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Review article

New Delhi metallo beta- Lactamase-1; Incidence and threats.

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ABSTRACT

Abstract: Carbapenems are among the few useful antibiotics against multidrug resistant Gram negative bacteria particularly those with extended spectrum beta-lactamase. Resistance to carbapenems is mediated by loss of outer membrane proteins and production of beta lactamase that is capable of hydrolyzing carbapenems. Many of the patients were hospitalized in Asia pacific region, had new type of metallo beta lactamase designated as New Delhi Metallo-1. Further, the broad resistance carried on these plasmids is a matter of concern for India. Although, beta-lactams have been widely used as the mainstay of treatment for several bacterial infections, carbapenems often becomes last resort. Carbapenem resistance due to acquired carbapenemases has emerged, spread since 2000, particularly from hospital-acquired infections. Carbapenemases differ from one another, including enzymes from class B (metallo-beta-lactamases, MBLs), class A and class D (serine carbapenemases). The most prevalent carbapenemase was in Enterobacteriaceae, KPC-type class-A carbapenemase, from *Klebsiella pneumonia*, especially in the United States, Asia, the United Kingdom, Israel and southern Europe. Globalization and population migration is one of the major factors in disseminating antimicrobial drug-resistant bacteria. The New Delhi metallo-beta-lactamase (NDM-1) is a novel type of MBL named after the city of origin. Since an alarming report was from Asia Pacific region, and worldwide dissemination of a new 'superbug' the issue is of concern. Since August 2010, spreading and dissemination has occurred, from all the continents, including the United States and Canada, European countries, Japan, Africa, Oman and Australia. Although, one reported death due to the bacterium carrying NDM-1, reported (Belgian man), concern is the casualty and the trip to these regions is a matter of question. In the present review paper we report the types of NDM-1, genes involved for the resistance, mechanism of resistance, epidemiology, Laboratory diagnosis, treatments and possible control measures of NDM-1.

1. Introduction

Antibiotic resistance of bacteria is commonly seen in daily medical practice. Among the different types of drug resistance, most microbiologists would agree that multi drug resistance Gram-negative bacteria pose the greatest threat to human health. In addition, there are fewer effective drugs developed specifically against Gram-negative bacteria [1]. During the past decade the increase of antibiotic resistance in enterobacteriaceae has become a major concern worldwide. An evolution of antibiotic resistant bacteria has resulted in the need for new antibiotics. β-lactam based drugs are the most predominantly prescribed antibiotics to combat bacterial infections. However, production of β-lactamases, which catalyzes the hydrolysis of the β-lactam bond of this class of antibiotics, by *Bacillus cereus*, *Escherichia coli*, *Klebsiella pneumoniae* are rendering them useless [2].

The wide spread use of carbapenems, the only agents reliably active against these bacteria, resulted in the emergence of a new antibiotic resistance mechanisms [3]. Carbapenem resistance due to acquired carbapenemases has emerged and spread world wide since the early 2000s [4] and the number of bacteria that produce metallo- β-lactamase (MBL) is on the rise [2]. The New Delhi Metallo- β-lactamase (NDM-1) is a novel type of MBL named after the city of origin, which has been recently criticized, following a common practice with transferable MBLs since VIM-1 was named after Verona, Italy. It was first reported in 2009 in Swedish patient .

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who travelled to New Delhi and acquired a urinary tract infection due to a carbapenem resistant *K. pneumoniae* strain resistant to all antibiotics tested except colistin [4]. Since then, there have been an increasing number of infections in patients from India, Pakistan and the United Kingdom [3]

2.Types of NDM-1

Extended-spectrum- β-lactamase (ESBLs) represent a major group of β-lactamases currently being identified world wide in large numbers, and are now found in significant percentage of *E .coli* and *K. pneumoniae* strains. They confer resistance through the genes residing in the plasmids [5]. Plasmid mediated β-lactamases conferring resistance to the third generation antibiotics like cephalosporins and monobactams. As a result, infections caused by such strains are very difficult to treat them and become a serious problem for hospitals worldwide [6]. Carbapenemases have been now studied in depth and widely differ from one another, including enzymes from class-B (Metallo-beta-lactamase, MBLs), Class A and class D (serine carbapenemase). The most prevalent carbapenemase so far in enterobacteriaceae is the KPC- type Class A carbapenemase, which has been found in *K. pneumoniae*, especially in United States, Asia, the United Kingdom, Israel and south Europe [4].

