- New Delhi Metallo-1 --NDM-1
- Metallo beta lactamases --MBLs
- Clinical and Laboratory Standards Institute -- CLSI
- Antibiotic Resistance Monitoring and Reference Laboratory --ARMRL
- Extended Spectrum Beta Lactamases -- ESBL

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2. National Health & Education Society, P. D. Hinduja National Hospital & Medical Research Centre

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