Archives of Pediatric Infectious Diseases. 2014 January; 2(1):188-91. DOI: 10.5812/pedinfect.5193

Published Online 2014 January 08. **Discussion**

Carbapenem-Resistant Bacteria and Laboratory Detection Methods

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Received: April 28, 2012; **Revised:** January 01, 2013; **Accepted:** July 02, 2013

Carbapenemase producing bacteria frequently possess resistance mechanisms to a wide-range of antibacterial agents, and are associated with high mortality and morbidity rates. Some enzymes like extended-spectrum β-lactamase (ESBL), New Delhi metallo-beta-lactamase-1 (NDM-1), *Klebsiella pneumoniae* carbapenemase (KPC), and OXA-producing *Enterobacteriaceae* are very important in frequently isolated nosocomial bacteria, which cause resistant to many classes of drugs. NDM-1 or KPCs, is a worldwide health problem which is slowly increasing. There is no vaccine to prevent infections produced by carbapenem-resistant bacteria.

Keywords: Beta-lactamase NDM-1; Plasmids; Drug Resistance, Microbial

1. Introduction

1.1. Emergence of Carbapenem Resistance

Carbapenemases, in gram-negative bacterial clinical strains are an increasing concern because they confer resistance to all beta-lactam antibiotics. The problem of dissemination of carbapenemases in several Mediterranean countries was observed mainly in *P. aeruginosa*. Later on, the most common carbapenemase in Europe among *Enterobacteriaceae* was Verona integron-encoded metallo-β-lactamase (VIM) among *K. pneumoniae* and *K. pneumoniae* carbapenemase (KPC). Other particularly problematic carbapenemases are the New Delhi metallo-β-lactamase (NDM), which is highly prevalent in the Indian subcontinent and the Middle East. The OXA-48-like enzymes have caused outbreaks in several European countries and are now spreading rapidly (1). Resistance to carbapenems has recently emerged in Enterobacteriaceae and has resulted in serious clinical consequences as carbapenems are practically the last option to treat infections due to these bacteria. The Ambler classes A, B, and D β-lactamases are classes of carbapenemases in *Enterobacteriaceae*. There are various classes of A carbapenemases: some of them are chromosome-associated (NmcA, Sme, IMI-1, SFC-1), and others are encoded by plasmid (*Klebsiella pneumoniae* carbapenemases [KPC], IMI-2, GES, derivatives). Anyway, Carbapenems are hydrolyzed by all of them and Clavulanic acid can partially inhibit the effect of these enzymes (2). Class B metallo-β-lactamases (MBLs) are mostly encoded by VIM and IMP types. In addition, the New Delhi metallo-β-lactamase-1 (NDM-1) has been added as a new metallo-β-lactamase type. In 1991, acquisition MBL and IMP-1 in Serratia marcescens had been reported for the first time. After that time MBLs have been reported worldwide. All β-lactams are hydrolyzing by these enzymes, exceptionally Aztreonam. Ethylenediaminetetraacetic acid (EDTA) can inhibit their activity but Clavulanic acid cannot do that (2,3). Class D types are most encoded by OXA-48 and OXA-181. Now, OXA-48 producers distribute in Europe, in the southern and eastern part of the Mediterranean sea, and Africa. But they have not been reported from the United States and Canada. OXA-48 producers were isolated from hospitalized patients who were hospitalized in endemic source. OXA-181 a point mutant analog of OXA-48 has been isolated from India or Indian origin. Carbapenems and broad-spectrum Cephalosporins, such as Ceftazidime, and Aztreonam are weakly hydrolyzed by OXA-48/OXA-18. EDTA or Clavulanic acid (resistance to Amoxicillin/Clavulanic acid) cannot inhibit their activ-

Implication for health policy/practice/research/medical education:
As remarkable results; the importance of these enzymes such as NDM-1 and KPC are comparable with HIV, tuberculosis and malaria. There is no vaccine to prevent infections produced by bacteria which have carbapenemases, yet. So, it is necessary to inspect phenotyping and genotyping NDM-1 resistant pattern world-wide.

ity. OXA-48 producers are most in *K. pneumoniae* and E. coli. Identification of OXA-48–type producers is difficult. Therefore, true prevalence estimation is difficult too (4, 5).

