New Delhi Metallo-β-Lactamases – the dawn of a post-antibiotic era?

Majewski P., Sacha P.*, Wieczorek P., Ojdana D., Michalska A., Tryniszewska E.

Department of Microbiological Diagnostics and Infectious Immunology, Medical University of Białystok, Poland

ABSTRACT

The increasing prevalence of acquired carbapenemases in Gram – negative bacteria is one of the biggest problems in the prevention and therapy of infectious diseases. NDM (*New Delhi Metallo-β-Lactamase*) is a recently discovered enzyme which has the ability to hydrolyze all β-lactam antibiotics, except aztreonam. Making that scenario more worrisome is the fact that mobile fragments of DNA carrying bla_{NDM} genes, also keeps a number of other genes encoding antibiotic resistance. NDM enzymes are currently present in different species of bacteria all over the world. NDM-producing bacteria are resistant to virtually all available antimicrobial agents except tigecycline, colistine and fosfomycine.

Key words: NDM carbapenemases, characteristics, epidemiology, susceptibility to antibiotics

***Corresponding author:**
Department of Microbiological Diagnostics and Infectious Immunology
Medical University of Białystok
Waszyngtona Str., 15A
15 - 269 Białystok, Poland
Tel.: 85 746 8571
Email: sachpt@umb.edu.pl **(**Paweł Sacha)

Received: 7.10.2012
Accepted: 28.11.2012
Progress in Health Sciences
Vol. 2(2) 2012 pp 153-160.

"Even the most outstanding achievements of engineering are embarrassingly awkward when compared with the level of precision that really miniaturized systems developed during the evolution of living beings."
Prof. Władysław J.H. Kunicki – Goldfinger

INTRODUCTION

Growing antibiotic resistance seems to be one of the biggest threats of modern medicine. Ten years ago attention was focused on resistant Gram-positive bacteria like *Methycillin Resistant Staphylococcus aureus* (MRSA) or *Vancomycin Resistant Enterococcus* (VRE), while today, multidrug-resistant Gram-negative pathogens (especially *Enterobacteriaceae*) are the main concern in bacterial infections. Dissemination of genes encoding antibiotic resistance mechanisms between Gram-negative bacteria is possible due to horizontal gene transfer. The exchange of genes encoding resistance mechanisms is possible owing to a mobile genetic element (e.g. plasmids, transposons, and integrons) which serves as a vehicle for these genes. Due to horizontal gene transfer, resistance can readily spread among Gram-negative bacteria [1].

Thus far, the β-lactam antibiotic class called carbapenems (like imipenem or meropenem) has been considered a fundamental therapy of serious infections caused by multidrug-resistant pathogens, especially Gram-negative bacteria producing *extended spectrum β-lactamases* (ESBL) [2]. Hence, the appearance of bacterial enzymes able to inactivate "last line" agents tends to be a serious therapeutic concern. Bacteria producing carbapenemases (e.g., KPC, VIM, and IMP) capable of hydrolyzing almost all β-lactam antibiotics are increasingly reported worldwide [3, 4].

One of the recently reported enzymes is the so-called NDM (*New Delhi Metallo-β-lactamase*) which is frequently encoded on plasmids almost always co-harboring other genes encoding mechanisms of resistance to agents other than β-lactams (e.g., fluorochinolones, tetra-cyclines). Bacteria producing NDM owe their "superbug" status to being resistant to virtually all available antimicrobial agents [5, 6].

Bacteria containing the $bla_{\text{NDM-1}}$ gene were first discovered in Sweden in 2008. A 59-year-old Swedish patient of Indian origin, with type II diabetes mellitus and multiple strokes was found to possess a highly resistant strain. In 2007, a patient was hospitalized in New Delhi where he acquired a urinary tract infection caused by *Klebsiella pneumoniae* which was found to be resistant to all antimicrobial agents except colistin. An isolate showed metallo-β-lactamase activity although it was negative for previously known metallo-β-lactamase genes [2, 5]. The first fatal case of infection caused by an NDM-producing strain was reported in Belgium 2010. A patient from Pakistan with diabetes died after uncontrolled sepsis due to an NDM-1-producing *Escherichia coli* [7]. Other fatal cases were described in India and the United Kingdom [8].

