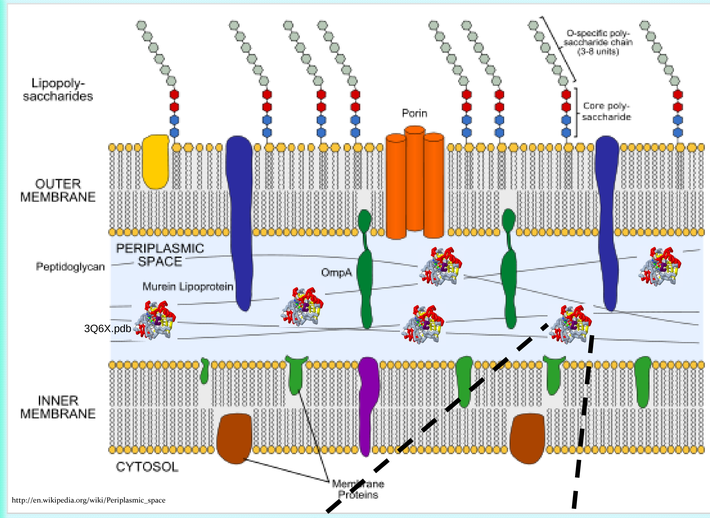
The Emergence of a Superbug: NDM-1 and Its Role in Carbapenem Resistance

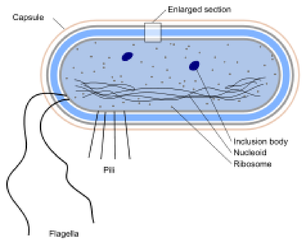
Milwaukee Academy of Science SMART Team: LaTyra Barnes, Cameron Bester, Camryn Burrows, Brandi Daubon, Derion Davis, Natalie Hall, Jonte Jackson, Jessie Jones, Tim Jones, Jailyn Kendrick, Virginia McCotry, Norbrianna Payne, Reiauna Taylor, Quintien Tyra, Eddie Walls, David Washington
Teachers: Kevin Paprocki and Tyler Reed
Mentors: Lance Presser, Ph.D. and Sanjib Bhattacharyya, Ph.D. City of Milwaukee Health Department Laboratory

