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NDM-1 Antibacterial resistance: A challange

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Abstract
Though antibacterial resistance is not a recent phenomenon, it is a critical health issue today. Much of the antibacterial resistance problem stems from the misuse or excessive use of antibiotics. Effective antibiotic stewardship is required to ensure that antibiotics are prescribed and used responsibly.
NDM-1 stands for *New Delhi metallo-beta-lactamase* enzyme. This enzyme destroys beta-lactam antibiotics including the penicillin's, cephalosporins, and carbapenems. It is also found that NDM-1 can easily jump from one strain of bacteria to another, presenting major challenge for clinicians in treating the infections, often demanding use of combination antibiotics. To reduce the risk of incidence of NDM-1, physicians and hospitals should ensure that antibiotics are used judiciously and appropriately.

Keywords: NDM-1, antibacterial resistance, beta-lactam antibiotics

Introduction
In nineteenth century, scientists discovered that microorganisms were the leading cause of several infections. In search of curing these infections, antibacterial drugs and then antibiotics were discovered. They saved many lives suffering from infections/diseases like pneumonia, rheumatic fever, bacterial meningitis etc. Despite saving many lives each year, their overuse and misuse reduced efficacy of antibiotics, leading to development of bacterial resistance. The need to invent more and more antibiotics arose due to the development of resistance. Further search led to the advent of antibiotics to provide efficacious and safe therapy to the patients. The structures of the antibiotics were modified to broaden their antimicrobial spectrum, increase potency and minimize the associated side effects. The antimicrobial spectrum was widened to include activity against not only gram positive bacteria but also gram negative species and they were classified as I, II, III, IV, V generation antibiotics.

History [5-9]
Sir Alexander Fleming accidently discovered the first antibiotic penicillin in 1929 which was followed by number of antibiotics till date. Scientific and commercial interest in the antibiotic field has led to the isolation and identification of antibiotic substances that may be numbered in thousands.

β-lactam antibiotics
In 1929, Fleming introduced penicillin which was active against strains of staphylococcus aureus. But this was actually used in 1940 for clinical purposes and proved its efficacy and safety worthwhile during World War II by saving lives of thousands of warriors. In 1943, drug companies began mass production of penicillin. The limited activity of the orally active penicillins like penicillin G and penicillin V, led to the search for number of derivatives of penicillin that could treat a wider range of infections. In 1961, the first major development was ampicillin, which was followed later by β-lactamase-resistant penicillins, including flucloxacillin, dicloxacillin, and methicillin. These were effective against β-lactamase-producing bacterial species, but were ineffective against the methicillin-resistant Staphylococcus aureus (MRSA) strains. In 1964, the antipseudomonal penicillins such as carbenicillin, ticarcillin, and piperacillin, active against Gram-negative bacteria and methicillin-resistant Staphylococcus aureus (MRSA) strains were developed.
In 1945, cephalosporins were discovered by G. Brotzn from *Cephalosporium acremonium* found in sea water and in 1948 Cephalosporin precursor was sent to Oxford for synthesis. In 1948, first generation cephalosporins namely Cephalexin, Cephradine and Cefadroxil were discovered. They have good antimicrobial activity against gram-positive bacteria but limited activity against gram-negative species. In 1979, second generation cephalosporins namely cefaclor, cefotetan, cefoxitin, cefprozil, Ceftin were discovered which have better activity against gram negative species. In 1984, the majority of third generation cephalosporins namely Cefdinir, Cefixime, Ceftibuten were discovered. The fourth generation cephalosporins have greater activity against gram-negative bacteria than the second and third generation and major compounds are Cefozopran, Cefclidine, Cefepime. In 5th generation cephalosporins there are only two drugs Ceftobiprole and Ceftaroline and these are also the only β-lactam antibiotics that are effective against methicillin-resistant-Staphylococcus-aureus (MRSA).