Extended-spectrum β-lactamases (ESBLs), such as the plasmid mediated Class- A TEM and SHV- derived enzymes have been reported by Tash and Bahar [7]. Although, ESBLs have been reported from round the globe and emerging as a serious problem, their ability to cause intense infections in endemic areas are yet to resolve. Today, over 150 different ESBLs have been described; almost all ESBLs are the derivative of the TEM or SHV enzymes. There are now more than 90 TEM derived β-lactamases and more than 25 SHV derived enzymes [7]. Recently, Espinal et al [8] identified a new variant of NDM-1 in *Acinetobacter baumannii* and designated as NDM-2. They reported that, the clonal dissemination of a NDM-2 producing *A. baumannii* was isolated in an Israeli rehabilitation ward. Kasse et al [23] revealed the sequence of NDM-2 by PCR and that variant had a C to G substitution at position 82 resulting in an amino acid substitution of proline to arginine at

3.Geographical Distribution

Rolain et al [4] suggested that the acquired carbapenemases have been mainly restricted to certain geographical areas and to specific bacterial species, and outbreaks as well as spread in other countries have been often associated with imported cases from countries where the bacteria have been endemic. Population mobility is known to be a main factor in globalization and in the same way spreading of antimicrobial drug-resistant organisms. For example, the emergence of KPC- producing enterobacteriaceae in the United States in 2001 could be later associated with the emergence of travel related outbreaks in other countries. A multidrug-resistant *Escherichia coli* isolate recovered in Australia produced a carbapenem-hydrolyzing β -lactamase. Molecular investigations revealed the first identification of the blaNDM-1 metallo- β -lactamase gene in that country. In addition, this E. coli isolate expressed the extended-spectrum β -lactamase CTX-M-15, together with two 16S RNA methylases, namely ArmA and RmtB, conferring high level of resistance to aminoglycosides Emergence of metallo-β-lactamase NDM-1-producing 3 multidrug resistant Escherichia coli in Australia [9].

4.Genes Responsible for Resistance

The rapid increase of drug resistance in enterobacteriaceae is mainly attributed to the genes located on plasmids that can subsequently and quickly spread in different bacterial species perhaps through genetic recombination [1]. The NDM-1 encoding gene is located on different positions of plasmids (a 180-kb plasmid for *K. pneumoniae* and 140 kb for *E. coli*) that are easily transferable to susceptible *E. coli* J53 at a high frequency [10]. These plasmids also harbor genes conferring resistance to almost all antibiotics, thus making their rapid dissemination in clinically relevant bacteria, resulting in a serious threat to therapy. Most plasmids detected in these bacteria were easily transferable and capable of wide rearrangement, suggesting a widespread transmission and plasticity among bacterial populations. Because this carbapenemase is encoded by a genetic element found on different plasmids that may duplicate or jump from bacteria to bacteria easily. Further, rapid dissemination and spread between different bacterial species by lateral gene transfer has below favored by globalization and travel represent a high risk of a world wide pandemic among enterobacteriaceae [4]. So far, NDM-1 carbapenemase has been detected in *K. pneumoniae*, E. coli, *Citrobacter freundii*, *Enterobacter cloacae*, and *Moraxella morganii* and has been resistant to nearly all classes of antibacterial agents, except polymixins and tigecycline.

A new EHEC serotype O104 *E. coli* strain has been reported and compared with the EAEC 55989 *E. coli* strain. The genome of this new *E. coli* was found to be around 5.2 Mb. As these genes conferring resistance to many antibiotics, have the ability to transfer from one bacterium to another intergenerically and pose a greater clinical threat than chromosomally encoded enzymes. Two metallo- β-lactamases genes imp and vim have been shown to be mobilized by integrons. The majority of resistance genes in enterobacteriaceae are carried on class-I integrons, and this is also true for the carbapenemases, such as VIM-1/- VIM-4. Majority of the mobile MBL genes are found as gene cassettes. These include bla-IMP, bla-GIM, bla -SIM and bla- KMH. The exception to these are genes encoding AIM-1 and SPM-1, which were adjacent to and thought to be mobilized by ISCR elements via a transcription event called rolling circle recombination [10]. As many as nine IMP variants have been reported throughout Southeast Asia and more recently in Europe and Canada. Three variants of VIM have also been documented. VIM-1 and VIM-2 have been reported in strains of *P. aeruginosa* across Europe and VIM-3 as in *P. aeruginosa* strain in Taiwan [11].

5.Mechanisms of Antibiotic Resistance

The resistance patterns of ESBL producing bacteria were remarkable for the high rate of coresistance to other classes of antibiotics. Approximately 60% of ESBL-producing *E. coli* was resistant to quinolones. The resistance of these bacteria to

piperacillin, tazobactam was about 63.3% which may reflect coexistence of Amp C type enzyme with ESBLs in some strains. Other mechanisms have also been postulated which may include ESBL-hyper production, change in outer membrane porins, or inhibitor-resistant TEM enzymes [6]. *Pseudomonas aeruginosa* is an opportunistic pathogen associated with a range of nosocomial infections. Carbapenems, including meropenem and imipenem, are the most effective antibiotic against this organism. However, resistance to carbapenems has emerged by altogether different mechanisms such as impermeability to drug due to loss of Opr D porin, the up-regulation of an active efflux pump system presenting the cytoplasmic membrane of these organisms. Production of metallo-beta-lactamases (MBLs) that hydrolyze all carbapenems [12, 13].

