The challenge of the New Delhi metallo-β-lactamase (NDM-1) and the carbapenemases

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Paradox

A small truth sitting on a large truth and running in the opposite direction

The lottery

The lottery makes people rich

The lottery makes people poorer
Antibiotic resistance and its cost: is it possible to reverse resistance?

Dan J. Andersson* and Diarmaid Hughes

Abstract. Most antibiotic resistance mechanisms are associated with a fitness cost that is typically observed as a reduced bacterial growth rate. The magnitude of this cost is the main biological parameter that influences the rate of development of resistance, the stability of the resistance and the rate at which the resistance might decrease if antibiotic use were reduced. These findings suggest that the fitness costs of resistance will allow susceptible bacteria to outcompete resistant bacteria if the selective pressure from antibiotics is reduced. Unfortunately, the available data suggest that the rate of reversibility will be slow at the community level. Here, we review the factors that influence the fitness costs of antibiotic resistance, the ways by which bacteria can reduce these costs and the possibility of exploiting them.

The introduction of antibiotic resistance is one of the most important medical innovations of the 20th century. It has been proposed that a reduction in antibiotic use (and, therefore, in the selective pressure for resistance) would benefit the fitter susceptible bacteria, enabling them to outcompete resistant strains over time. Experimental studies and theoretical modeling support this basic concept, but other processes such as compensatory evolution and genetic evolution complicate the picture and make reversibility less probable in real-life settings. This Review focuses on experimental studies of the factors (in particular, the fitness cost) that influence the development of resistance and the prospects for reversibility. We describe the outcomes of laboratory studies and clinical interventions to reduce the frequency of resistant bacteria in individual patients as well as at the community level and explain how reversibility, if it occurs at all, proceeds so slowly that, in most cases, it is unlikely to be of practical importance.

The development of resistance and its reversibility. The rate of appearance of resistant bacteria is determined by the frequency of resistance and the selective pressure exerted on it. This is a result of the fact that it is possible to measure the frequency of resistance directly. Therefore, the rate of elimination of resistant strains can be measured using this approach. The rate of reversibility can be measured using the frequency of resistant strains directly. This is particularly useful in the evaluation of treatment outcomes, where the frequency of resistant strains is used to determine the effectiveness of a treatment. The rate of reversibility can also be estimated using indirect approaches, such as the frequency of resistant strains during treatment, which is used to determine the effectiveness of a treatment. The rate of reversibility can be measured using the frequency of resistant strains directly. This is particularly useful in the evaluation of treatment outcomes, where the frequency of resistant strains is used to determine the effectiveness of a treatment. The rate of reversibility can also be estimated using indirect approaches, such as the frequency of resistant strains during treatment, which is used to determine the effectiveness of a treatment.
Dynamics of fitness during resistance acquisition

- Survival of the least impaired
- Compensatory mutations
- sick
Over expression of GroEL causes increased fitness

Genomic buffering mitigates the effects of deleterious mutations in bacteria

Sophie Maisnier-Patin¹, John R Roth², Åsa Fredriksson³, Thomas Nyström³, Otto G Berg⁴ & Dan I Andersson¹,⁵

Nature Genetics
Volume 37 number 12
December 2005

We measured the decrease in fitness caused by increasing mutation number in the bacterium Salmonella typhimurium using a regulated, error-prone DNA polymerase (polymerase IV, DinB). As mutations accumulated, fitness costs increased at a diminishing rate. This suggests that random mutations interact such that their combined effect on fitness is mitigated and that the genome is buffered against the fitness reduction caused by accumulated mutations. Levels of the heat shock chaperones DnaK and GroEL increased in lineages that had accumulated many mutations, and experimental overproduction of GroEL further increased the fitness of lineages containing deleterious mutations. These findings suggest that overexpression of chaperones contributes to antagonistic epistasis.
Carbapenem resistance in *Klebsiella Pneumoniae*

2005

2010

http://www.ecdc.europa.eu
Trends in proportion of imipenem-resistant *Klebsiella pneumoniae* isolates in hospitals in Greece, 2000-2006

- % of imipenem-resistant *K. pneumoniae* in wards
- % of imipenem-resistant *K. pneumoniae* in ICU
- % of hospitals with imipenem-resistant *K. pneumoniae*