An abundant group of infections caused by NDM-producing bacteria correlates with prior travel to India. Originally it was thought that only patients admitted to hospitals in India acquired such infections. However, *Walsh et al.* demonstrated that multiple NDM-producing bacteria are circulating in the Indian community [9]. Hence, the source of infection could be environmental rather than purely nosocomial.

Factors contributing to an increased incidence of carbapenemase-producing pathogens are worthy of emphasis. Long-term improper disposal of β-lactam agents has contributed significantly to the growing prevalence of ESBL in India. To date, India has an extremely high prevalence of ESBL in *Enterobacteriaceae* at 70-90% which is one of the highest percentages worldwide [1]. High incidence of ESBL is a major factor in the enormous reliance on carbapenems (e.g., therapy of uncomplicated urinary tract infection). Misinformed antibiotic policy influenced not only the selection of carbapenem-resistant subpopulations but also the production of β-lactamases (e.g., imipenem is a strong inducer of AmpC cephalosporinase) [8, 10].

Overall characteristics of metallo-β-lactamases

Bacterial enzymes capable of hydrolyzing the amide bond in the β-lactam ring are divided on the basis of the amino acid sequence. They fall into four classes – A, B, C, and D. The class B includes metalloenzymes, whose activity is determined by the presence of a divalent zinc ion in the active site. Classes A, C and D rely on serine in the active site [11].

Metallo-β-lactamases (hereinafter referred to as MBL) were first described in the mid-1960's, twenty-five years after the discovery of the first serine β-lactamase. Initially, MBLs were found to be produced only by low-virulent bacteria. For over a decade, the increasing incidence of MBLs encoded mostly on plasmids was observed in *Enterobacteriaceae* and other Gram-negative bacteria [12]. At present, acquired MBLs (e.g., IMP, VIM and the newly discovered NDM) are one of the main factors determining multidrug resistance. Apart from the difference in the structure of the active site (and thus the mechanism

of action), MBLs differ from serine β-lactamases in the hydrolytic spectrum. MBLs lack of activity against monobactams and efficiently hydrolyze carbapenems, penicillins and cephalosporins. Another feature distinguishing MBLs from serine enzymes is resistance to clinical inhibitors of β-lactamases [13].

Metallo-β-lactamases (class B) falls into three subclasses, divided on the basis of differences in amino acid sequence and structure of the active site. Subclasses B1 and B3 require two divalent zinc ions, while subclass B2, with the narrower spectrum of activity, requires only one ion. NDM is a new representative of subclass B1 [2] and to date, there are seven described variants of NDM [14].

Genotypic and phenotypic characteristics of NDM

Yong et al. characterized a genomic library of *Klebsiella pneumoniae* 05-560 strain (which is the first described NDM-producing isolate) in order to gather all genes conferring resistance to β-lactam antibiotics. Apart from the $bla_{\text{CMY-4}}$ gene they found a new gene $bla_{\text{NDM-1}}$, consisting of a 1350-bp DNA fragment encoded by 807 nucleotides. Among contiguous genetic material, the former element is distinguished by low guanine and cytosine content at 57%, while adjacent genetic material contains 62-65% of these nucleotides [2].

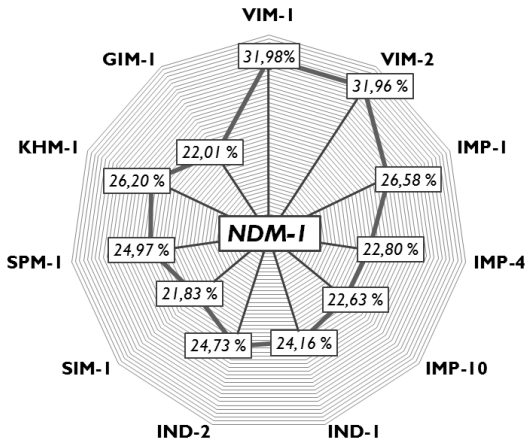

Figure 1. The identity of other metallo-β-lactamases compared to NDM-1. The alignment was prepared with the CLUSTALW application. The Gen Bank accession numbers for the sequences are as follows: VIM-1 EU118150, VIM-2 GQ288399, IMP-1 AB753459, IMP-4 AF244145, IMP-10 AB074433, IND-1 EF394436.1, IND-2 EF394438.1, SIM-1 GQ288397.1, SPM-1 AJ492820.1, KHM-1 AB364006, GIM-1 AJ620678.1.

