Vol 2 | Issue 1 | Jan –Jun 2011 | 17-21. **Print ISSN – 2229 7502**

Review Article

PHARMACOLOGICAL AND BIOCHEMICAL ASPECTS OF NEW DELHI METALLO-BETA-LACTAMASE (NDM-1)-A SUPERBUG: AN OVERVIEW

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ABSTRACT

New Delhi Metallo-beta-lactamase (NDM-1) is an enzyme which makes bacteria resistant to a broad range of beta-lactam antibiotics. This includes antibiotics of the carbapenem family, which are a mainstay for the treatment of antibiotic-resistant bacteria. The gene for NDM-1 is one member of a large gene family that encodes beta-lactamase enzymes called carbapenemases. Bacteria which carry such genes are often referred to in the news media as "superbugs", since infections with these bacteria are very hard to treat successfully. NDM-1 was first identified in December 2009 in a patient hospitalised in New Delhi with an infection caused by *Klebsiella pneumoniae*. It was later detected in bacteria in India, Pakistan, the United Kingdom, the United States, and Canada. The most common bacteria that make this enzyme are Gram negative such as *Escherichia coli* and *Klebsiella pneumoniae*, but the gene for NDM-1 can spread from one strain of bacteria to another by horizontal gene transfer. 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). This article presents a brief review of NDM-1 with an emphasis on its various pharmacological and biochemical aspects. The article also focuses on concept of antibiotic resistance.

Keywords: New Delhi Metallo-beta-lactamase 1, NDM-1, Superbug NDM-1, beta-lactum antibiotics, New Delhi Metallo β Lactamase 1.

INTRODUCTON

Beta-lactamases are enzymes produced by some bacteria and are responsible for their resistance to antibiotics like penicillins, cephamycins, and carbapenems (ertapenem). Cephalosporins are relatively resistant to beta-lactamase.).

These antibiotics have a common element in their molecular structure: a four-atom ring known as a beta-lactam. The lactamase enzyme breaks that ring open, deactivating the molecule's antibacterial properties. Beta-lactam antibiotics are typically used to treat a broad spectrum of Gram-positive and Gram-negative bacteria. Beta-lactamases produced by Gram-negative organisms are usually secreted. Penicillinase is a specific type of β-lactamase, showing specificity for penicillins, again by hydrolysing the beta-lactam ring. Molecular weights of the various penicillinases tend to cluster near 50kD. Penicillinase was the first β-lactamase to be identified: it was first isolated by Abraham and Chain in 1940 from Gram-negative *E. coli* even before penicillin entered clinical use [1] but penicillinase production quickly spread to bacteria that previously did not produce it or only produced it rarely. Penicillinase-resistant beta-lactams such as methicillin were developed, but there is now widespread resistance to even these. Functionally Beta-lactamases are classified into various groups [2], the Group-I include cephalosporinases not inhibited by clavulanic acid, Group 2 are penicillinases, cephalosporinases, or both inhibited by clavulanic acid, Group 3 are the zinc based or metallo {beta}-lactamases, corresponding to the molecular

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class B, which are the only enzymes acting by the metal ion zinc as well as Group 4 are penicillinases that are not inhibited by clavulanic acid, and they do not yet have a corresponding molecular class. The molecular classification of β-lactamases is based on the nucleotide and amino acid sequences in these enzymes. To date, four classes are recognised (A-D), correlating with the functional classification. Classes A, C, and D act by a serine-based mechanism, whereas class B or metallo-β-lactamases need zinc for their action [3].

New Delhi Metallo-beta-lactamase (NDM-1)

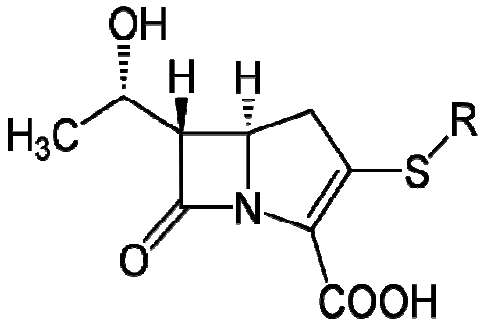
New Delhi metallo-beta-lactamase (NDM-1) is an enzyme that makes bacteria resistant to a broad range of beta-lactam antibiotics. These include the antibiotics of the carbapenem family, which are a mainstay for the treatment of antibiotic-resistant bacterial infections. The gene for NDM-1 is one member of a large gene family that encodes beta-lactamase enzymes called carbapenemases.
Bacteria that produce carbapenemases are often referred to in the news media as "superbugs" because infections caused by them are difficult to treat. Such bacteria are usually only susceptible to polymyxins and tigecycline. NDM-1 was first identified in December 2009 in a patient hospitalised in New Delhi with an infection caused by *Klebsiella pneumoniae*. It was later detected in bacteria in India, Pakistan, the United Kingdom, the United States, and Canada. The most common bacteria that make this enzyme are Gram negative such as *Escherichia coli* and *Klebsiella pneumoniae*, but the gene for NDM-1 can spread from one strain of bacteria to another by horizontal gene transfer [4].

