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SUPERBUG: ANTIMICROBIAL RESISTANCE DUE TO NDM-1

Kamaljit Singh Sandhe[1]*, Neha Arora[2], Inderjit Singh[3], Tarun Garg[4]

1. Global Institute of regulatory affairs, Pune.
2. Department of Quality Assurance, I.S.F. College of Pharmacy, Moga, Punjab.
3. Department of Pharmaceutical Analysis, I.S.F. College of Pharmacy, Moga, Punjab.
4. Department of Pharmaceutics, Seth G.L.Bihani S.D. College of Tech. Edu, Sriganganagar

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For Correspondence:

Kamaljit Singh Sandhe

Global Institute of Regulatory Affairs, Pune.

E-mail:

purveshpatel24@yahoo.com

INTRODUCTION

Resistance to antimicrobial drugs is increasing worldwide. This resistance is, at least in part, associated with high antimicrobial usage. The extensive use or misuse of antimicrobial agents, not only as treatment in humans and veterinary medicine but also as growth promoting substances in livestock production, has greatly promoted the appearance of antimicrobial resistant bacteria. Antimicrobials have been designated as 'societal drugs' because giving these agents to one person affects not only the microorganisms in the person being treated, but also in the people and the environment around that person[1]. Gram-positive bacteria like coagulase-negative staphylococci (CoNS), S aureus, and Enterococcus species continue to be important causes of infection in the hospital environment and, according to the most recent report from the Surveillance and Control of Pathogens of Epidemiologic Importance (SCOPE) project, they account for 60% of healthcare associated bloodstream monomicrobial infections in US hospitals[2]. The emergence of resistance in gram-negative organisms is perhaps of even greater concern than resistance in gram-positive bacteria[3]. Despite increasing awareness little attention is being paid to the problem. In August 2010, when WHO announced that swine flu H1N1 are no longer pandemic, just 24 hours later A new threat in the form of Superbug (as named by news media) blinked against health protection associations. World's newest antibiotic resistant microbial forms were brought in public's attention. These superbugs are highly resistant to all antibiotics including carbapenems. The development of antimicrobial resistance in bacteria renders some infections untreatable today and antimicrobial resistance is now a major health concern.

WHAT A SUPERBUG IS?

The superbug is a microbe with high resistance to antibiotics. It is a type of resistance that has been spreading among different bacteria. The bacteria that pick up this resistance become resistant to virtually all good antibiotics. Carbapenems (imipenem, meropenem, ertapenem) are regarded as most powerful antibiotics which are used as last resort for number of infections such as E.coli and Klebsiella. These bacteria are called as superbugs because the infections caused by them are resistant to wide spectrum antibiotics and are difficult to treat. Now the questions arise where such a resistance come from? How the bacteria acquire power to resist even the strongest antibiotics? How the resistance pass to other microbes and other strains and in which form the transmission take place? Several mechanisms have been developed by bacteria in order to deal with antibiotics but all require either the modification of existing genetic material or acquisition of new genetic material. The development of resistance is progressive, evolving from low levels through intermediate to high levels. With the exception of the direct transfer of genetic information, which can result in immediate high resistance, the development of resistance through stepwise incremental remodeling of the microbe often appears as gradually increasing minimal inhibitory concentrations (MICs)[4]. Organisms that are resistant to one drug are likely to become resistant to others. At least two phenomena contribute to this multi-drug resistance: cross-resistance within a class of antibiotics and genetic loci which can regulate resistance to multiple classes of antibiotics.

Cross-resistance is the phenomenon whereby bacteria that develop resistance to one antibiotic of a class also are resistant to other members of that class to which they may have never been exposed. The mechanisms of resistance by spontaneous mutations have been discarded due to rare mutation events. For every 10^7-10^8 bacteria only one single base pair is expected to change. According to British medical journal "Lancet", the superbug has a gene that codes for enzyme from the category of lactamases that destroy antibiotic property of drug thus making the bacteria highly resistant. The Enzyme was named as NDM-1 and gene coding for it was named as bla $_{NDM-1}$.

