Leis Kamil, Mazur Ewelina, Szyperski Paweł, Kaczor Patryk, Kolan Michalina, Wiśniewski Bartłomiej, Jabłońska Magdalena, Kulczyńska Aleksandra, Baska Aleksandra, Kazik Jakub, Bojczuk Daria, Grzelakowska Klaudyna, Kałużny Krystian, Gałązka Przemysław. Metallo-Beta-Lactamases: NDM. Journal of Education, Health and Sport. 2019;9(6):27-40. eISNN 2391-8306. DOI http://dx.doi.org/10.5281/zenodo.3236053
http://ojs.ukw.edu.pl/index.php/johs/article/view/6977
https://pbn.nauka.gov.pl/sedno-webapp/works/914931

The journal has had 7 points in Ministry of Science and Higher Education parametric evaluation. Part B item 1223 (26/01/2017).
1223 Journal of Education, Health and Sport eISSN 2391-8306 7

The authors declare that there is no conflict of interests regarding the publication of this paper.

Received: 05.05.2019. Revised: 25.05.2019. Accepted: 31.05.2019.

METALLO-BETA-LACTAMASES: NDM

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Abstract: New Delhi, an enzyme belonging to the Metallo-beta-lactamases and Carbapenemases group, is most commonly found in Klebsiella pneumoniae and Escherichia

coli. It determines these bacteria resistance to the majority of known antibiotics. NDM-1 was discovered in 2008 in a man treated in New Delhi for E. coli infection. Since the first appearance, 17 subtypes of this enzyme have been discovered. Its occurrence has been reported in Europe (Great Britain, France, Belgium - where even a national alert has been issued) as well as in the United States and in Africa. Most cases of New Delhi enzyme bacterial infections are reported in India or in people who have traveled to this country. There is no treatment method described so far. Therapy is based on antibiotic susceptibility test results. Colistin shows the highest effectiveness against NDM. There are also tests suggesting that Isomargololone and Nimbolide may be effective in most cases of NDM infection.

Key words: metallo beta lactamases, New Delhi, carbapenemes, E. coli

1. Introduction

New Delhi Metallo-beta-lactamases (NDM) are the enzymes that are found mainly in Enterobacteriae - Klebsiella pneumoniae and Escherichia coli, but may also be present in other genera. The most common is NMD type 1 (NDM-1). Its presence determine these bacteria resistance to all beta-lactam antibiotics. NDM was first described in 2008. India is the main source of infection, but the problem with pathogens carrying these enzymes affects the whole world.

2. Metallo-beta-lactamase

These enzymes belong to carbapenemases, which contain metal-zinc in their structure. Hydrolysis is its mechanism of resistance to penicillins, cephalosporins, and carbapenems. However, they are still susceptible to monobactams. Most of them are resistant to beta-lactamase inhibitors, however, they are inhibited by EDTA (edetic acid). This susceptibility is not demonstrated by other beta-lactamases because they lack metal component in their structure **[30, 41]**. Metallo-beta-lactamases are classified according to the 1995 proposal of Bush et al. into group 3 and molecular class B **[3, 31]**. The internal classification of carbapenemases, based on a number of functional and molecular features, divides it into groups A, B, C and D. Table 1 depicts all the groups, together with their representatives **[41]**.

TAB. 1

Table 1. Division of carbapenemases

Carbapenemases	Representatives
A	MSP NMC IMI KPC GES
B (metallo- beta- lactamases)	**NDM** VIM
C	CMY
D (CHDL-carbapenem-hydrolyzing class D β-lactamases)	OXA

[18, 22, 29, 31, 40, 41]

3. NMD-1 structure

The bla NMD-1 gene encodes a single chain polypeptide, built of 269 amino acids with molecular mass of about 27.5 kDa. It is actively present as a monomer with an approximate mass of 28 kDa. The enzyme contains a leader peptide in its structure. It contains a unique additional sequence at positions 162-166 bla NMD-1 numbering. On the one hand, it has hardly common features with other Metallo-beta-lactamases, but on the other, it contains zinc like the rest of them. It is the most closely related to VIM-1 **[14]** and VIM-2 **[54]** produced by K. pneumoniae and Acinetobacter baumanii. The identity is 32.4% **[23, 59]**.