Sulphonamides
In 1935, the antibacterial sulphonamides were introduced by Domagk *et al* to treat allergies and cough. They are also found to have antifungal and antimalarial activity. Later, numbers of sulphonamides were discovered like sulfamethoxazole, sulfadiazine, sulfacetamide, sulphadoxine etc. for the treatment and prophylaxis of Pneumocystis carinii pneumonia, cerebral toxoplasmosis, respiratory and urinary tract infections.

Aminoglycosides
In 1944, streptomycin, the first aminoglycoside antibiotic, was obtained from soil bacterium Streptomyces griseus by

Waksman and associates. Later in 1949 neomycin sulfate was discovered from Streptomyces fradiae. In 1957, Kanamycin was isolated by Umezawa and coworkers from Streptomyces kanamyceticus. In 1963, Gentamycin was discovered by Weinstein *et al* from Micromonospora purpurea. Later numbers of derivatives were discovered including Tobramycin, Netilmicin and sisomicin etc.

Chloramphenicol

In 1947, chloramphenicol was isolated from *Streptomyces venezualae* by Ehrlich *et al*. to treat bacterial infections like meningitis, plague, cholera, and typhoid fever.

Macrolides

In 1950, picromycin, the first antibiotic of this group was discovered. In 1952, erythromycin was derived from Streptomyces erythreus while in 1956 vancomycin was introduced for penicillin-resistant Staphylococcus. Later numbers of other antibiotics were discovered including clarithomycin, azithromycin, dirithromycin etc.

Tetracyclines

In 1948, chlortetracycline, from Streptomyces aureofaciens was discovered by Duggar. This is active against gram-positive and gram-negative bacteria, spirochetes, mycoplasma and chlamydiae. In 1950, oxytetracycline was discovered by Finlay *et al* from Streptomyces rimosus. Later number of derivatives like rolitetracycline, methacycline, and doxycycline were discovered.

Quinolones

In 1962, nalidixic acid, the quinolone antimicrobial, was discovered by George Lesher and coworkers for treatment of urinary tract infections. In 1978, norfloxacin was discovered while in 1986, ciprofloxacin and ofloxacin were discovered which were followed by other derivatives like levofloxacin, temafloxacin, moxifloxacin.

Miscellaneous antibiotics

In 2000, a newer synthetic agent linezolid, the oxazolidinone type antibacterial, was introduced. In 2003, daptomycin, the lipopeptide antibiotic, derived from actinomycete, was introduced. In 2007, ratapamulin, a topical antibiotic, belonging to the class of pleuromutilin was discovered. In 2011, fidaxomicin, a tiacumicin antibiotic was discovered. In 2012, bedaquiline, a bactericidal drug which belongs to a new class of antibiotics diarylquinolines was introduced. In May 2014, dalbavancin, a novel second-generation lipoglycopeptide antibiotic was approved by FDA and has been recommended by FDA for approval to the European Medicines Agency (EMA). In August 2014, oritavancin, a vancomycin derivative was approved by the FDA, and has also been recommended for approvalto the European Medicines Agency (EMA). In 2014, tedizolid, oxazolidinone derivative, discovered by Dong-A was the third antibiotic approved by the FDA. In February 2015, Ceftazidime, a third generation cephalosporin was approved by FDA for the treatment of complicated intra-abdominal infections.