Gene encoding NDM was found to disseminate between bacterial populations following this unique mechanism. Most of the genes encoding drug resistance in *Enterobactericaeae* are localized on mobile gene cassettes – components of class I integrons or adjacent to *ISCR* elements using rolling-circle replication [15]. The $bla_{\text{NDM-1}}$ gene, is the only one thus far described as gene encoding MBLs not carried in a class I integron or adjacent to *ISCR* elements. Expression of the $bla_{\text{NDM-1}}$ gene is driven by an intact promoter sequence that appears to be acquired along with the gene [2].

Plasmids of different size serve as vehicles for the $bla_{\text{NDM-1}}$ gene. Some plasmids bearing resistance genes can be efficiently transferred only between closely related bacteria, while others have the ability to replicate in a wide range of hosts. The gene encoding NDM is mostly located on the plasmids belonging to several "incompatibility groups" (IncL/M, IncFII, IncA/C), although it is generally the IncA/C plasmid that is characterized by an especially wide range of potential hosts [1, 16].

The case of the first Swedish patient inferred the transfer of the $bla_{\text{NDM-1}}$ gene *in vivo*. Apart from *Klebsiella pneumoniae* 05-506 carrying the $bla_{\text{NDM-1}}$ gene (180-bp plasmid) which was isolated from urine, the patient possessed *Escherichia coli* isolated from the gut which was found to carry a 140-kb plasmid containing the $bla_{\text{NDM-1}}$ gene. Different sizes of plasmids would suggest additional rearrangement *in vivo* [2].

A value of at least 30% amino acid diversity is used as a cutoff for classification of a new metallo-β-lactamase [17]. NDM-1 proved to be a unique molecule, as compared to closely related VIM-1/VIM-2, which shows 68% diversity (Fig.1). Compared to the other subclass B1 enzymes, the amino acid sequence of NDM-1 shows an additional insert between residues 162 and 166 not present in other MBLs as well as low similarity in methionine-67 – glycine-71 loop region [18-20].

The location of the first unique sequence far away from the active site seems to exclude its direct effect on the catalytic ability of the enzyme. The second unique region is considered to play a crucial role in NDM substrate and inhibitor binding. It is assumed that this loop region has more mobility during substrate binding. Thus, NDM-1 may exhibit a different substrate interacting mode and enzymatic mechanism than related MBLs [19].

MBL active sites need both stability and flexibility to perform the substrate hydrolysis. Two unusual residues, Lys-125 and Tyr-229, were shown to play critical roles in stabilizing the conformation of the active site, through formation of a specific hydrogen bond network (with residues Asn-76, Asp-90, Thr-91, His-122 and Ser-249) and hydrophobic interactions (with residues Leu-209,

Table 1. The worldwide occurrence of NDM- producing isolates

GENUS	COUNTRY	REFERENCES
Achromobacter spp.	India	[9]
Acinetobacter baumanii	Indi, Germany, Egypt	[23, 29]
Aeromonas caviae	India	[30]
Citrobacter braakii	India	[30]
Citrobacter freundii	India, France, United Kingdom	[1, 23, 30]
Enterobacter spp.	India, United Kingdom	[1, 29]
Escherichia coli	India, Belgium, Sweden, France, Canada, Austria, Germany, Norway, United Kingdom	[1, 2, 13, 16, 23, 31]
Kingella denitrificans	India	[9]
Klebsiella oxytoca	India	[1]
Klebsiella pneumoniae	India, Sweden, Kenya, Canada, France, Finland, Denmark, Belgium, Austria, Germany, Italy, Netherland, Norway, Spain, Slovakia, United Kingdom, Australia	[1, 2, 12, 23, 31]
Morganella morganii	India, Belgium, United Kingdom	[1, 6, 32]
Proteus mirabilis	France	[23]
Providencia rettgeri	India	[9, 29]
Pseudomonas aeruginosa		
Pseudomonas oryzihabitans		
Pseudomonas pseudoalcaligenes		
Pseudomonas putida		
Shigella boydii		
Stenotrophomonas maltophilia		
Sutonella indologenes		
Vibrio cholerae		

Leu-218, Leu-221 and Leu-269) [18]. Furthermore, it has been established that the deep cavity of the NDM-1 active site provides a larger volume than other reported MBLs and thus could provide more space for adopting substrates [19].