4. Discovery

In 2008, Young et al. presented a report on the IDSA-Infectious Diseases Society of America about the identification of a new Metallo-beta-lactamase group. It was isolated from a 59-year-old patient treated in India, New Delhi, hence the name of the enzyme. He was diagnosed with a Klebsiella pneumoniae urinary tract infection. Following the results of susceptibility testing, it was found that the isolate was resistant to all beta-lactam antibiotics. It was susceptible to only one beta-lactam- aztreonam plus colistin and fluoroquinolones. After microbiological analysis, the bacterial enzyme was classified into the metal-lactamase B1 subgroup **[44, 55, 59]**.

5. Threat

Consecutive cases of infections caused by bacteria containing abovementioned enzymes were reported in the UK **[33]** and in the US **[6]** in 2010 and in South Africa in 2011 **[2]**. Although patients had previously visited India in the first two cases, disease is not confined to this one particular region but affects the whole world. In Belgium, a national alert was issued in 2010 due to the NDM isolation **[1]**. In subsequent years there have been reports of bacteria producing those enzymes in various parts of the world **[12, 19, 39]**. Travelling from India to other countries may play an important role in NMD spreading **[28]**.

This enzyme is also a significant problem because of the asymptomatic carriers, lack of effective pharmacotherapy and its potential presence among many Gram-negative bacteria. Its existence has been identified in for instance: Providencia spp. (for example P. rettegri) **[25]**, Proteus spp., Klebsiella oxytoca, Citrobacter freundii, Morganella morgani **[44]**, Salmonella enterica **[4]** and Acinetobacter baumanii **[17]**. In 2010, Kumarasamy et al. presented a report about 180 cases of New Delhi's (NMD-1) isolates. 111 of them were identified in K. pneumoniae and 36 in E. coli **[24]**.

Bacteria with NMD-1 may be responsible not only for urinary tract infections but also diarrhea, peritonitis, lung infections, sepsis, and soft tissue infections **[37]**. The disease is not age specific. There are cases of infection among pediatric patients **[32]**, including newborns **[45]**. Seema et al. found that the majority (35.1%), of NDM bacterial infections, occur in patients hospitalized in intensive care units **[46]**. The youngest patient in their study, infected with NDM, was one day old, and the oldest was 85 years old.

NDM-containing pathogens are also capable of infecting animals **[47]**. Yousfi et al. described the case of an E. coli NDM-5 infection in Algerian dog **[60]**.

Walsh et al. published the results of water tests from New Delhi. They collected tap water, seepage and sewage effluent samples. The NMD-1 gene was detected in two of 50 drinking-water samples and in 51 of 171 seepage samples. This indicates the capital of India as a particularly dangerous source of pathogens containing NMD-1 enzyme **[56]**.

In addition, it was found that the carriage by Enterobacteriae spp., Aeromonas spp. and Vibrio cholerae was stable and associated with resistance patterns typical for NMD-1. Unstable gene transmission occurred in non-fermenting bacteria. The best temperature for transfer turned out to be 30 ° C. Researchers identified 20 different strains of microorganisms in the samples, 12 of which carried genes on plasmids and two of them (Vibrio cholerae and Aeromonas caviae) on chromosomes **[56]**.