NDM-1ENZYME

As the enzyme was first detected from a Swedish patient of Indian origin in 2008, It was named as "New Delhi metallo-beta lactamase (NDM)" after the name of New Delhi, the capital city of India[5]. Later on it was detected in many Asian and other European countries. The gene bla_{NDM-1} which codes for enzyme was discovered by 'Yong and team'[5]. The most common bacteria that make this enzyme are gram negative bacteria. Citrobactor freundii, E.coli and Klebsiella pneumoniae commonly carry bla_{NDM-1} gene[6]. Deshpande and colleagues has found the NDM-1 enzyme clinically in Enterobacteriaceae and Acinetobacter baumannii. The worldwide spread of Enterobacteriaceae that carries carbapenemases producing genes, including bla_{NDM-1}, is a significant threat to human health[7]. Many strains carry bla_{NDM-1} gene on plasmid, which by horizontal gene transfer enable the gene to be transferred between different strains of bacteria. Horizontal gene transfer (HGT) is the movement of genetic material between bacteria other than descent in which information travels through the generation. HGT can take place mainly by 3 mechanisms such as transformation, conjugation and transduction. In conjugation the conjugal plasmids mediate the transfer of DNA and process requires cell to cell contact. This mode of gene transfer can occur even in distinctly related bacteria. The flow of resistance genes to other strains occur in same way. Long DNA segments can be transferred by conjugation. NDM-1 is regarded as new subclass of group1 of MBLs which possesses novel amino acids near to active site. Dongeum yong et al has shown that it also possesses a tyrosin at position 222 instead of universally conserved tryptophan. The ^{bla}NDM-1 open reading frame encodes a putative protein of 269 amino acids with a molecular mass of approximately 27.5 kDa. NDM-1 is actively present as monomer which is shown by Yong and team by NDM-1 sequencing and behaviour through gel filtration and mass spectroscopy[5].

Lactamases

β-lactamases are the enzymes produced by bacteria and are capable of inactivating certain type of antibiotics. β-lactamases form a diverse group of enzymes; some have affinities for the structures of a limited number of antimicrobial agents whereas others are extended- or broad-spectrum β-lactamases that are able to degrade a wide array of antibiotics[8]. MBLs mediate resistance to β-Lactams by cleaving amide bond of β-Lactam ring. MBLs possess a distinct set of amino acids forming active site to coordinate with zinc ions, which in turn

coordinate two water molecules necessary for hydrolysis[9]. The enzyme secretions may be present in large quantities and most bacteria produce only one form of enzyme. The enzymes can be grouped into following four classes.

Table 1. Group of enzymes

Class-A	Include extended spectrum β- lactamases (ESBLs). Degrade penicillin, cephalosporins.
Class-B	These are Zn^{2+} dependent enzymes that destroy all β-lactamase except Aztreonam (a carbapenems).
Class-C	Active against cephalosporins.
Class-D	Include cloxacillin degrading enzyme.

Gram positive bacteria produce large amount of β-lactamase and most of these enzymes are penicillinase. About 1% of dry weight of bacterium can be penicillinase. Gram negative bacteria produce relatively small amount of β-lactamases. These are located in perplasmic space between the inner and outer cell membrane. The carbapenems which were considered highly active to overcome the effect of lactamases are now under threat by gene ^{bla}NDM-1 producing NDM-1 enzyme which is a carbapenemases beta lactamase. This enzyme hydrolyzes and neutralise the activity of carbapenems antibiotics. Thus the bacterium carrying the NDM-1 enzyme is most powerful superbug around and there are no current antibiotic to combat NDM-1. The matter of further higher concern is that the DNA code can easily jump from one bacterium strain to another through horizontal gene transfer. The spread of mobile carbapenemases among bacterial pathogens is of great concern. It is because these are not only resistant to various β-lactam antibiotics but are also resistant to multiple other classes of antibiotics, leaving very few treatment options available.