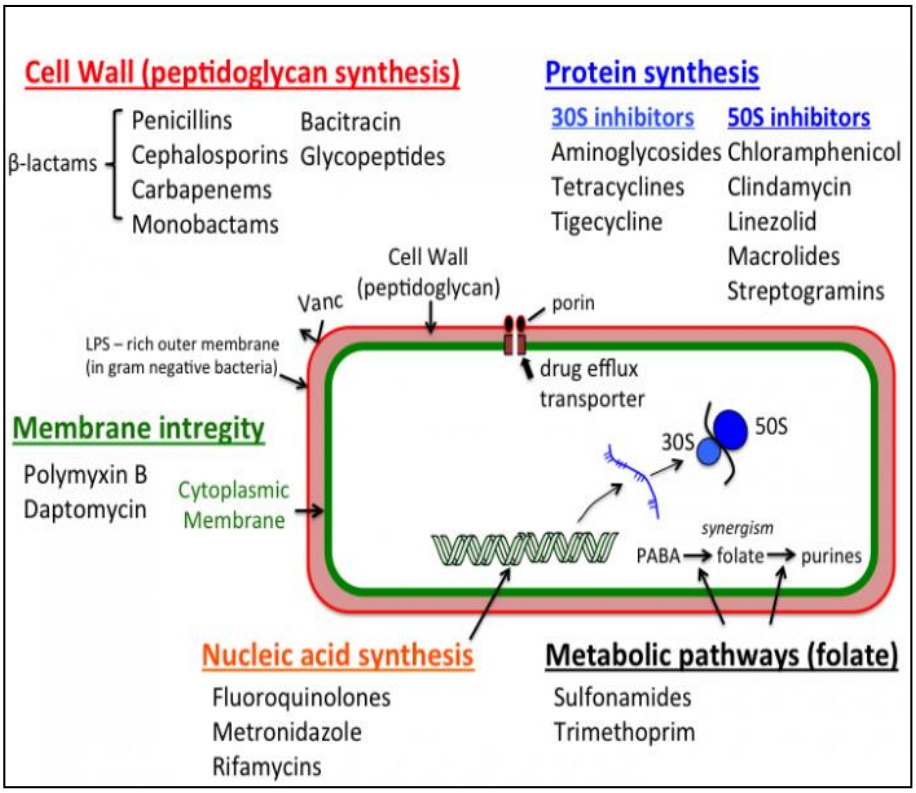


Fig 1: Types of Antibiotics with mechanism of action

Antibiotic resistance [10]

Antibiotic resistance is a natural phenomenon. Antibiotic resistance occurs when an antibiotic loses its ability to effectively control or kill bacterial growth. Antibiotic resistance is the ability of bacteria to resist the effects of an antibiotic by developing combating mechanisms in its body. In other words we can say Antibiotic resistance is the ability of a microorganism to survive in the presence of an antibiotic.

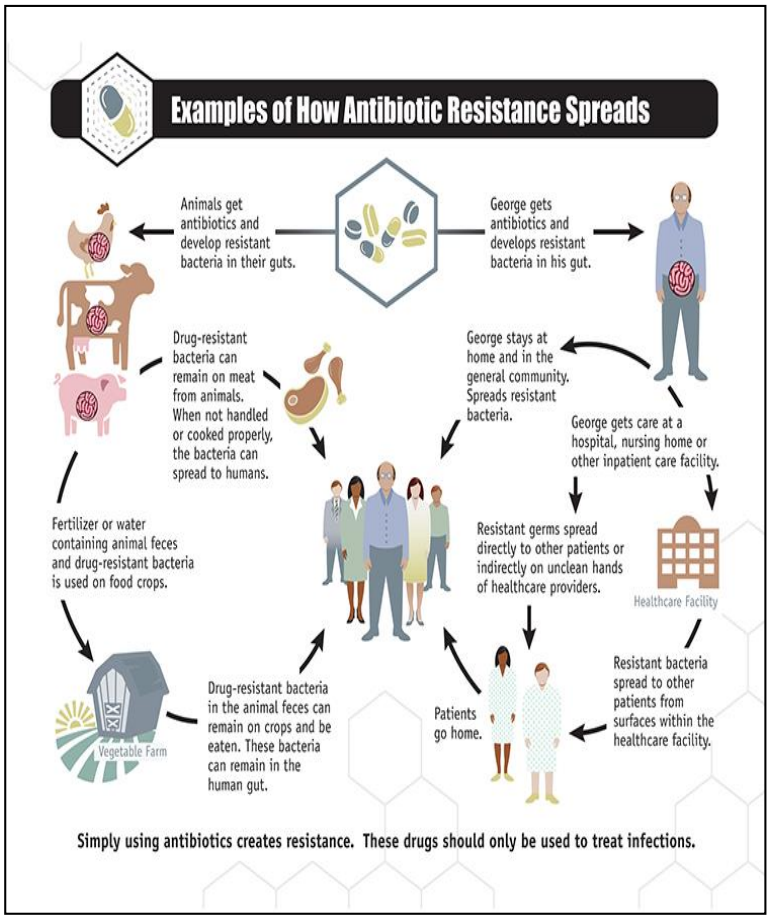
Reasons for development of resistance [11]