6.Epidemiology

There is a need to study the epidemiology of extended spectrum β-lactamase producing enterobacteria (ESBL-PE) as antibiotic resistance is increasingly problematic in health care institutions. Novel ESBLs gene variants have also been emerged. Most ESBLs are variants of the classical TEM-1 and SHV-1 β-lactamases, with one or more amino acid substitutions that confer resistance to broad spectrum cephalosporins and monobactams. Almost all SHV coding ESBL genes have G/A mutations, which would indicate glycine/serine and glutamate/lysine substitutions at amino acid 238 and 240 (SHV-5) confer a large increase in resistance to ceftazidime. These mutations have been documented in clinical isolates of *K. pneumoniae* from hospitals in Mexico and have been implicated in outbreaks with mortality. Further 101 different SHV mutants have been reported worldwide [14].

Most plasmids detected in these bacteria were easily transferable and capable of wide rearrangement suggesting a widespread transmission among these bacterial populations. Rolain et al [4] suggested that, among the 25 patients detected in the UK, 17 patients had traveled to India or Pakistan within one year and 14 had been hospitalized, showing a world wide dissemination of a new "super bug" from a local source in Asia. Indeed, since August 2010, spreading and dissemination has occurred, with several cases being reported by national and international media from other countries in all continents, including US, Canada, Europe, Japan, Africa, Oman and Australia. Further, recent report suggested that a *K. pneumoniae* NDM-1 positive strain has been isolated from a patient repatriated in Marseille, France in April 2010, after an accident in New Delhi. To date there is only one reported death attributed to an infection with a bacterium carrying NDM-1 occurring in a Belgian being repatriated in Belgium after a car accident during a trip in Pakistan [4]. Kumarasamy et al [15] reported that not all patients infected with NDM-1 positive bacteria have a history of hospital admission in India, and extended spectrum beta lactamases are known to be circulating in the Indian community through drinking water and seepage samples. A group of scientists collected around 171 seepage samples and 50 tap water samples from New Delhi and 70 sewage effluent samples from Cardiff waste water treatment works and subjected to analysis for the presence of NDM-1 gene. They found that, there are two of 50 drinking water samples contain bla (NDM-1) and 51 of 171 seepage samples from New Delhi; the gene was not found in any sample from Cardiff. The presence of NDM-1 β-lactamase producing bacteria in environmental samples in New Delhi has important implications for people living in the city who are reliant on public water and sanitation facilities. *E. coli* O104 first emerged as a pathogen in a small outbreak in Helena in early 1994. Four people developed abdominal cramps and bloody diarrhea. Experts at the U.S. Center for Disease Control and Prevention (CDC) in Atlanta identified a serotype called O104:H21 was the etiological agent. CDC investigation later found that among 18 patients, most of them were women and the median age was 36 years. The source of infection in Germany is still unclear, and the rare serotype could make it harder for authorities to find it. "O104 is very hard to distinguish from normal, non-pathogenic *E. coli*," reported by Lothar Beutin, head of the National Reference Laboratory for E .coli at the Federal Institute for Risk Assessment (Disease Control and Prevention). Epidemiological surveillance data from Europe (EARS-Net) and other parts of the world indicates that antibiotic resistance was increasing high in enterobacteriaceae members like E. coli, and K. pneumoniae. These bacteria which are the part of normal human gut flora are also commonly causing several infections such as urinary tract infections, bloodstream infections or health care infections (Epidemiological update, 2010) (Fig.1).

Fig 1. Global spread of the New Delhi metallo-beta-lactamase encoding gene (NDM-1).

(http://onlinelibrary.wiley.com/doi/10.1111/j.1469-0691.2010.03385.x/full) [22]

7.Serotypes among NDM-1 bacteria

According to the results of the current draft assembly the estimated genome size of this new *E. coli* strain is about 5.2 Mb. Sequence analyses indicated that the bacterium was EHEC serotype O104 *E. coli* strain; however, this was conclude as new serotype earlier not involved in any E. coli outbreaks. Comparative analysis showed that this bacterium has 93% sequence similarity with the EAEC 55989 *E. coli* strain, which was isolated from Central African Republic and known to cause serious diarrhea. This new strain of *E. coli*, however, has also acquired specific sequences that appear to be similar to those involved in the pathogenicity of hemorrhagic colitis and hemolytic-uremic syndrome. The acquisition of these genes may have occurred through horizontal gene transfer. The analysis further showed that this deadly bacterium carried several antibiotic resistance genes, including resistance to aminoglycoside, macrolides and Beta-lactam

which makes antibiotic treatment extremely difficult [16]. Center for Disease Control and Prevention in Atlanta had identified a serotype called O104:H21 an etiological agent, causing abdominal cramps and bloody diarrhea.