The $bla_{\text{NDM-1}}$ gene open reading frame encodes a protein consisting of 269 amino acids with a molecular weight of approximately 27,5 kDa [2]. When compared to VIM-2, NDM-1 displays strong binding ability to cephalosporins and penicillins. Interestingly, NDM does not bind carbapenems as strongly as IMP-1 or VIM-2, but hydrolyzes them at a rate similar to these enzymes. Generally, NDMs compared to other MBLs have a high affinity for the substrate which is probably the result of the specific structure of the active site [2, 19].

Epidemiology

Since 2008, the medical community has noticed the spread of NDM-producing bacteria from India to Europe (mainly the United Kingdom), the United States, Canada, and Australia, and multi - drug resistant pathogens across Europe [9]. Currently it is believed that India stands as a major reservoir of NDM-producing bacteria. Recent studies also point out new sources of pathogens: the Balkans Montenegro, Serbia and Kosovo) and the Middle East (Oman, Iraq) [8].

Bacteria from the *Enterobacteriaceae* family (usually *Klebsiella pneumoniae* and *Escherichia coli*) and Gram-negative non-fermentative bacteria (such as *Acinetobacter baumannii* and *Pseudomonas aeruginosa*) are considered as major producers of NDM (Tab.1), although environmental analysis (analysis of tap water and groundwater) conducted by *Walsh et al.* has shown that NDM is also produced by strictly pathogenic microorganisms such as *Shigella boydii* and *Vibrio cholerae*. Experimental conjugation between the studied environmental strains and reference *Escherichia coli* J53 strain conducted by *Walsh et al.* showed the greatest efficiency of the process at 30°C, suggesting a much greater role in environmental gene transfer than previously thought. In India, temperatures reach 30°C for seven months a year, and this period coincides with the monsoon season, rich in flooding.

Such conditions create a perfect opportunity for multidrug-resistant pathogens to circulate in the environment [9].

The prevalence of the bla_{NDM-1} gene among *Escherichia coli*, the main component of human intestinal flora, is established at a disturbingly high level. It is likely that the intestinal flora of hundreds of thousands citizens of South Asia are rich in NDM-producing bacteria. Thus, resistant pathogens can very easily spread by the "colonized" hands, water, or everyday objects. This condition creates a risk of high prevalence of community-acquired urinary tract infection or diarrhea [8, 9]. Another factor contributing to the spread of multidrug-resistant bacteria is considerable overcrowding and poor sanitation. As the UN News Centre revealed in India, the second most populous country in the world, only 30% of the population (about 366 million people) has full access to sanitation [21]. The high prevalence of NDM-producing bacteria in environment and poor sanitation could contribute to importing highly resistant subpopulations into Indian hospitals and other countries. Unfortunately, the situation in a large number of Indian hospitals favors further selection of multi-drug-resistant microorganisms. Many units do not follow hygiene and infection control standards [22]. To make matters worse, India is a country with widespread over-the-counter usage of antimicrobial agents [6].

In 2008, about 5 million tourists visited India and Pakistan and about 10 million citizens have traveled from India. Considerable efflux of people into India is associated to some extent with the phenomenon of medical tourism (e.g., cosmetic surgery, transplants) owing to much lower costs and shorter waiting times than in Europe and America. Thus, there is a high risk of permanent transfer of NDM-producing strains from India [22, 23].

Therapeutic options

NDM-producing pathogens appear to be resistant to virtually all available antimicrobial agents which is a major concern (Tab.2).