A. Vatopoulos  Eurosurveillance Vol 13 issue 1-3 Jan-march 2008  
www.eurosurveillance.org
# Mobile Carbapenemases in Enterics

<table>
<thead>
<tr>
<th>Molecular class</th>
<th>Minor</th>
<th>Major</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>A</strong> (Serine active site)</td>
<td>GES-4,5, NMCA, SME 1-3, IMI</td>
<td><strong>KPC</strong></td>
</tr>
<tr>
<td><strong>B</strong> Metallo-β-lactamases (Metal active site)</td>
<td>IMP, GIM-1, KHM, SMB</td>
<td><strong>NDM, VIM-1,</strong></td>
</tr>
<tr>
<td><strong>D</strong> (Serine active site)</td>
<td>OXA-162, OXA-181</td>
<td><strong>OXA-48,</strong></td>
</tr>
</tbody>
</table>

Generally carbapenemases are difficult to detect in enteric bacteria.
Resistant to everything except colistin

5 $\beta$-lactamases

- **OXA-48**
- **OXA-47**
- **SHV2a**
- **TEM-1**
- **SHV**

**PROPERTIES**

- Hydrolyses penicillins and carbapenems but not 3$^{rd}$ generation cephalosporins
- Strong carbapenemase: 10X the activity of OXA-40, 3X activity of KPC
- Inhibitor resistant
IMPORTANCE OF METALLO-\(\beta\)-LACTAMASES

- Broad spectrum of activity.
- No clinical inhibitor available.
- Closely associated with Multi-Drug and Pan Resistance.
- They are emerging rapidly.
Location of Mobile MBL Containing Organisms 1995
Lahey website lists 31 IMP, 32VIM and 6 NDM variants (September 2011)
Carbapenemase +ve Enterobacteria referred to ARMRL, 2003-May 2011

Klebsiella pneumoniae >> E. coli >> other species
Carbapenemases in France

<table>
<thead>
<tr>
<th>Bacterial species</th>
<th>Number of episodes by type of carbapenemase</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OXA-48</td>
<td>KPC</td>
</tr>
<tr>
<td>Klebsiella pneumoniae</td>
<td>12</td>
<td>14</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>9</td>
<td>1</td>
</tr>
<tr>
<td>Enterobacter cloacae</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>Enterobacter aerogenes</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Citrobacter freundii</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Proteus mirabilis</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Serratia marcescens</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>23*</td>
<td>16</td>
</tr>
</tbody>
</table>

6/7 NDM-1
Travel history to India

~80% of OXA-48
Have travel history to Turkey or Morocco

193 patients
29% infected
71% colonized

Similar story in other European countries: Grundmann et al Eurosurveill 2010,
Hentschke et al 2011 DMID
Emergence of High Levels of Extended-Spectrum-β-Lactamase-Producing Gram-Negative Bacilli in the Asia-Pacific Region: Data from the Study for Monitoring Antimicrobial Resistance Trends (SMART) Program, 2007

Stephen P. Hawser, Samuel K. Bouchillon, Daryl J. Hoban, Robert E. Badal, Po-Ren Hsueh, and David L. Paterson

Asia pacific region (not including India)

HAI 31.5%  ESBL rate
CAI 13.7%  ESBL rate

China (E. coli)

HAI 64.7% ESBL rate
CAI 36%  ESBL rate

India (E. coli)

HAI 78.9%
CAI 79%

9 sites in India lowest ESBL E. coli rate was 54.5% and the highest 94.1%
“Rate of acquisition of ESBL-producing strains was highest for travellers visiting India.”

88% ESBL acquisition rate

With 2 week stay in India (p=0.001)
Index isolate
59 year old Swedish National of Indian descent who often travelled between India and Sweden For medical treatment.