The main reasons for development of resistance to antibiotics is their misuse, unnecessary prescription of antibiotics for viral infections, frequent prescription of broad-spectrum antibiotics, inadequate use by the patient, not respecting either dosage or duration of the treatment and self-medication. The availability of counterfeit drugs has further exacerbated drug resistance in the developing world.

Mechanisms of antibiotic resistance [12]

Mechanisms involved in development of resistance are:

- ***Phenotypic resistance:*** Changes in the bacterial physiological state such as the stationary phase and the dormant phase develop antibiotic persisters.
- ***Genetic resistance:*** Develops due to the transfer of genetic material among bacteria by several means like conjugation, transduction and transformation.
- ***Natural or intrinsic resistance:*** Develops due to spontaneous gene mutation in the lack of selective pressure due to presence of an antibiotic.
- ***Acquired resistance:*** It is often caused by mutation in the chromosomal genes or by the acquisition of mobile genetic elements such as plasmids or transposons, which carry the antibiotic resistant gene.



Methods for determining antibiotic susceptibility [13]

The methods routinely used for testing of antibiotic susceptibility of the microorganisms are Kirby-Bauer (disk diffusion) method, Stokes method, E-test, agar and broth dilution method. They are used for the determination of minimum inhibitory concentration (MIC).The E-test, mainly used for determination of phenotypic resistance, is a popular quantitative technique for the determination of antimicrobial susceptibility.

How to prevent emergence of resistance [14]

Prevention of emergence of antibiotic resistance during treatment is an important goal while prescribing antimicrobials. To achieve this we should understand the following principles.

- Take antibiotics exactly as per the directions of the physician.
- Do not skip doses.
- Complete the prescribed course of treatment, even when you start feeling better.
- Only take antibiotics prescribed for you, do not share or use leftover antibiotics.
- Do not save antibiotics for the next illness. Discard any leftover medication once the prescribed course of treatment is completed.
- Document the dose, duration and indication for every antibiotic prescription.
- Professionals should follow hand hygiene and other infection control measures with every patient.
- Antibiotics without prescription must be restricted.

Ndm-1 enzyme [15-17]

β-lactamases are the enzymes which destroy the β-lactam antibiotics. There are many types of beta-lactamases. Most of

them hydrolyse older beta-lactam antibiotics, making them inactive. But they cannot hydolyse newer agents like the carbapenems.

NDM-1 stands for *New Delhi metallo-beta-lactamase*, which is an enzyme produced by certain strains of bacteria. New Delhi metallo-β-lactamase (NDM) enzymes are the latest carbapenemases to be recognized since 2008. They have been reported worldwide, mostly in bacteria from patients epidemiologically linked to the Indian subcontinent, where they occur widely in hospital and community infections, and also in contaminated urban water. Majorly the strains of *Klebsiella pneumoniae*, *Escherichia coli*, and *Acinetobacter baumannii* genera of bacteria are known to possess the gene for NDM-1. The NDM1 gene is also found to be present in *Morganella morganii,* E. cloacae *and Enterobacteriaceae* strains.

This enzyme makes bacteria resistant to a broad range of beta-lactam antibiotics. This is an enzyme that destroys not only older beta-lactam antibiotics like penicillins, cephalosporins but also the newer carbapenems.