Table 2. Antibiotic susceptibility of the NDM-producing isolates

ANTIMICROBIAL AGENT	N° OF TESTED STRAINS	SUSCEPTIBLE STRAINS [%]	REFERENCES
Imipenem	223	1,34	[1, 2, 23, 29 – 31, 33 – 39]
Meropenem	188	18,08	[1, 2, 23, 30, 31, 33 – 35, 37, 38]
Etrapenem	112	0	[2, 23, 29 – 31, 34, 35, 37, 38]
Ciprofloxacin	220	10,91	[1, 2, 23, 29 – 31, 33, 35, 37, 38]
Ampicillin	76	0	[1, 23, 30, 31, 35, 37, 39]
Cefotaxime	217	0	[1, 2, 23, 30, 33, 35, 37, 39]
Gentamicin	189	8,99	[1, 23, 30, 31, 33 – 35, 37 – 39]
Netilimicin	14	0	[29, 33 – 35, 39]
Colistin	246	85,77	[1, 2, 23, 29 – 31, 33, 35 – 39]
Tigecycline	248	63,71	[1, 23, 29, 30, 31, 33 – 39]
Fosfomycin	92	91,30	[30, 36]

The coexistence of different antibiotic resistance genes in NDM-producing bacteria is widespread. Plasmids containing the bla_{NDM-1} gene frequently include many other antibiotic resistance genes (Tab.3). Many authors have shown high *in vitro* activity of colistin, tigecycline, and fosfomycin. One of the major drawbacks of colistin (polymyxin group) is its nephrotoxicity (causing acute renal failure) and neurotoxicity (causing paresthesia, ataxia, confusion, respiratory muscles paralysis) [24]. It absorbs very little and poorly diffuses into body fluids and tissues. It also shows no activity against the Proteus group (*Proteus, Morganella and Providencia*) [12].

Tigecycline, the first representative of the glicylocyclines, exhibits high *in vitro* activity against many multidrug-resistant strains [25], but at the same time shows no activity against the Proteus group and *Pseudomonas aeruginosa*. It cannot be used in urinary tract infections and there are no specific data confirming the safety of tigecycline under 18 years of age [26].

Unfortunately, each of these agents has considerable pharmacological limitations.

Table 3. The presence of other antibiotic resistance genes in NDM-producing strains

ANTIMICROBIAL AGENTS	RESISTANCE GENES	REFERENCES
β-lactam antibiotics	*bla*$_{TEM-1}$, *bla*$_{OXA-1}$, *bla*$_{OXA-10}$, *bla*$_{OXA-23}$, *bla*$_{OXA-24}$, *bla*$_{OXA-28}$, *bla*$_{OXA-51}$, *bla*$_{OXA-58}$, *bla*$_{CTX-M-15}$, *bla*$_{CMY-2}$, *bla*$_{CMY-4}$, *bla*$_{CMY-6}$ *bla*$_{DHA}$, *bla*$_{SHV-2A}$, *bla*$_{SHV-11}$, *bla*$_{SHV-12}$, *bla*$_{KPC-2}$	[1, 2, 29, 33, 34]
Chinolones	*qnrB1*	[32]
Aminoglycosides	*armA, rmtA, rmtB, rmtC, rmtD, npmA, aadA1*	[2, 33, 34]
Rifampicin	*arr-2*	[2]
Sulfonamides	*sul-2*	
Chloramphenicol	*cmlA*	
Macrolides	*ereC*	

Fosfomycin, an almost forgotten antimicrobial agent may be one of the therapeutic options for the treatment of infections caused by NDM-producing bacteria. However, in monotherapy it is useful only in urinary tract infection [11, 12].

Current pharmaceutical research includes very few novel antimicrobial agents acting on Gram-negative bacteria [1]. D-captopril is one of the currently developed agents, which showings affinity to the active site of NDM and is considered a potential inhibitor of these enzymes; however, the dosage able to inhibit the MBLs presents a high cytotoxicty [19].

Research is also being conducted on ACHN-490, a sisomycin derivative that exhibits activity against bacteria resistant to carbapenems. However, it is ineffective in the presence of 16S rRNA methylase which confers resistance to aminoglycosides [26, 27].

Unfortunately, NDM-producing bacteria almost always possess genes encoding 16S rRNA methylase.

Another antimicrobial agent, apramycin (aminoglycoside analog), used in veterinary medicine, has shown *in vitro* activity against all NDM-producing strains with co-produced 16S rRNA methylase. Unfortunately, apramycin is not suitable for use in therapy because of its narrow therapeutic index. However, its unique structure may serve in the future as a prototype for new molecules resistant to both NDM and methylases [27].