Carbapenems (imipenem, ertapenem, meropenem, doripenem) are a class of beta-lactam antibiotics with a broad spectrum of activity against gram-positive, gram-negative, and anaerobic bacteria. Carbapenemase enzymes belonging to Ambler molecular classes A to D have been detected in various clinical isolates. Of these the class B enzymes are clinically the most significant. They are the metallo-beta-lactamase (MBL) enzymes of the IMP or VIM series that have been reported worldwide. MBL enzymes, whose genes are plasmid and integron located, hydrolyze virtually all beta-lactams except aztreonam [5]. Many of the carbapenemase producers are frequently resistant to fluroquinolones and aminoglycosides.

NDM-1 was first detected in a *Klebsiella pneumoniae* isolate from a Swedish patient of Indian origin in 2008. The gene coding for this unique enzyme *blaNDM-1* was found in one of the three resistance-carrying regions of an integron. NDM-1 shares very little identity with other MBLs. As well as possessing unique residues near the active site, NDM-1 also has an additional insert between positions 162 and 166, which is not present in other MBLs. NDM-1 has a molecular mass of 28 kD and is monomeric[6]. NDM-1 have been isolated from *K pneumoniae*,*Escherichia coli*,*Citrobacterfreundii*, *Enterobacter cloacae,* and *Morganella morganii.* Other classes of carbapenemases have already been found in *K pneumoniae, E cloacae, Pseudomonas aeruginosa,* and *Acinetobacter baumannii* [7]. The first clue to the presence of a carbapenemase comes from the increased minimum inhibitory concentration (MIC) values or frank resistance of the enterobacteriaceae to ertapenem, imipenem, or meropenem. NDM-1 is inhibited by EDTA like other MBL enzymes; this has been demonstrated by the EDTA-disc synergy test. The carbapenemase activity can be screened for by the modified Hodge test [8]. Further characterization and identification of the enzyme can be done only by molecular methods. The Structure of the carbapenem backbone is given in figure 1.

Treatment of infections caused by pathogens producing carbapenemases, including NDM-1, poses a serious challenge as these infections are resistant to all commonly used antibiotics. Treatment of patients should be guided by the susceptibilities of the individual pathogens, and clinical laboratories must test for a wide range of antibiotics, including tigecycline, colistin, polymyxin, and aztreonam. The use of antibiotic combinations may have to be considered in desperate cases. Carbapenems are the only reliably active antibiotics against many multiresistant gram-negative pathogens, particularly those with extended-spectrum beta-lactamases (ESBLs) and *AmpC* enzymes [9]. The emergence and diversity of carbapenemase-producing strains is therefore a major concern and one that Indian microbiologists cannot afford to ignore.

Figure 1: Structure of the Carbapenem backbone



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The gene for NDM-1 is one member of a large gene family that encodes beta-lactamase enzymes called carbapenemases. Bacteria that have these genes are often referred to in the news media as "superbugs" because infections caused by them are difficult to treat. Indeed, the United Kingdom's Health Protection Agency has reported that "most isolates with NDM-1 enzyme are resistant to all standard intravenous antibiotics for treatment of severe infections.NDM-1wasfirst identified in December 2009 in a patient hospitalised in New Delhi with an infection caused by Klebsiella pneumoniae. It was later detected in bacteria in India, Pakistan, the United Kingdom, the United States, and Canada. The most common bacteria that make this enzyme are Gram negative such as Escherichia coli and Klebsiella pneumoniae, but the gene for NDM-1 can spread from one strain of bacteria to another by horizontal gene transfer.

