Emergence and Spread of NDM-1 Reveals Novel Superbugs

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Introduction

Overuse of prescription antibiotics and poorly established medical facilities in underdeveloped countries are contributing factors to the proliferation of antibiotic resistant bacteria and superbugs. The plasmid-borne *bla*NDM-1 gene produces New Delhi Metallo-beta-lactamase-1 (NDM-1), whose spread among bacteria has led to global outbreaks of bacteria resistant to all carbapenems. Structurally, NDM-1's uniquely large and flexible active site contributes to its ability to catalyze a wide array of antibiotics by hydrolyzing the beta-lactam ring. Since carbapenems are one of the last resort antibiotics to treat Gram negative bacterial infections, bacteria that possess the NDM-1 gene are especially difficult to treat.

Background

A Swedish patient was the first person to be infected with an NDM-1, a Metallo-Beta-Lactamase (MBL) harboring strain of *Klebsiella pneumoniae* that was resistant to all antibiotics except colistin. Because it is believed that the strain emerged in New Delhi, India, the MBL was named New Delhi Metallo-Beta-Lactamase-1. The diagram below shows the suspected route of spread of the NDM-1 gene.

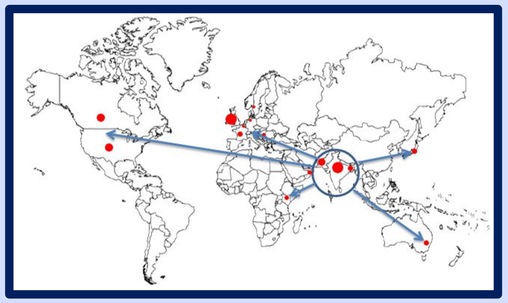


Fig. 1. Worldwide Proliferation of NDM-1

Currently, Beta-lactamases are grouped into four classes based on their amino acid sequence. NDM-1 is a class B Beta-Lactamase, the only group that coordinates with a metal cation in the active site. NDM-1 belongs to the class B_1 and as a result can exist in either a mono-zinc or di-zinc form.

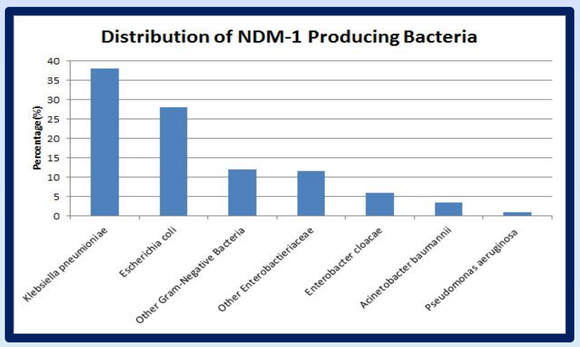
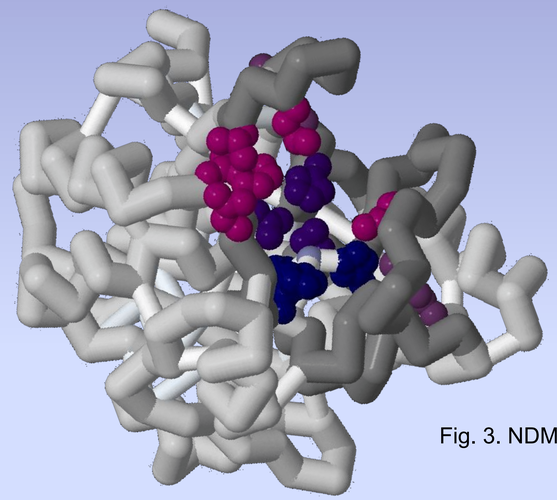


Fig. 2. Distribution of NDM-1 Producing Bacteria

NDM-1 (3SFP) Molecular Structure



NDM-1 is composed of a single 270 amino acid polypeptide oriented in a αβ/βα fold. The active site is located in a groove between the two β sheets surrounded by several loops. Loops 1 and 2 form the base of the active site while loops 3, 4, and 5 form the roof and walls. Loops 1 and 4 are shifted outward, increasing the accessibility to ligands attempting to bind to NDM-1's active site. Within the active site are two critical zinc cofactors that facilitate catalytic activity.

Fig. 3. NDM-1 (3SFP)

Active Site Neutralizes ß-Lactams

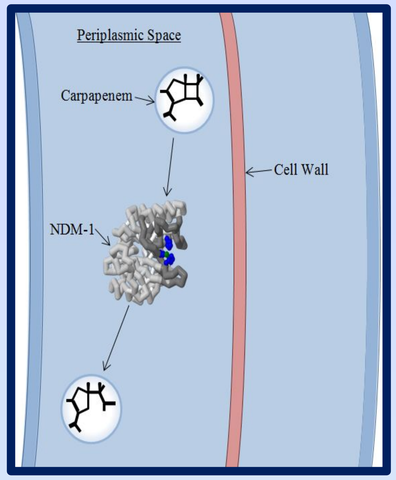


Fig. 4. Hydrolytic Site

All beta-lactams, including carbapenems, have a common ring structure referred to as a beta-lactam ring. The second zinc molecule within the active site cavity recognizes the carboxylate group of the beta-lactam ring. A salt bridge is formed between one of the carboxyl oxygens and the N_z molecule of Lys211.