One of the more innovative approaches is called “antiplasmidic therapy” or “plasmid targeting”. This novel approach is based on molecules imitating plasmidic ctRNA (counter-transcribed RNA) involved in replication control of plasmids, including the induction of their elimination. Such futuristic therapy could restore effectiveness of a large group of antimicrobial agents, although the utility of plasmid targeting is still only a concept that requires further studies [28].

CONCLUSIONS

Hostile environments (e.g., antibiotic pressure) accelerate the selection of features which give particular advantage to bacterial populations. Due to the aforementioned selections, extremely efficient mechanisms of gene transfer have developed. Genes encoding NDM with many others resistance genes are currently disseminating between different species with unprecedented ease. In contrast to currently known resistance mechanisms (e.g., ESBL or MBL), NDM could rise to worldwide concern for reasons beyond nosocomial infections. To date there is no therapeutic option for putative infection caused by NDM-producing bacteria. Pathogens producing *New Delhi Metallo-β-lactamases* may be the dawn of post-antibiotic era.

Conflicts of interest

We declare that we have no conflicts of interest.

Acknowledgments

We thank Steven J. Snodgrass for editorial assistance.

REFERENCES

1. Kumarasamy KK, Toleman MA, Walsh TR, Bagaria J, Butt F, Balakrishnan R, Chaudhary U, Doumitch M, Giske CG, Irfan S, Krishnan P, Kumar AV, Maharjan S, Mushtaq S, Noorie T, Paterson DL, Pearson A, Perry C, Pike R, Rao B, Ray U, Sarma JB, Sharma M, Sheridan E, Thiurunrayan MA, Turton J, Upadhyay S, Warner M, Welfare W, Livermore DM, Woodford N. Emergence of a new antibiotic resistance mechanism in India, Pakistan and UK: a molecular, biological and epidemiological study. Lancet Infect Dis. 2010 Sep; 10(9): 597-602.
2. Yong D, Toleman MA, Giske CG, Cho HS, Sundman K, Lee K, Walsh TR. Characterization of a New Metallo-β-lactamase Gene, bla_{NDM-1} and a Novel Erythromycin Esterase Gene Carried on a Unique Genetic Structrure in *Klebsiella pneumoniae* Sequence Type 14 from India. Antimicrob Agents Chemother. 2009 Dec; 53 (12): 5046-54.
3. Sacha PT, Wieczorek P, Hauschild T, Żórawski M, Olszańska D, Tryniszewska E. Metallo-beta-lactamases of *Pseudomonas aeruginosa* – a novel mechanism resistance to beta-lactam antibiotic. Folia Histochem Cytobiol. 2008; 46 (2): 137-42.
4. Sacha PT, Ostas A, Jaworowska J, Wieczorek P, Ojdana D, Ratajczak J, Tryniszewska E. The KPC type beta-lactamases: new enzymes that confer resistance to carbapenems in Gram-negative bacilli. Folia Histochem Cytobiol. 2009; 47 (4): 537-43.
5. Deshpande P, Rodrigues C, Shetty A, Kapadia F, Hedge A, Soman R. New Delhi Metallo-β-lactamase (NDM-1) in *Enterobacteriaceae*: Treatment options with Carbapenems Compromised. J Assoc Physicians India. 2010 Mar; 58: 147-9.