Information first presented at ICAAC in 2008

A Novel Subgroup Metallo-β-lactamase (MBL), NDM-1 Emerges in Klebsiella pneumoniae (KPN) from India. Yong, D., Toleman, M. A., Giske, C., Walsh, TR
Internet search for NDM-1 beginning of August 2010

Only 3 hits

Original paper

UK ARMRL resistance alert

Noodles Corbis who plays an Ibanez NDM1 guitar

August 6th >5 million internet hits numerous TV, newspaper reports

Offspring guitarist and former high school janitor called “Noodles-corbis”
New Delhi metallo-beta-lactamase

The Washington Post

New 'superbug' found in UK hospitals

New 'superbugs' 

Urinary tract infections, ailments caused by germ the power to destroy ant of a fresh generation of

Taiwan reports first case

Super-bug NDM-1: first known fatality, more infections worldwide

Drug-resistant 'Superbug' sparks global fears

Debt problems? Want to know if you can wipe out all the debt you can't afford?
Emergence of a new antibiotic resistance mechanism in India, Pakistan, and the UK: a molecular, biological, and epidemiological study


Summary
Numerous species, strains and plasmids

Multiple incA/C plasmids, non-typeable multiple incF, incQ and numerous others numerous species
Indian Response to LID paper

“It is disturbing, in context, to read calls in the popular press for UK patients to opt for corrective surgery in India with the aim of saving the NHS money. As our data show, such a proposal might ultimately cost the NHS substantially more than the short-term saving and we would strongly advise against such proposals.”

Lancet infectious diseases received 26 letters of complaint

- **New Delhi metallo-beta-lactamase 1 reply**
Indian government response

**Linking superbug to India 'totally irrational': Govt**

Agencies  Posted online: Thu Aug 12 2010, 15:22 hrs

**Govt rubbishes report linking superbug to India**

CNN-IBN  Posted on Aug 12, 2010 at 18:57 | Updated Aug 13, 2010 at 10:10

New Delhi: The Government on Thursday rejected the report that claimed that India was home to a "superbug".

**Superbug: Govt to counter UK**

New Delhi August 12, 2010

The linking of the antibiotics-resistant 'superbug' to India has sprung the government into action.

**MNCs may be behind superbug 'propaganda', say MPs**

August 12, 2010 13:48 IST

New Delhi: Members in the Rajya Sabha on Thursday suspected hands of multinational pharmaceutical and hospital companies behind British scientists' claims of 'superbug'.

**Govt says unfair to blame India on alarm over 'New Delhi' bug**

Teena Thacker  Posted online: Thu Aug 12 2010, 08:12 hrs

**Parts of NDM report doctored: Indian co-author of study**

The Indian co-author of the medical study linking hospitals in the country to a "superbug" dismissed as hypothetical this conclusion and said interpretations were made without his knowledge.

**Frightening picture of superbug is incorrect confirmed by Superbug author**

Submitted by Piyush Diwan on Sat, 08/14/2010 - 02:51.

**Government says superbug not linked to India**

12 August 2010

**Govt trashes reports of NDM-I's India links**

Says there could be an 'ulterior motive' in circulating such reports

PTI | New Delhi | August 13 2010
Drug controller general of India??

Now, the Drug Controller General of India (DCGI) has sent letters to the Indian scientists in the research team, asking for details about the “form and manner adopted in collecting human and biological material from various sites within the country and transferring them or exporting them to another country”.

The scientists have been given 15 days to list the rules, regulations and guidelines they followed while carrying out the study.

Dr V M Katoch, chairman of the screening committee under the Health Ministry, said the Indian...

Are we in Iran or Pakistan

By: raj | 02 Sep 2010

It is shocking member of Parliament who we voted with a limited mandate to govern and give the best for next 5 years, take themselves the responsibility as permanent guardians and gods of the people who put them there. It is also equally shocking government agencies taking the cue is harassing this poor doctors. Let me tell you one thing - where I live a very nice place in Chennai. The sewer over flows and runs in the main road. This is a common sight in many places in Chennai. School children try to side step and walk around. This sight will never be seen any part of the world. I am sure diseases. Our patri which they have be politicians and parl

Dr

By: Mark Tolenean | 03-Sep-2010

It seems very confusing at least to me that the Drug Controller General of India appears to be more interested in controlling scientists than he is at controlling the indiscriminate use of antibiotics in India - one must question whether he is in the right Job!!

Instead fund thes

By: Witheld | 02-Sep-2010

The fact that the superbug has been discovered is a great contribution to science, as it will pave way for another phase of research to fight this killer bacteria. It is very difficult to understand the sentiments and resistance attached to using 'New Delhi' into it's description. The fact, that the scientists have been given a notice of regulatory breach(s), reminds us of the dark ages. Decades ago when I attempted to report Cholera like illness in rural M.P. while still a fresh medical graduate; I had to face a policeman sent by the officials, and was asked grilling questions regarding the 'proof' it's existence. This superbug is a killer, and it can not be fought 'politically'. The scientists (and their thoughts) do not have 'borders'. Let them work uninterrupted, and even if they did any omission, it can be easily
Drug resistant superbug threatens UK hospitals

Thursday 28 October 2010

Efforts to contain a new strain of superbug threatening the NHS and other health authorities around the world may almost be unachievable, exclusive research by Channel 4 News reveals.