2. What is NDM-1?

Some bacteria carry a gene (DNA code) named NDM-1, which stands for New Delhi metallo-beta-lactamase-1. NDM-1 producing bacteria are resistant to nearly all antibiotics, including carbapenem antibiotics which are also known as antibiotics of the last resort. Because, NDM-1 gene makes the bacterium produce an enzyme which neutralizes the activity of carbapenem antibiotics. These bacteria are the most powerful superbug around. Such bacteria are usually susceptible only to "Polymyxin" polymyxins and “Tigecycline" tigecycline. Recently, emergence of NDM-1 enzymes is considered as a major threat, because bacteria which possess this beta-lactamase are resistant to almost all β-lactam drugs (6 ,7) like Fluoroquinolones and Aminoglycosides (8), except Aztreonam (7,9), Tigecycline and Colistin. However, bacteria could be resistant to the last three drugs (10) and also Fosfomycin (11). In 2009, the NDM-1 enzyme was reported for the first time. It was detected in a Swedish patient who had traveled to India and was hospitalized in New Delhi during 2007. He acquired urinary tract infection (UTI) due to carbapenem resistant *Klebsiella pneumoniae* (12). *Enterobacteriaceae* NDM-1 producers cause a wide range of infections such as septicemia, UTI, diarrhea, pulmonary infections, peritonitis, devices- associated and soft tissue infections (9). The bacteria with NDM-1 enzyme are known as superbugs and public health must pay more attention to them (13), and centers for disease control and prevention (CDC) have guidelines to control strains with NDM-1 (6). The most common types of infections with NDM-1 producer's bacteria have been reported in adults, but blaNDM-1 in two strain of *K. pneumonia* was reported from a neonatal intensive care unit (NICU) in India (14). More than 6 different NDM allotypes are known (Table 1).

Table 1. NDM-type β-Lactamases (Reprinted from www.lahey.org/studies/).

Enzyme	Nucleotide	Reference
NDM-1	FN396876	AAC 53:5046-5054, 2009
NDM-2	JF703135	JAC 66:1260-1262, 2011
NDM-3	Assigned	
NDM-4	Assigned	
NDM-5	JN104597	
NDM-6	JN967644	

The NDM-1 gene is carried on a plasmid or chromosome. The rapid emergence of the NDM-1 gene has been related to movable plasmids which can move among different bacteria, subsequently, this gene can spread throughout the world (7). Plasmids with the NDM-1 gene can be transmitted to other bacteria, even to the human intestinal flora. There are about 10 – 100 trillion bacteria as human normal flora which can be causative of many problems in human. It has been estimated that about 100 million Indian carry bacteria with NDM-1 gene as normal gut flora (15). The blaNDM-1 gene is located on different plasmids and can harbor a large number of resistance genes. These genes are carbapenemase genes (OXA 48, OXA 181 and VIM). Aminoglycoside resistance genes (16s rRNA methylase), plasmid- associated cephalosporinase genes (CMY-16, CMY- 58), class A genes (KPC), ESBL genes (TEM,CTX-M-15,SHV-12), macrolides resistance genes (esterase), qnr genes (qnrAB, qnr B1, qnr B2) and Rifampin resistance gene. The above-mentioned plasmids (with blaNDM-1) do as multidrug resistance (MDR) pools and cause pan-drug resistance (16,17). A paper in lancet infectious disease journal (2010) showed that NDM-1 gene cases had been isolated from India, Pakistan and the UK by molecular, epidemiological and biological studies (12). About 70 cases have been identified in the UK, 150 in India and Pakistan, 4 in Australia, 20 in Austria, 2 in Belgium, 4 in Canada, 3 in China, 1 in Germany, 1 in Hong Kong, 1 in Singapore, 1 in Sweden and cases in Taiwan, America, France, Kenya and Italia were 1, 3, 2, 7 and 1 case (s), respectively (9). Recently, CDC has suggested NDM-1 as a transmissible agent, especially in patients who have been hospitalized in Pakistan and India. There are NDM-1 pathogens in acquired infections from hospital environment and community (12).