6. Moellering RC. NDM-1 – A Cause for Worldwide Concern. N Engl J Med. 2010 Dec; 363 (25): 2377-9.
7. Bogaerts P, Bouchahrouf W, de Castro R, Deplano A, Berhin C, Pierard D, Denis O, Glupczynski Y. Emergence of NDM-1-producing *Enterobacteriaceae* in Belgium. Antimicrob Chemother. 2011 Jun; 55 (6): 3036-8.
8. Nordmann P, Poirel L, Toleman MA, Walsh TR. Does broad-spectrum β-lactam resistance due to NDM-1 herald the end of the antibiotic era for treatment of infections caused by Gram-negative bacteria? J Antimicrob Chemother. 2011 Apr; 66 (4): 689-92.
9. Walsh TR, Weeks J, Livermoore DM, Toleman MA. Dissemination of NDM-1 positive bacteria in the New Delhi environment and its implications for human health: an environmental point prevalence study. Lancet Infect Dis. 2011 May; 11 (5): 355-62.
10. Jacoby GA. AmpC β-Lactamases. Clin Microb Rev. 2009 Jan; 22 (1):161-82.
11. Bush K, Jacoby GA. Updated Functional Classification of β-Lactamases. Antimicrob Agents Chemother. 2010 Mar; 54 (3): 969-76.
12. Cornaglia G, Giamarellou H, Rossolini GM. Metallo-β-lactamases: a last frontier for β-lactams? Lancet Infect Dis. 2011 May; 11 (5): 381-93.
13. Struelens MJ, Monnet DL, Magiorakos AP, Santos O'Connor F, Gieseceke J. New Delhi metallo-beta-lactamase 1-producing *Enterobacteriaceae*: emergence and response in Europe. Euro Surveill. 2010 Nov; 15 (46): 1-8.
14. Lahey Clinic – available at http://www.lahey.org/Studies/ [cited 2012 October 10].
15. Toleman MA, Bennet PM, Walsh TR. ISCR Elements: Novel Gene-Capturing Systems of the 21st Century? Microbiol Mol Biol Rev. 2006 Jun; 70 (2): 296-316.
16. Potron A, Poirel L, Nordmann P. Plasmid-mediated transfer of the bla_{NDM-1} gene in Gram-negative rods. FEMS Microbiol Lett. 2011 Nov; 324 (2): 111-6.
17. Cornaglia G, Akova M, Amicosante G, Canton R, Cauda R, Docquier JD, Edelstein M,. Frere JM, Fuzi M, Galleni M, Giamarellou H, Gniadkowski M, Koncan R, Libisch B, Luzarro F, Miriagou V, Navarro F, Nordmann P, Pagani L, Peixe L, Poirel L, Souli M, Tacconeli E, Vatopoulos A, Rossolini GM. Metallo-beta-lactamases as emerging resistance determinants in Gram-negative pathogens: open issues. Int J Antimicrob Agents. 2007 Apr; 29 (4): 380-8.
18. Zhang H, Hao Q. Crystal structure of NDM-1 reveals a common β-lactam hydrolysis mechanism. FASEB J. 2011 Aug; 25 (8): 2574-82.
19. Guo Y, Wang J, Niu G, Shui W, Sun Y, Zhou H, Zhang Y, Yang C, Lou Z, Rao Z. A structural view of the antibiotic degradation enzyme NDM-1 from a superbug. Protein Cell. 2011 May; 2 (5): 384-94.
20. Liang Z, Li L, Wang Y, Chen L, Kong X, Hong Y, Lan L, Zheng M, Guang-Yang C, Liu H, Shen X, Luo C, Li KK, Chen K, Jiang H. Molecular basis of NDM-1, a New Antibiotic Resistance Determinant, PLoS One. 2011 Aug; 6 (8): 1-8.
21. Un News available at http://www.un.org/apps/news/story.asp?NewsID=34369&Cr=mdg& Cr1 [cited 2012 January 10].