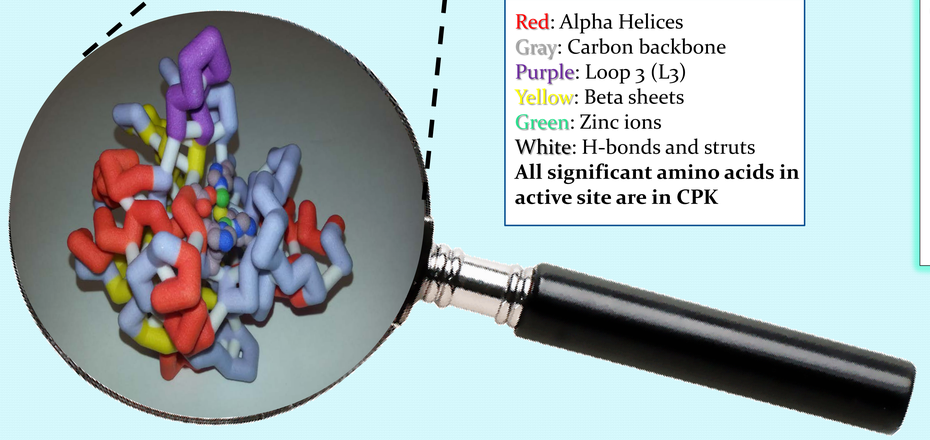
Abstract

Imagine going to the doctor to be treated for a normally treatable infection only to find that no effective treatments exist because all conventional antibiotics are ineffective. In some regions of the world, antibiotic prescription isn't regulated and overuse has lead to antibiotic resistance. Carbapenems are a class of antibiotics that inhibit bacterial cell wall synthesis and are often used as a last resort treatment for bacterial infections. New Delhi Metallo-ß-lactamase-1 (NDM-1) is an enzyme that occurs in several types of bacteria and conveys resistance against Carbapenems. The Milwaukee Academy of Science SMART Team (Students Modeling A Research Topic) modeled the NDM-1 protein using 3D modeling technology. NDM-1 is a single-chain polypeptide consisting of 270 amino acids found in the bacterial periplasmic space. The NDM-1 active site consists of two loops (L10 and the highly flexible L3) and two zinc ions. These zinc ions are held in place by three histidine amino acids (H120, H122, H189) on L3 and a triplet of amino acids on L10. The zinc ions bind to and sever the ß-lactam ring on Carbapenems, inhibiting its antibiotic properties. It's the flexibility of L3 that gives NDM-1 the ability to hydrolyze the full spectrum of Carbapenems. Researchers are concerned because the gene for NDM-1 is located on a plasmid that's frequently passed via horizontal gene transfer among various species of bacteria. An understanding of NDM-1's structure and function may prevent an outbreak of bacteria equipped with the NDM-1 enzyme.



Gram Negative Bacterial Cell Wall

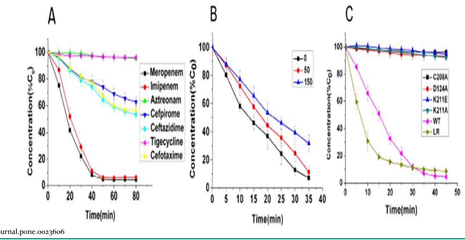




Red: Alpha Helices
Gray: Carbon backbone
Purple: Loop 3 (L3)
Yellow: Beta sheets
Green: Zinc ions
White: H-bonds and struts
All significant amino acids in active site are in CPK

Hydrolytic Properties of NDM-1 on Carbapenems

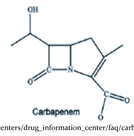
The series of graphs below depicts three separate experiments to determine the hydrolytic properties of NDM-1 on various carbapenems. **Graph A.** In this experiment, various carbapenems were combined in a solution with NDM-1 and their changes in concentration were measured over the course of 80 minutes. The carbapenems tigecycline and aztreonam remained unaffected, while imipenem and meropenem were almost completely hydrolyzed. **Graph B.** In this experiment, the researchers were examining the effects of the concentration of tigecycline (a carbapenem unaffected by NDM-1 in Graph A) on the hydrolysis of meropenem (a carbapenem almost completely hydrolyzed by NDM-1 in Graph A). Higher concentrations of tigecycline resulted in a slower decomposition rate of meropenem, hinting at tigecycline's ability to somewhat inhibit the effects of NDM-1 on susceptible carbapenems. **Graph C.** In this experiment, researchers genetically modified NDM-1 in single amino acid substitutions to determine which amino acids were significant to the hydrolytic properties of NDM-1 on meropenem. Whereas changes to the three amino acids (C208, D124, and K211) that are suspected to hold the second zinc ion (Zn2) in place in the active site completely rendered NDM-1 ineffective at hydrolyzing meropenem, other single amino acid substitutions retained NDM-1's potency, thereby solidifying our understanding of exactly which amino acids are significant in NDM-1's active site and hydrolytic properties.



http://www.plosone.org/article/info%3Adoi%2F10.1371%2Fjournal.pone.0023606

Carbapenems

Carbapenems are a class of intravenously administered antibiotics that are typically used to treat infections where other antibiotics have failed. They are characterized by the presence of a β-lactam ring at the core of their molecular structure much like penicillin but have chemical differences that make them resistant to most β-lactamases. This makes them an ideal candidate for treatment for bacterial infections that possess β-lactamases that confer resistance to penicillin. Carbapenems function in much the same way as penicillin; they bind to Penicillin Binding Proteins (PBPs) within a bacterium which in turn prevents that bacterium from catalyzing the formation of peptidoglycan, a key component of the bacterial cell wall. With no ability to synthesize peptidoglycan, the cell wall eventually dissolves and rapidly kills the bacterium. Peptidoglycan's universal use in bacteria makes carbapenems effective against a large variety of both Gram-positive and Gram-negative bacteria. The first carbapenem, thienamycin, was discovered in 1976 and served as a model for all subsequent carbapenems.