In 2011, a Canadian woman was diagnosed with K. pneumoniae, after visiting India. Initially, diarrhea appeared during her stay in this Asian country. Then congestive heart failure and hypertension developed. She was readmitted to the hospital later due to unspecified encephalitis, decreased consciousness, urinary tract infection, and ongoing diarrhea. There was also a fever and a blood pressure 100/80 mm Hg. Urine culture grew highly drug-resistant K. pneumoniae (colistin-sensitive) and stool specimen was positive for Clostridium difficile. After some time, E. coli beta-lactamase (+) co-infection was also confirmed. Ultimately, after the attempts of combined treatment with antibiotics, the patient died. This is a proof that NDM-1 pathogens infections can be very severe, involving many systems, and that the co-infection may occur **[34]**.

Another case was 75-year-old patient from the UAE. He presented with pneumonia, respiratory failure, stroke and septic shock. The causative agent was NMD-5 producing K. pneumonia and was contained by colistin and meropenem **[8]**.

Some bacteria containing the bla NDM gene, have a ble MBL gene encoding a BRP MBL protein that determines resistance to bleomycin **[10]**. This gene, which determines antibiotic resistance, may also coexist with the bla OXA 48 and bla OXA 181 genes, respectively forming enzymes: OXA 48 and OXA 181 **[5, 11, 50]**.

6. NDM variants

NDM occurs in several variants. The main type is NDM-1, but recently there has been also NDM-2, NDM-3 **[43]**, NDM-4 (differing from NDM-1 by a single amino acid substitution at position 154) **[35]**, NDM-5 described. Hornsey et al. described a case of multidrug-resistant E. coli ST648 isolate, designated EC405. Researchers have come to the conclusion that they stumbled upon a new subtype of New Delhi Metallo-beta-lactamase. This variant of the enzyme differed from existing enzymes due to amino acids substitutions at positions 88 and 154, and reduced susceptibility to antibiotics. This discovery took place in 2011 in the UK (the patient was previously hospitalized in India). In addition, EC405 was found to contain part of IS Aba125, most likely derived from Acinetobacter baumanii **[16]**.

NDM-2 was isolated from Acinetobacter baumanii. The first reports of this variant appeared in 2011. The bla NDM-2 gene is surrounded by two copies of IS Aba 125. Compared to NDM-1 in NDM-2 there is an amino acid substitution at positions 28 and 82.

Espinal et al. reported the identification of this variant in Israel and Kaase et al. described it in Egypt in patients with no previous travel or hospitalization on the Indian subcontinent **[13, 20]**.

Rahman et al. asserted that up until 2014, seven types of NDM (1-7) had been identified. They examined 464 samples from India and claimed that NDM 5-7 is more common in E. coli, and more common in patients hospitalized for urological reasons. The NDM-7 enzyme also appeared in Europe – f.eg. in France and in Germany **[9, 15, 42]**.

NDM-8 have been found in E. coli isolated from the respiratory tract of Nepali patient. This variant differed from NMD-1 by substitution at positions 130 and 154 **[51]**.

In 2014, Wang et al. described the new NDM-9 variant. The enzyme was detected in K. pneumoniae in China **[57]**.

In 2016, Yao et al. reported NDM-9 detected in E. coli (colistin resistant), which also produced MCR-1 and Khajuria et al. detected NDM-10 in K. pneumoniae isolate **[21, 58]**.

NDM-12 and NDM-13, produced by E. coli, have also been described, both in Nepal **[49, 52]**. The first variant showed differences in the amino acid sequences (from NMD-1) at positions 154 and 222. The NDM-12 bla gene was located on a plasmid. The second subtype gene, NDM-13, was found on chromosomes. Changes when comparing to NDM-1 were at positions 95 and 154. NDM-14 was also isolated from Acinetobacter lwolffi in China **[61]**. It demonstrates a single change (vs. NMD-1) in the amino acid sequence – at a position 130.

In 2017 a variant 17 of the New Delhi enzyme was discovered. It was identified in E.coli strain from chicken in China. Compared to NDM-1, NDM-17 had three amino acid substitution (positions 88, 154, 170) that significantly increased their carbapenemase activity **[27]**.