22. Arya SC, Agarwal N. International travel with acquisition of multi-drug resistant Gram-negative bacteria containing the New Delhi etallo-beta-lactamase gene, bla_{NDM-1}.Trav Med Infect Dis. 2011 Jan; 9 (1): 47-8.
23. Koh TH, Leverstein-Van Hall MA, Hammerum AM, Bogaerts P, Stuart JC, Khoo CT,. Toleman MA, Verrkonen A, Wijaya L, Hansen F. Global spread of New Delhi metallo-β-lactamase 1. Lancet Infect Dis. 2010 Dec; 10 (12): 828-32.
24. Mendes CA, Burdmann EA. Polymyxins – a review focusing on their nephrotoxicity. Rev Assoc Med Bras. 2010 Dec-Nov; 56 (6): 752-9.
25. Poulakou G, Kontopidou FV, Paramythiothou E, Kompoti M, Katsiari M, Mainas E, Nicolau C, Yphantis D, Antoniadou A, Trikka-Graphakos E, Roussou Z, Clouva P, Maguina N, Kanellakopoulou K, Armaganidis A, Giamarellou H. Tigecycline in treatment of infections from multi-drug resistant Gram-negative pathogens. J Infect. 2009 Apr; 58 (4): 273-84.
26. Giamarellou H. Multidrug-resistant Gram-negative bacteria: how to treat and for how long. Int J Antimicrob Agents. 2010 Dec; 36 Suppl. 2: 50-4.
27. Livermore DM, Mushtaq S, Warner M, Zhang JC, Maharjan S, Doumith M, Woodford N. 2011. Activity of aminoglycoside, including ACHN-490, against carbapenem-resistant *Enterobacteriaceae* isolates. J Antimicrob Chemother. 2011 Jan; 66 (1): 48-3.
28. DeNap JC, Hergenrother PC. Bacterial death comes full circle: targeting plasmid replication in drug-resistant bacteria. Org Biomol Chem. 2005 Mar; 3 (6): 959-66.
29. Lascols C, Hackel M, Marshall SH, Hujer AM, Bouchillon S, Badal R, Hobban D, Bonomo RA. Increasing prevalence and dissemination of NDM-1 metallo-β-lactamase in India: data from the SMART study (2009), J Antimicrob Chemother. 2011 Sep; 66 (9): 1992-7.
30. Perry JD, Naqvi SH, Mirza IA, Alizai SA, Hussain A, Ghirardi S, Orenga S, Wilkinson K, Woodford N, Zhang J, Livermore DM, Abassi SA, Raza MW. Prevalence of fecal carriage of *Enterobacteriaceae* with NDM-1 carba-penemase at military hospitals in Pakistan, and evaluation of two chromogenic media. J Antimicrob Chemother. 2011 Oct; 66 (10): 2288-94.
31. Kus JV, Tadros M, Simor A, Low DE, McGeer AJ, Willey BM, Larocque C, Pike K, Edwards IA, Dedier H, Melano R, Boyd DA, Mulvey MR, Louie L, Okeahialam C, Bayley M, Whitehead C, Richardson D, Carr L, Jinnah F, Poutanen SM. New Delhi metallo-β-lactamase-1: local acquisition in Ontario, Canada, and challenges in detection. Can Med Assoc J. 2011 Aug; 183 (11): 1257-61.
32. Nordmann P, Couard JP, Sansot D, Poirel L. Emergence of an Autochthnous and Community-Acquired NDM-1-Producing *Klebsiella pneumoniae* in Europe. Clin Infect Dis. 2012 Jan; 54 (1): 150-1.
33. Kumarasamy K, Kalyanasundaram A. Emergence of *Klebsiella pneumoniae* isolate co-producing NDM-1 with KPC-2 from India. J Antimicrob Chemother. 2012 Jan; 67 (1): 243-4.
34. Karthikeyan K, Thirunarayan MA, Krishnan P. Coexsistence of bla_{OXA-23} with bla_{NDM-1} and armA in clinical isolates of *Acinetobacter baumanii* from India. J Antimicrob Chemother. 2010 Oct; 65 (10): 2253-70.
35. Stone NR, Woodford N, Livermore DM, Howard J, Pike R, Mushtaq S, Perry C, Hopkins S. Breaktrough bacteraemia due to tigecycline-resistant *Escherichia coli* with New Delhi metallo-β-lactamase (NDM-1) successfully treated with colistin in a patient with calciphylaxis. J Antimicrob Chemother. 2011 Nov; 66 (11): 2677-8.
36. Berçot B, Poirel L, Dortet L, Nordmann P. In vitro evaluation of antibiotic synergy for NDM-1-producing *Enterobacteriaceae*. J Antimicrob Chemother. 2011 Oct; 66 (10): 2295-7.
37. Samuelsen Ø, Thilesen CM, Heggelund L, Vada AN, Kümmel A, Sundsfjord A. Identification of NDM-1 producing *Enterobacteriaceae* in Norway. J Antimicrob Chemother. 2011 Mar; 66 (3): 670-2.
38. Kaase M, Nordmann P, Wichelhaus TA, Gatermann SG, Boninn RA, Poirel L. NDM-2 carbapenemase in *Acinetobacter baumannii* from Egypt. J Antimicrob Chemother. 2011 Jun; 66 (6): 1260-2.
39. Roy S, Viswanathan R, Singh AK, Das P, Basu S. Sepsis in neonates due to Imipenem-resistant *Klebsiella pneumoniae* producing NDM-1 in India. J Antimicrob Chemother. 2011 Jun; 66 (6): 1411-3.