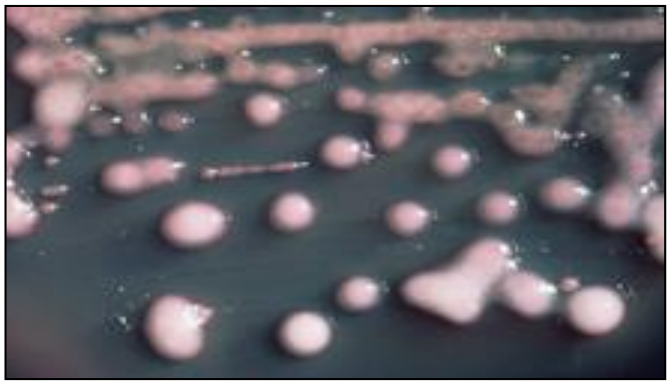


Fig 2: *Klebsiella pneumoniae*, the bacterium in which NDM-1 was first identified

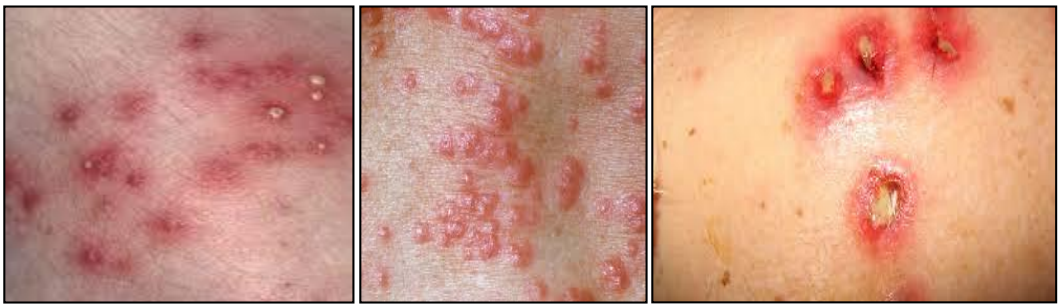


Fig 3: Infections caused by NDM-1

The gene for NDM-1 is found on plasmids (DNA strands), which can easily spread from one strain of bacteria to another, particularly in patients receiving antibiotic treatment. Infections caused by NDM-1 bacterial pathogens are difficult to treat, however it doesn't by itself make pathogens more virulent or transmissible. These infections have ranged from mild to severe, though some have been fatal. The patient's immunocompromised status could be a risk factor for these infections. NDM-1 positive pathogens cannot lead to pandemics like bird or swine flu, but diseases caused by NDM-1 positive pathogens could result in clinical complications.

Origin and spread [18-25]

The NDM-1 enzyme was named after New Delhi, the capital city of India. It was first described by Yong *et al* in December 2009, when he came across a Swedish patient having antibiotic-resistant bacterial infection, acquired in Delhi. The infection was unsuccessfully treated in a New Delhi hospital. After the patient's repatriation to Sweden, a carbapenem-resistant *Klebsiella pneumoniae* strain, bearing the novel gene, was identified. The authors concluded that the new resistance mechanism arose in India.

Muir A, Weinbren MJ *et al*. in March 2010, a study in a hospital in Mumbai found that most carbapenem-resistant bacteria carried the novel NDM-1 gene. In May 2010, a case of infection with *E. coli* expressing NDM-1 was reported in Coventry in the United Kingdom. The patient was a man of Indian origin who had undergone dialysis during his visit to India, 18 months prior to the detection. In initial bioassays, it was found that the bacteria was fully resistant to all antibiotics, while further investigation showed that it was susceptible to tigecycline and colistin. In July 2010, a team from New Delhi reported three cases of NDM-1 *bearing Acinetobacter baumannii*, that were found in the intensive care unit of a hospital in Chennai, India in April 2010.

A study by a multi-national team was published in the August 2010 issue of the journal "*The Lancet Infectious Diseases"* which reported 37 cases in the United Kingdom, 44 in Chennai, 26 in Haryana, and 73 in various other states of India. It was found that *Klebsiella pneumoniae*, *Escherichia coli*, and *Acinetobacter baumanniiare the causal bacterias.* The gene isolates obtained from these causal bacterias were resistant to different classes of antibiotics, including beta-lactam, fluoroquinolones, and aminoglycosides but were susceptible to the colistin, a polymyxin antibiotic published by Mark A Toleman *et al*.