Carbapenemases

These enzymes fall into 3 of ambler classes of beta-lactamases A, B and D class. These include the following[10]

- Klebsiella pneumonia carbapenems (KPC:Class-A).
- Four Serine carbapenems (SME, NMC-A, IMI, GES:Class-A).
- several metallo β-lactamases (IMI,VIM:Class-B).

Class-B metallo β-lactamases (MBL carbapenemases) have wide spectrum of activity and are presenting a serious threat because of their profile dissemination. Karthikeyan Kumarasamy and colleagues have analysed the prevalence of NDM-1 in carbapenems-resistant enterobacteria isolates from patients in India, Pakistan, and UK from 2003 to 2009[11]. Metallo β -lactamases constitute the molecular class B of Ambler[12] and fall in group 3 according to Bush jacoby medeireos functional classification[8]. Most MBLs are broad spectrum enzymes which also hydrolyze a varity of penicillins and cephalosporins. The class B is further identified in 3 subclasses on the basis of known sequences as subclass B1, B2 and B3.

- Subclass B1:- It includes the enzymes; possess zinc coordinating residues of three histidines and one cysteine. Examples include VIM protein in some P.aerouginosa isolates, IMP protein from P.aerouginosa isolates, IND-1 enzyme from chryseobacterium indalogenes etc[13].

- Subclass B2:- It includes the enzymes which possesses an asparagine instead of histidine at first place of principal zinc binding motif, NXHD. B2 subclass includes enzymes produced by various species of aeromonas. Examples include CphA, Imis and CphA2[9, 10, 13].
- Subclass B3:- Include L_1 protein from Stenotrophomonas matophila. L_1 is unique among all β-lactams in being tetramer while all other B1, B2 and other B3 enzymes are monomer.

WHY RESISTANCE ACQUISITION RATE IS INCREASING

There are more than 150 antibiotics available to public, but the smart bacteria are acquiring resistance with greater rate. The pharmaceutical companies concentrate more on finding antimicrobial which are similar to one already found. Thus companies avoid themselves from high cost of developing new drug. Such profit seeking companies also tend to keep themselves away from the risk of producing unmarketable drug. So, for the bacteria already resistant to one antimicrobial drug easily acquires resistance to similar antimicrobial. Widespread misuse of antibiotics also accelerates the rate of resistance acquisition. The main causes which may enhance resistance development include:

- Antibiotics in animal feed.
- Unnecessary and unfinished antibiotic prescriptions.

Antibiotics in animal feed are delivered to healthy animals as well. The bacteria which lives in symbiotic relationship with animals like cows and chicken acquire resistance gene and can horizontally transfer gene to other bacteria. In few cases of viral infection, antibiotics are also prescribed unnecessarily.

VARIOUS REPORTED CASES…

1) In December 2009: First case detected in a Swedish national who acquired Klebsiella pneumonia infection while treatment in India in 2007[24].
2) March 2010: Study in Mumbai found the bla_{NDM-1} gene carried by bacteria isolated from a patient.
3) May 2010: A case of infection with E.coli carrying resistant gene was reported in Coventry.
4) June 2010: Three cases reported of Enterobacteriaceae having antibiotic resistance in United States.
5) July 2010: Three cases of Acinetobacter baumannii bearing bla_{NDM-1} were reported by a team in New Delhi.
6) August 2010: Journal “The Lancet infectious diseases” published a study by multinational team which prepared a list of such cases as fellow[11]:

Table 2. Reported cases in different sites

Site	Reported cases	Reference
United kingdom	37	11
United states	03	
France	02	
Austria	02	14
Belgium	02	15

Denmark	01	16
Sweden	01	05
Chennai	44	
Haryana	26	
Other sites in India and Pakistan	73	

The wide spread of bla_{NDM-1} in Indian environment is vividly indicated by the fact that most of Indian isolates from Chennai and Haryana were from community-acquired infections [11, 17].