In addition, another strain of bacteria linked to NDM-1 in UK was *Klebsiella*, present in the gut which contained the superbug NDM-1. *Klebsiella pneumoniae* symptoms include sudden onset of high fever and hemoptysis (currant jelly sputum). Anderson et al [17] reported that particular serotypes and virulence genes in *K. pneumoniae* using real-time PCR for serotyping and detection of virulence genes. PCR targeted the serotypes like K1, K2, K5, K20, and K57 and the virulence genes-rmpA and wcaG. Further, they reported that the presence of mucoid phenotypes was typical. Among these the most commonly encountered serotype was K2 and the most frequent virulence gene was wcaG.

8.Symptoms

Symptoms due to infections caused by NDM-1 producing bacteria reflect the site of infection. Common sites of infection include blood, urinary tract, lungs and wounds. Symptoms do not differ between bacteria that produce NDM-1; however, those affected are more difficult to treat and are at a higher risk of complications. The general symptoms of *E. coli* include, diarrhea usually associated with pain and cramping in the abdomen, nausea and vomiting, urinary tract infections and *K. pneumoniae* symptoms include, fever (can be accompanied by dizziness and chills), cough (usually present with sputum), upper respiratory tract infection, congestion, wheezing, nausea and vomiting.

9.Laboratory diagnosis

The detection of antimicrobial resistance in NDM-1 bacteria has done mainly by phenotypic and genotypic methods [18]. Phenotypic diagnosis of isolates that carry metallo-β-lactamases (MBLs) has been performed using the modified Hidge test, MBL Etest, or Imipenem-DETA Double Synergy Test, antimicrobial disk diffusion methods and determination of minimum inhibitory concentrations (MICs) are the major techniques employed [19]. Genotypic methods could detect the presence of particular genes conferring resistance to specific antimicrobials. An example is the presence of NDM-1 gene, encoding for the New Delhi metallo-beta-lactamase, conferring resistance to carbapenems. Genetic testing can determine the locus and architecture of the resistance mechanism, its transferability and the co-existence of resistance mechanisms of other antimicrobials [18].

Enterobacterial isolates expressing the carbapenemase NDM-1 are emerging worldwide. Twenty-seven NDM-1-positive isolates of worldwide origin were to identify Although susceptibility to carbapenems varied, a combined test (IMP/IMP + EDTA), the Etest MBL, and automated susceptibility testing by Vitek2 (bioMérieux) identified those NDM-1 producers as verified by PCR using specific primers. Screening for carriers of NDM-1 producers may be based on media such as the ChromID ESBL culture medium routinely used to screen for extended-spectrum β-lactamase producers, which gives excellent detection levels with low limits of detection ranging from 8 x 100 to 5 x 102 CFU/ml. The CHROMagar KPC culture medium had higher limits of detection (1 x 101 to 5 x 105 CFU/ml) and may be proposed for the follow-up of outbreaks of infections with NDM-1 producers. Colonies growing on these screening media can be verified as NDM-1 producers with conventional molecular methods as described herein [20].

10.Treatment

Treatment would be guided by the antibiotic sensitivity pattern of the bacteria. Currently it appears that most NDM-1 strains are sensitive to colistin and tigecycline. But, as with all infections, patients should undergo treatment only under the supervision of a physician. The physician would explore the best possible options suited for the individual patients and the type of infection [21].

11.Problems

The problems associated with NDM_{-1}. This may include lack of a routine standardized phenotypic test for MBL detection, the consequent probable high prevalence of unrecognized asymptomatic carriers allowing an international dissemination of such bacteria, the scarcity of available effective antibiotics so far and the possibility to disseminate in many different Gram negative bacteria.

12.Strategies

The few control strategies as follows [4].

1. Early detection by molecular methods, especially real time PCR should be used for the detection of this specific carbapenemase encoding gene, specially for patients returning from Asia, to limit the propagation and dissemination of these alarming "Super bugs".

2. A systematic monitoring of both infected and possible asymptomatic carriers, should be the rule to implement control strategy policies such as contact isolation procedures.

3. Further, it is optimized that all expatriates needs to be examined for the persistence of NDM-1 to enable to limit the spread of the super bug.

An extensive effort is required to document the prevalence of NDM-1 lactamase not only in Asian countries but also globally.

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