Nordmann P and Poirel L *et al*, on 6th September 2010, Japan detected its first ever case of the NDM-1 enzyme, in a Japanese man who had returned from India in May 2009. Hospital officials confirmed the presence of *Klebsiella pneumoniae* containing NDM-1 enzyme.

An environmental point prevalence study was conducted by research team from University of Cardiff, UK, from September 26 to October 10, 2010. This study found *Klebsiella pneumoniae and Acinetobacter baumannii* with the NDM-1 gene in drinking water and seepage samples in New Delhi. During the study 50 tap water samples and 171 seepage samples were collected from sites within 12 kms of central Delhi. From these samples 51 out of 171 seepage samples and 2 out of 50 tap water samples were found to contain NDM-1 gene expressing *Klebsiella pneumoniae and*

Acinetobacter baumannii. This was reported in April 2011 issue of British Medical Journal "Lancet" by Walsh Timothy R, Janis Weeks, and David M Livermor *et al*.

Payal Deshpande and Camilla Rodrigues *et al*. in June 2012 studied carbapenem resistant isolates of Enterobacteriaceae and found that, out of 24 carbapenem resistant Enterobacteriaceae 22 was NDM producers while 2 were NDM non-producers. Amongst the 22 NDM producing organisms 10 were Klebsiella species, 9 were Escherichia coli, 2 were Enterobacter species and 1 was Morganella morganii. This high number of NDM1 containing strains compromises the treatment options with the carbapenems.

In the study by Poirel L and Yilmaz M *et al*. in April 2013, Twenty-two consecutive carbapenem-resistant enterobacterial isolates were recovered from patients hospitalized in different units at a university hospital in Istanbul, Turkey. These were Klebsiella pneumoniae isolates producing the carbapenemases NDM-1, and KPC-2 (Klebsiella pneumoniae carbapenemase), and Escherichia coli isolates producing NDM-1.

Jain A and Hopkins KL *et al*. published a study conducted in 83 laboratories of UK from February 2008 to July 2013, confirmed 326 NDM-positive isolates from 250 patients.

Johnsan AP and woodford N *et al*. study in November 2013 reported that NDM carbapenemases have been reported from 40 countries worldwide.

Gharout-Sait A and Alsharapy A *et al* in October 2014 investigated the presence of ten carbapenem-resistant Enterobacteriaceae (eight Klebsiella pneumoniae isolates and two Enterobacter cloacae) isolates from Yemen. All of the 10 carbapenem-resistant Enterobacteriaceae were resistant to β-lactam antibiotics, tobramycin, ciprofloxacin and cotrimoxazole.

Cailin liu and Hui Xu *et al*. reported in August 2015 that a study was conducted to determine the prevalence of New Delhi metallo-β lactamase-1 (NDM-1) producing *Enterobacteriaceae* in Henan province, China. This study reports a high incidence and endemic spread of NDM-1-producing carbapenem-resistant *Enterobacter cloacae* isolates in Henan province, China. Eight (72.7%) out of eleven carbapenem-resistant *E. cloacae* isolates collected between June 2011 and May 2013 were identified and found as NDM-1 positive.

Wafaa Y. Jamal and M. John Albert *et al*. reported in March 2016 that a study was conducted to determine the prevalence of New Delhi metallo-β lactamase-1 (NDM-1) producing *Enterobacteriaceae* in Kuwait over a one year period. *Enterobacteriaceae* isolates with reduced susceptibility to carbapenems were collected from four government hospitals in Kuwait from January–December 2015. This study reveals that carbapenem resistance among *Enterobacteriaceae* mediated by NDM-1 production is becoming a significant occurrence in several hospitals in Kuwait. The results indicate that NDM-1 was present in 2.7% of all *Enterobacteriaceae* in this study and in about 34.4% of all CRE (carbapenem-resistant Enterobacteriaceae).

Clinical presentation of NDM- 1 bacterial infection [26, 27]

There are no common symptoms as the resistance can be expressed by a number of different types of bacteria, and symptoms vary with the site of infection. The bacteria cause opportunistic infections in hospital patients, with common sites of infection including: the blood, urinary tract, lungs, and wounds. The severity of infections involving NDM-1 can vary from mild to fatal. The resistance makes the infections much harder to treat.