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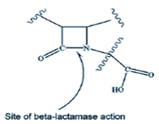

http://www.uic.edu/pharmacy/centers/drug_information_center/faq/carbapenem.php

The "square" structure in the middle is the β-lactam ring that is both the characteristic structure of all carbapenems and the target of NDM-1. The R's are simply the side chains that vary from carbapenem to carbapenem; the core of the molecule is pictured above and is consistent for all carbapenems.

http://en.wikipedia.org/wiki/File:Penicillin_spheroplast_generation_horizontal.svg

In the diagram at left, the cell wall synthesis inhibitor (CWSI) such as a carbapenem is applied to a solution containing bacteria. The bacterium attempts to remodel its cell wall by destroying some peptidoglycan while rebuilding it, but the CWSI prevents the bacterium from synthesizing peptidoglycan. As a result, the cell wall is breached and the cytoplasm leaks out of the cell wall, thereby killing the bacterium.

Anatomy of NDM-1

Anatomy of NDM-1 New Delhi metallo-β-Lactamase, (NDM-1), is a reoccurring enzyme in bacteria that conveys resistance to carbapenems. NDM-1 is a single chain polypeptide containing 270 amino acids found in a bacterium's periplasmic space which is located between the outer and inner cell membranes of a Gram Negative bacterium. The NDM-1 active site consists of two loops (L10 and the highly flexible L3) and two zinc ions. The zinc ions found in the active site are held in place by three histidines which are H120, H122, and H189. The binding zinc ions sever the β-lactam ring in carbapenems which inhibit its antibiotic properties. It is the flexibility of L3 helps gives NDM-1 the ability to hydrolyze the full spectrum of carbapenems.

Evolution of NDM-1

New Delhi Metallo β-lactamase (NDM-1) is an enzyme found in some bacteria that can make infections untreatable by most carbapenem antibiotics. It was first discovered in December, 2008 in a Swedish man with *Klebsiella pneumonia* who had been treated in New Delhi when he contracted an infection that was resistant to a broad range of β-lactam antibiotics. In many regions of India, prescription medicine isn't regulated, and prescription drugs can be purchased at will and used in any amount for any period of time. Misuse of these antibiotics in countries such as this has enabled bacteria to evolve into the deadly superbug it is today. Vacation and business travel have played a large role in introducing the NDM-1 enzyme into countries outside of the Indian subcontinent. Cases have now been detected in many countries including Great Britain, Canada, Sweden, Australia, Japan, and now the United States. Its first appearance in the USA was June, 2010 in Americans who had recently sought medical care in India. Some researchers in Northern China have even extracted bacterial DNA from various water sources that showed a great deal of NDM-1. In one of the treatment plants, the total number of NDM-1 genes leaving the plant was greater than that coming in due to horizontal gene transfer. A better understanding of NDM-1 can help to both prevent future breakouts and potentially find a cure.

Horizontal Gene Transfer

Horizontal gene transfer (HGT) is the movement of genes between organisms that defer from any traditional reproduction. It refers to the movement of genetic information without bearing any novel offspring. The gene for NDM-1, for example, is passed along through horizontal gene transfer. There are several ways HGT can happen. This includes transformation (a cell taking in genetic material) and transduction (a bacteria gives DNA to another bacteria). The gene for NDM-1 is found on a ring of bacterial DNA known as a plasmid that is commonly transferred between bacteria. This phenomenon is responsible for NDM-1's appearance in several different species of bacteria. HGT is the primary reason bacteria can resist drugs, playing a major role in the evolution of bacteria.

Conclusion

- ❑Carbapenems are category of antibiotics typically used as a last resort in persistent infections
- ❑Overuse/improper use of antibiotics gives bacteria an opportunity to evolve resistance to them
- ❑NDM-1 is an bacterial enzyme that confers resistance to most carbapenems
- ❑Zinc ions within NDM-1's active site bind and sever the β-lactam ring on carbapenems, inhibiting their antibiotic properties
- ❑NDM-1 is located on a plasmid that is typically transferred by horizontal gene transfer from one bacteria to another
- ❑Understanding the function of NDM-1 could help prevent a potential outbreak of dangerous bacterial

"The SMART Team Program is supported by the National Center for Advancing Translational Sciences, National Institutes of Health, through Grant Number 8UL1TR000055. Its contents are solely the responsibility of the authors and do not necessarily represent the official views of the NIH."