The proposed mechanism of action (shown below) involves a bulk water molecule that transfers a proton to the oriented hydroxide ion which attacks the carbonyl group of the substrate. Through the addition of a proton, the carbonyl group becomes a carboxylate group. Finally, the proton is transferred to the N4 nitrogen of the beta lactam ring from a separate bulk water molecule, causing the cleavage between the C7 carbon and N4 nitrogen.

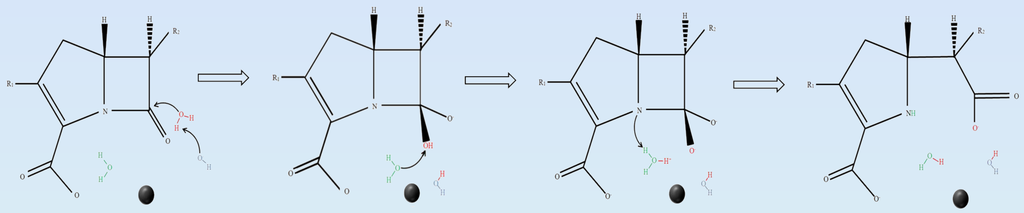


Fig. 5. Reaction Mechanism

Divergence from Other Metallo-ß-lactamases

Compared to the two most closely related Metallo β-Lactamases, VIM-2 and IMP-1, NDM-1 has a mere 32% sequence identity. Structurally, the main differences between NDM-1 and other Metallo β-Lactams are its larger active site, increased hydrophobicity, and more flexible loops. Specific mutations within Loop 1 increase the flexibility of the loop and facilitate substrate binding. Another structural difference is NDM-1's longer N-terminus, forming two more beta strands. This strengthens the hydrophobicity of Loop 3 and facilitates substrate binding. Furthermore, the alanine occurrence within NDM-1 is 15.2% higher than the average for all proteins, contributing to the lower sequence identity. All of these differences result in a broader range of substrate binding and more potent hydrolysis.

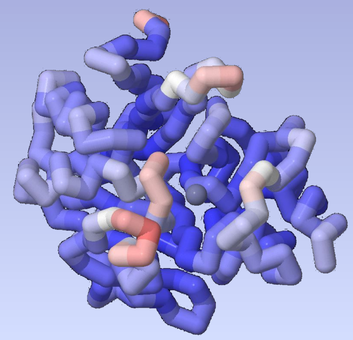


Fig. 6. Variability of NDM-1's structure (red indicates greater variability)

Clinical Identification

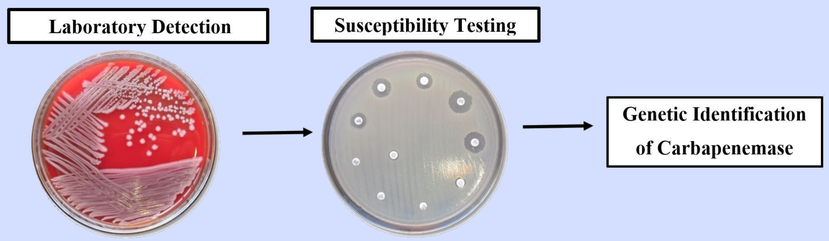


Fig. 7. Clinical Identification of Carbapenemase

Future Outlook

Despite NDM-1's ability to confer resistance against what are traditionally the most efficacious antibiotics, beta-lactams, there are alternative solutions. For instance, most NDM-1 containing bacteria are susceptible to tigecycline, colistin, and to some extent, fosfomycin. However, their severe side effects limit dosage and pragmatic usage. Another problem lies in current technology for detection of NDM-1, most commonly Polymerase Chain Reaction. PCR is expensive, and its specificity renders it unable to identify new variations of carbapenemases such as NDM-1 without whole genome sequencing. As a result, there is a dire need for an inexpensive, fast, sensitive, and accurate test for the detection of carbapenemases.

References

Kim, Youngchang, Christine Tesar, Joseph Mire, Robert Jedrzejczak, Andrew Binkowski, Gyorgy Babnigg, James Sacchettini, and Andrzej Joachimiak. "Structure of Apo- and Monometalated Forms of NDM-1--A Highly Potent Carbapenem-Hydrolyzing Metallo-ß-Lactamase." *PLoS ONE* 6.9 (2011): 1-10. Print.

Kim, Youngchang, Mark A. Cunningham, Joseph Mire, Christine Tesar, James Sacchettini, and Andrej Joachimiak. "NDM-1 the Ultimate Promiscuous Enzyme: Substrate Recognition and Catalytic Mechanism." *The FASEB Journal* (2013): 1917-1927. Print.

Zhang, HongMin, and Quan Hao. "Crystal Structure of NDM-1 Reveals a Common ß-lactam Hydrolysis Mechanism." *The FASEB Journal* (n.d.): 2574-2581. Print.

Rolain, J. M., Parola, P. and Cornaglia, G. (2010), New Delhi metallo-beta-lactamase (NDM-1): towards a new pandemia?. Clinical Microbiology and Infection, 16: 1699–1701. doi: 10.1111/j.1469-0691.2010.03385.x

Yong, Dongeun, Mark A. Toleman, Christian G. Giske, Hyun S. Cho, Kristina Sundman, Kyungwon Lee, and Timothy R. Walsh. "Characterization of a New Metallo-β-Lactamase Gene, BlaNDM-1, and a Novel Erythromycin Esterase Gene Carried on a Unique Genetic Structure in Klebsiella Pneumoniae Sequence Type 14 from India." *PMC*. NCBI, National Center for Biotechnology Information, 21 Sept. 2009. Web. 13 Mar. 2016.