3. How Untreatable Is NDM-1 Producing Bacteria?

NDM-1 is spreading in India and Pakistan. It has reached Europe, the USA, Canada and Australia. NDM1 producing bacterial strains are resistant to all antibiotics. Physicians in the UK have managed to fight these infections with a combination of several different drugs. Currently, surveillance, prompt identification, isolation of infected patients, disinfecting hospital equipment, and thorough hand-hygiene procedures in hospitals are the only ways to combat the spread of NDM-1. This is going to be a challenge and will require international cooperation (9).

4. How Is NDM-1 Detected?

Some pathogens with NDM-1 and KPC may not be recognized by common laboratory tests; consequently, unaffected antibiotics are prescribed in the wrong way, therefore causes emergence and spread of more resistant bacteria (15,18). The E- test strips are recommended to identify MBL producing bacteria; it is based on metallo-β-lactamases inhibition by EDTA. These strips have two parts Imipenem and Imipenem-EDTA. The E- test MBL is reliable for detection of resistance, except in *Enterobacter cloacae* and *K. pneumoniae* (19). It can be substituted by DDST after applying an Imipenem disc 10 mm apart from a disc containing approximately 1900 mg of EDTA. If Imi-

penem-EDTA disc inhibition zone is more than 4 mm and the inhibition zone is not seen for Imipenem disc, strain is considered as metallo-β-lactamase positive (such as NDM-1). The Hodge-test has low specificity, therefore, it is not recommended for metallo-β-lactamase identification (20). Carbapenemase production is detected by the Modified Hodge test (MHT) when the test isolate produces the enzyme and allows the growth of a carbapenem susceptible strain (*E. coli* ATCC 25922) towards a Carbapenem disk. The result is a characteristic cloverleaf-like indentation. (21). Except class D carbapenemases, Meropenem associated inhibitor is very specific and sensitive to detect carbapenemases. The microarray is a very precise method to detect multidrug *Enterobacteriaceae* β-lactamase positive (22). Also, Real time-PCR method is a useful technique. Advantages of this method are specificity and sensitivity, rapid detection of NDM-1 producers in less than 2 hours; also it can be used to detect KPC, OXA, VIM and IMP-type (23). The other technique; Loop-mediated isothermal amplification (LAMP) was used to detect NDM-1 producers in 2011 (24). To screen carbapenemases such as NDM and KPC enzymes, the rapid multiplex- PCR (less than 4 hours) is reliable (25). Pulsed-filed gel electrophoresis (PFGE) and multilocus sequence typing (MLST) are two techniques to determine the identity and genotype of β-lactamase-related plasmid genes like NDM (7,26,27). So far, in Iranian studies on *P. aeruginosa*, the emphasis was on identification of Ambler class A and Ambler class D serine OXA and also three reports on Ambler class B beta-lactamases (27-32). As remarkable results; NDM-1 or KPCs, is a worldwide health problem which is slowly becoming as important as HIV infection, extremely drug-resistant tuberculosis and malaria. There is no vaccine to prevent infections produced by Carbapenem-resistant bacteria. So, it is necessary to inspect phenotyping and genotyping of NDM-1 and KPC resistant pattern world-wide.

Acknowledgements

With all due respects, this review is presented to researchers who work on drug-resistant bacteria.

Authors' Contribution:

All the authors contributed in writing this article.

Financial Disclosure:

The authors declare no competing financial interests.

Funding/Support:

There is no support for this study.

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