Enzyme Function

Carbapenems are a class of beta-lactam antibiotics which are capable of killing most bacteria by inhibiting the synthesis of one of their wall layers. The carbapenems were developed to overcome antibiotic resistance mediated by bacterial beta-lactamase enzymes. However, the bla_{NDM-1} gene produces NDM-1, which is a carbapenemase beta-lactamase - an enzyme that hydrolyzes and inactivates these carbapenem antibiotics. Carbapenemases are particularly dangerous resistance mechanisms, since they can inactivate a wide range of different antibiotics [10]. The NDM-1 enzyme is one of the class B metallo-beta-lactamase; other types of carbapenemase are class A or class D beta-lactamases [11].(The class A*Klebsiella pneumoniae* carbapenemase (KPC) is currently the most common carbapenemase, which was first detected in North Carolina, USA, in 1996 and has since spread worldwide [12]. A later publication indicated that Enterobacteriaceae that produce KPC were becoming common in the United States. [13]) The resistance conferred by this gene (bla_{NDM-1}) therefore aids the expansion of bacteria that carry it throughout a human host, since they will face less opposition/competition from populations of antibiotic-sensitive bacteria, which will be diminished by the original antibacterial treatment.

Origin and Spread

The NDM-1 enzyme was named after New Delhi, the capital city of India, as it was first described by Yong et al. in December 2009 in a Swedish national who fell ill with an antibiotic-resistant bacterial infection that he acquired in India. The infection was unsuccessfully treated in a New Delhi hospital and after the patient's repatriation to Sweden; a carbapenem-resistant *Klebsiella pneumoniae* strain bearing the novel gene was identified. The authors concluded that the new resistance mechanism "clearly arose in India, but there are few data arising from India to suggest how widespread it is. In March 2010 a study in a hospital in Mumbai found that most carbapenem-resistant bacteria isolated from patients carried thebla_{NDM-1} gene [14]. In May 2010 a case of infection with *E. coli* expressing NDM-1 was reported in Coventry in the United Kingdom [15]. The patient was a man of Indian origin who had visited India 18 months previously, where he had undergone dialysis. In initial assays the bacteria was fully resistant to all antibiotics tested, while later tests found that it was susceptible to tigecycline andcolistin.

The authors warned that international travel and patients' use of multiple countries' healthcare systems could lead to the "rapid spread of NDM-1 with potentially serious consequences". As of June 2010, there were three reported cases of Enterobacteriaceae isolates bearing this newly described resistance mechanism in the US, the CDC stated that "All three U.S. isolates were from patients who received recent medical care in India." However, US experts have stated that it is unclear if this strain is any more dangerous than existing antibiotic-resistant bacteria such as methicillin-resistant Staphylococcus aureus, which are already common in the USA. In July 2010 a team in New Delhi reported a cluster of three cases of *Acinetobacter baumannii* bearing bla_{NDM-1} that were found in the intensive care unit of a hospital in Chennai, India in April 2010. As previously, the bacteria were fully resistant to all the aminoglycoside β-lactam and quinolone antibiotics, but were susceptible to tigecycline and colistin.

This particularly broad spectrum of antibiotic resistance was heightened by the strain bearing expressing several different resistance genes in addition to bla_{NDM-1} [16]. In early August 2010 a chemical compound GSK 299423, was found to significantly fight against antibiotic-resistant bacteria by making such bacteria unable to reproduce, citing a likely treatment to the NDM-1 strain. On September 6, 2010 Japan detected its first ever case of the NDM-1 enzyme. In May 2009, a Japanese man in his 50's who had recently returned from holidaying in India was struck with a fever and hospitalized, later making a full recovery. Hospital officials have confirmed that tests carried out after the patient's recoveries were positive for the NDM-1 enzyme [17].

Antibiotic resistance

Antibiotic resistance is a type of drug resistance where a micro organism is able to survive exposure to an antibiotic. Genes can be transferred between bacteria in a horizontal fashion by conjugation, transduction, or transformation. Thus a gene for antibiotic resistance which had evolved via natural selection may be shared. Evolutionary stress such as exposure to antibiotics then selects for the antibiotic resistant trait. Many antibiotic resistance genes reside on plasmids, facilitating their transfer. If a bacterium carries several resistance genes, it is called multiresistant or, informally, a superbug. The primary cause of antibiotic resistance is antibiotic use both within medicine and veterinary medicine.