7) August 2010: The first reported death of a Belgian man in a hospital in Pakistan.
8) August 2010: Canada witnessed its first confirmed case at Brampton in Ontario province. Similar cases were also reported from British Columbia and Alberta provinces.
9) September 2010: First case in Japan carrying NDM-1 enzyme was detected.

DOUBTFUL FINDINGS, CONCLUSIONS AND SUGGESTIONS BY INVESTIGATORS

- **Overestimation of prevalence**
  Studies performed at various locations show that the biases were not eliminated accurately and multiplicity of data exists. So the measured prevalence rates cannot be regarded as accurate. In many cases the overestimation was due to the isolates obtained from same patient at different time intervals or from different sources at same time like from blood as well as urine. It is very much clear from the study on suspected NDM-1 infected patients in United Kingdom. Total 37 isolates were obtained from 29 patients[11] and it is vividly mentioned by investigators. More than one sample was collected from each patient.
- **Blaming Indian sub continental hospitals**
  In most of the studies regarding carbapenems resistance, the authors suggested or restricted foreigner to travel Indian sub continental hospitals for surgical treatment. So one would expect that subject patients acquired infection and resistance in hospitals. However the isolates from Chennai and Haryana were primarily infected from community acquired infections and they were admitted to hospitals latter on[11].
- Age group, interval between two isolates is not clearly mentioned.
- The types of hospitals where the isolates were obtained from are also not mentioned. The hospitals in India which the foreigners generally opt for medical tourism meet the world class specifications. General interpretation as made by various investigators and authors regarding not visiting the Indian hospitals seems to be unduly alarming.

CONTROVERSIES OVER NAME NDM

Resistance to antibiotics is worldwide occurrence and major factor is considered to be the indiscriminate use of antibiotics. Transmissible resistance of bacteria to many drugs is not new. In Greece bacteria resistant to colistin existed much earlier than in India subcontinent. NDM (New Delhi metallo lactamase) was assigned because the first reporting was linked to Indian capital city New Delhi. Major controversies between investigators, mass media, politicians and authors aroused over the name. To blame a region for its spread might not be

fair. Such resistance could be present in other parts of the world also. Non-reporting does not mean that it does not exist there. About 41% of patients who were found positive for NDM-1 had no travel or any other link to Indian subcontinent. The investigators could link only 17 of 37 U.K patients harbouring the bla_{NDM-1} gene to Indian subcontinent while others resided as well as treated only in U.K. The first KPC was isolated in North Carolina in 1996 and it had spread throughout the world. Other MBLs carbapenems such as IMP and VIM were also discovered far before the NDM-1. VIM-1 was first described in Verona, Italy in 1997 and found resistant to series of β-lactams[9]. It is not yet proved which form is more virulent whether previously discovered wide spread IMP, VIM or recently highlighted NDM-1. The sudden wakeup call and the way the story was presented in media led to fear among public. The Indian council for medical research have expressed concerns about such conclusions.

DISCUSSIONS

The prudent use of antimicrobial agents is a necessary control measure to reduce resistance. Shorter courses of therapy and less prophylactic use may be ways to reduce antimicrobial use infections. This creates the potential for increased morbidity, mortality and health care costs associated with infections which have, until now, been simply and cheaply treated. Such resistant bacteria are being isolated with increasing frequency and there is concern that the world is entering an era in which antimicrobials will no longer be effective treatment for a wide range of common bacterial infections[18, 22]. In an effort to decrease the appearance of antimicrobial-resistant bacteria the European Union (EU) initiated several actions including the removal of all antimicrobials used as growth-promoting substances in the livestock industry (Regulation EC 1831/2003)[19]. Despite the urgency of the problem, the achievement of these goals has not been simple or straightforward, and accomplishments to date have been insufficient. Role of media in dissemination of information and awareness is worth appreciation but it is not create panic and controversial issues in public. It also requires support and leadership from the government and a willingness to address complex and sometimes controversial scientific, medical, and economic issues as it is needed in case of NDM-1 controversy.

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