7. Treatment

This enzyme determines bacteria's resistance to all beta-lactam antibiotics. In some cases, they are susceptible to aztreonam, but usually, they produce beta-lactamase that hydrolyzes it **[48]**.

In the treatment of NDM-containing bacteria, there is no particular algorithm or standard antibiotic therapy. In the enzyme presence - susceptibility test should be made, to look for effective treatment. There are some examples of infections incidents and treatment listed below.

In 2013, Rogers et al. reported two cases of urinary tract infection with Enterobacteriaceae harboring New Delhi bla NDM-1 genes (Enterobacter cloacae) and previously undescribed enzyme variant bla NDM-3 (Escherichia coli). Both patients lived in Australia and traveled to the Indian Subcontinent. Oral therapy with nitrofurantoin was successful in one case, while a combination of colistin and rifampicin was required in the second patient. **[43]**.

Muir et al. described a case of NMD-1 bacterial infection in the United Kingdom. After performing susceptibility testing, they found that the microorganism was completely resistant to all antibiotics except for colistin and tigecycline **[33]**.

In 2012, Nordmann et al. described a case of a woman from southern France, with cystitis due to K. pneumoniae, harboring NDM-1 and CTX-M-15 genes located on two different plasmids. Neither this patient nor her husband had traveled to any country in the previous 3 years, including countries with a high prevalence of NDM-1 producers. She also denied having a diet containing Indian food. It may correspond to the ultimate spread of NDM-1 producers worldwide - outside its main reservoir. The Vitek-2 automated susceptibility testing showed multidrug resistance. However, the pathogen exhibited susceptibility to antibiotics such as fosfomycin, colistin, tetracycline, amikacin, and tigecycline **[36]**.

Peirano et al. presented a case of a young man who reported, as did the other patients mentioned above in this review, a trip to India in an interview. Pyelonephritis and prostatitis caused by NDM-1 producing E.coli were diagnosed. Antimicrobial drug susceptibility determined with the VITEK 2 and Etest AB Biodisk confirmed the multiresistant nature of these bacteria. Fortunately, the patient was successfully treated with ertapenem and fosfomycin combination therapy (even though in the Vitek-2 E. coli MH01 test showed resistance to ertapenem and sensitivity to tigecycline and fosfomycin) **[38]**.

Chen et al analyzed more than 11,000 E. coli, K. pneumoniae, A. baumanii and P. aeruginosa isolates. The bla NDM-1 gene was detected on plasmids of 4 strains of A. baumanii. Analysis showed that three of them were sensitive to aminoglycosides, fluoroquinolones and colistin **[7]**.

Lascols et al. analyzed the effects of antibiotics on strains carrying the bla NDM-1 gene. 72.7% were susceptible to colistin and 69.7% to tigecycline. Whereas 9.1% were resistant and 51.5% were susceptible to both of them **[25]**.

Abovementioned findings suggest that each NMD producer is sensitive to different antibiotics, thus each infection should be treated individually accordingly to antibiotic susceptibility testing result. Although colistin is the most effective drug, it is not a gold standard and there are cases of resistance as in NDM-9 **[26, 57]** due to the presence of MCR-1.

Currently, there are ongoing studies to find a drug effective in New Delhi enzymes producers treatment. Tests have shown that Nimbolide and Isomargololone have a high affinity to NDM, and may be effective NDM inhibitors **[53]**.

8. Summary

Bacteria carrying NDM enzymes are an important clinical problem. Recently discovered Metallo-beta-lactamase New Delhi group is more commonly occurring and differ in its variants. Up to date, infections by several forms had been described. Due to its multidrug resistance, New Delhi infections are associated with severe clinical outcomes, often resulting in death and making it difficult to find effective treatment. Moreover, these enzymes, though predominantly occurring in E. coli and K. pneumoniae, can be produced by many bacteria and the infections may have a different course and affect any age group. However, there is an ongoing search to find an inhibitor that offers the chance for effective treatment.

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