The major sign or symptoms of a person infected with bacteria carrying NDM-1, depends upon the part of the body invaded by the organism. For example

- Urinary infections
- Pneumonia, or wound infections.
- Fever and fatigue.
- Patients may go into shock when the bacteria containing NDM-1 enters the bloodstream.
- Patients will not respond to most of the conventional antibiotics.

Prevention of superbug NDM-1 [28]

One can prevent spread of Superbug NDM-1 carrying Bacteria by taking following measures

- Provision of good hygienic conditions, with adequate cleanliness and sanitization is the best bet against further spreading of this superbug.
- Reporting any incidence of Superbug NDM-1 and isolating that patient at the earliest is essential and helps in preventing the further spread of the organisms.
- Antibiotics should never be shared with friends or family, even if they have the same infection. Let the doctor decide and write a new prescription.

Controlling the spread of Superbug NDM-1 is largely depends on stopping of further mutation the gene that causes it. Since it is largely confined to the hospitals, its further spread can be prevented by ensuring that, those dealing with such patients; wear long sleeved disposable gowns and use disposable gloves.

Treatment and future prospective for an NDM-1 [28, 29]

NDM-1 strains are resistant to almost all antibiotics. No antibiotic is available in the market which is specifically effective against NDM-1 containing strains. Physicians are treating these patients by using following antibiotics

1. **Colistin**: an old antibiotic. Colistin was not used much in recent decades, because of its higher toxicity as compared to other antibiotics. But now this antibiotic is found to be active against NDM 1 containing Klebsiella pneumoniae, *Escherichia coli*, and *Acinetobacter baumannii*. Recommended therapeutic dose of colistin for infections caused by bacteria carrying NDM-1 is 2.5 to 5 mg/kg a day
2. **Tigecycline**: When a patient is not responding to colistin then another antibiotic tigecycline (Tygacil) is used. Tigecycline is also active against *Klebsiella pneumoniae*, *Escherichia coli*, and *Acinetobacter baumannii*, but should be used cautiously in serious infections because it does not achieve high levels in the bloodstream due to protein binding. Recommended therapeutic dose for infections caused by bacteria carrying NDM-1 is initial dose of 100 mg intravenously, followed by 50 mg intravenous every 12 hours.
3. **Combination of Colistin and Tigecycline**: Mahableshwar Al bur *et al*. studied the Bactericidal Activity of Multiple Combinations of Tigecycline and

Colistin against NDM-1-ProducingEnterobacteriaceae. They have reported that Colistin sulfate and methane sulfonate alone showed good early bactericidal activity, often with subsequent regrowth. Tigecycline alone had poor activity. Addition of tigecycline to colistin does not produce increased bacterial killing; instead, it may cause antagonism at lower concentrations.

4. **Aztreonam:** This antibiotic is also found to be active against *Escherichia coli.* The recommended dose for moderately severe infections is 1 to 2 g intravenously or intramuscularly every 8 to 12 hours and for severe infections it is 2 g intravenously every 6 to 8 hours.

Current research: Researchers from GlaxoSmithKline have identified a new antibiotic compound that may inhibit NDM-1 containing bacteria's, so the bacterial replication is inhibited or stopped. Unfortunately, the compound has not gone through any clinical trials and is not commercially available.

Conclusion

Development of NDM-1 containing bacteria, have posed a challenge to the physicians in treating bacterial infections. New Delhi metallo-beta-lactmase-1 is an enzyme that confers antibiotic resistance to bacteria and is thus a serious threat to human health. Strains spread from person to person through contact with contaminated items. Good hand hygiene practices will reduce the risk of spreading or acquiring NDM-1. In hospitals, hand hygiene is critical and patients with NDM-1 should be placed in private rooms and health-care workers should use gowns and gloves when entering the room. In addition, newer and more effective antibiotics need to be discovered to control these infections.

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