The greater the duration of exposure the greater the risk of the development of resistance irrespective of the severity of the need for antibiotics. The widespread use of antibiotics both inside and outside of medicine is playing a significant role in the emergence of resistant bacteria. Antibiotics are often used in rearing animals for food and this use among others leads to the creation of resistant strains of bacteria. In some countries antibiotics are sold over the counter without a prescription which also leads to the creation of resistant strains. In supposedly well-regulated human medicine the major problem of the emergence of resistant bacteria is due to misuse and overuse of antibiotics by doctors as well as patients. Other practices contributing towards resistance include the addition of antibiotics to the feed of livestock. Household use of antibacterials in soaps and other products, although not clearly contributing to resistance, is also discouraged (as not being effective at infection control).Also unsound practices in the pharmaceutical manufacturing industry can contribute towards the likelihood of creating antibiotic resistant strains.

Certain antibiotic classes are highly associated with colonisation with superbugs compared to other antibiotic classes. The risk for colonisation increases if there is a lack of sensitivity (resistance) of the superbugs to the antibiotic used and high tissue penetration as well as broad spectrum activity against "good bacteria" [18].

DISCUSSIONS AND CONCLUSION

It may be concluded that New Delhi Metallo-beta-lactamase (NDM-1) is an enzyme which makes bacteria resistant to a broad range of beta-lactam antibiotics. This includes antibiotics of the carbapenem family, which are a mainstay for the treatment of antibiotic-resistant bacteria. NDM-1, which stands for New Delhi metallo-beta-lactamase-1 is a gene (DNA code) carried by some bacteria. If a bacteria strain carries the NDM-1 gene it is resistant to nearly all antibiotics, including carbapenem antibiotics - also known as antibiotics of last resort. Carbepenems are the most powerful antibiotics, used as a last resort for many bacterial infections, such as *E. coli* and *Klebsiella*. The NDM-1 gene makes the bacterium produce an enzyme which neutralizes the activity of carbepenem antibiotics. The gene for NDM-1 is one member of a large gene family that encodes beta-lactamase enzymes called carbapenemases. Bacteria which carry such genes are often referred to in the news media as "superbugs", since infections with these bacteria are very hard to treat successfully. Indeed, the United Kingdom Health Protection Agency has stated that "most isolates with NDM-1 enzyme are resistant to all standard intravenous antibiotics for treatment of severe infections." The virtual non-existence of antibiotic policies and guidelines in India to help doctors make rational choices with regard to antibiotic treatment is a major driver of the emergence and spread of multidrug resistance in India. This is augmented by the unethical and irresponsible marketing practices of the pharmaceutical industry, and encouraged by the silence and apathy of the regulating authorities.

The only way to currently combat the spread of NDM-1 is through surveillance, prompt identification and isolation of infected patients, disinfecting hospital equipment, and thorough hand-hygiene procedures in hospitals. This is going to be a challenge and will require international cooperation. Microbiologists in India have a very important role in the prevention of spread of these dreaded multiresistant pathogens across the world. They should actively participate in the clinical decision making with regard to the treatment of infections, influence the policies and approach to infections and antimicrobials by the government, develop guidelines for antibiotic therapy in their local hospitals, become infection-control doctors, set up surveillance systems for drug-resistant organisms, and educate healthcare workers and the general public about the dangers of multidrug resistant organisms, including hospital-acquired infections. So far, doctors in the UK have managed to fight these infections with a combination of several different medications. However, scientists have detected some bacterial strains that are resistant to all antibiotics. The only way to currently combat the spread of NDM-1 is through surveillance, prompt identification and isolation of infected patients, disinfecting hospital equipment, and thorough hand-hygiene procedures in hospitals. This is going to be a challenge and will require international cooperation.

Actions and Alert

- Be alert to the increase in carbapenemase-producing Enterobacteriaceae, and the growing importance of NDM - 1 enzyme.
- Recognise exposure to healthcare systems in India and Pakistan as additional major risk factors for infection or colonization with multiresistant, carbapenemase-producing Enterobacteriaceae
- Patients infected with producers should be isolated to prevent onward transmission in hospitals; carriage in the patient's faecal flora should be examined for producers of the same or different species; similar screening of close unit contacts should be strongly considered.

ACKNOWLEDGEMENT

The authors are sincerely thankful to Mr. Dilip Singh, honourable Chairman and Secretary of Aligarh College of Pharmacy for their valuable guidance, co-operation and help in providing facilities to utilize the library and internet in the college.

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