The bacteria, which has been linked to patients treated in hospitals in the Indian sub-continent, is resistant to nearly all antibiotics. Though first only found in patients being treated in hospital - we've found evidence that it may be widespread in the community in
Example of bacterial growth from a New Delhi Environmental sample

Incubate at 35°C - 37°C for 16 - 24 hrs. Read results.

- Pink/Red
- Turquoise/Blue-Green
- Dark Blue/Purple
- Brown halo
- Brown/Green
- Non-pigmented

E. coli Enterococcus spp. coliforms Proteus Morganella Providencia Pseudomonas spp. staphylococci streptococci
Dissemination of NDM-1 positive bacteria in the New Delhi environment and its implications for human health: an environmental point prevalence study

Timothy R Walsh, Janis Weeks, David M Livermore, Mark A Toleman

Summary

Background Not all patients infected with NDM-1-positive bacteria have a history of hospital admission in India, and also extended-spectrum β-lactamases are circulating in the Indian community. We therefore measured the prevalence

Findings From Sept 26 to Oct 10, 2010, 171 seepage samples and 50 tap water samples from New Delhi and 70 seepage samples from Cardiff Wastewater Treatment Works were collected. We detected blaNDM-1 in two of 50 drinking-water samples and 51 of 171 seepage samples from New Delhi; the gene was not found in any sample from Cardiff. Bacteria with blaNDM-1 were grown from 12 out of 171 seepage samples and two out of 50 water samples, and included 11 species in which NDM-1 has not previously been reported, including Shigella boydii and Vibrio cholerae. Carriage by enterobacteria, aeromonads, and V cholerae was stable, generally transmissible, and associated with resistance patterns typical for NDM-1; carriage by non-fermenters was unstable in many cases and not associated with typical resistance. 20 strains of bacteria were found in the samples, 12 of which carried blaNDM-1 on plasmids, which ranged in size from 140 to 400 kb. Isolates of Aeromonas caviae and V cholerae carried blaNDM-1 on chromosomes. Conjugative transfer was more common at 30°C than at 25°C or 37°C.

Interpretation The presence of NDM-1 β-lactamase-producing bacteria in environmental samples in New Delhi has important implications for people living in the city who are reliant on public water and sanitation facilities. International surveillance of resistance, incorporating environmental sampling as well as examination of clinical isolates, needs to be established as a priority.
Positive NDM-1 isolation sites within 12km of central New Delhi
1 in 5 Delhiites drink impure water  

(March 11\(^{th}\) 2011)

The toxic cocktail included Entero Bacter, E.Coli and Salmonella Typhae bacteria. These bacteria enter drinking water if it is contaminated with raw sewage, which contains human excreta.

The result is a virtual bio weapon, which can cause gastrointestinal diseases like typhoid, cholera, gastroenteritis, or jaundice.

Indian standards allow up to 10 CFU per 100 ml. The standard prescribed...
Again Indian government denial

Don't spread panic on superbug, says Sheila Dikshit

Superbug: Sheila Dikshit plays down threat as Delhi govt offers chlorine tablets

New Delhi: Chief Minister Sheila Dikshit today appealed people in the city not to panic in the wake of reports that drug resistant bacteria was found in Delhi's public water supply and said water agency Delhi Jal Board (DJB) had rejected the findings of international scientific journal 'Lancet'.

"Delhi Jal Board has very categorically said that this is not the case. I am in touch with the CEO and he said that it is not so. So please don't spread panic when there is no (need to) panic," Dikshit...
NATIONAL POLICY FOR
CONTAINMENT
OF
ANTIMICROBIAL RESISTANCE
INDIA

Directorate General of Health Services
Ministry of Health & Family Welfare
Nirman Bhawan, New Delhi
Hospital detected cases of NDM are the tip of the iceberg

Prevalence of faecal carriage of Enterobacteriaceae with NDM-1 carbapenemase at military hospitals in Pakistan, and evaluation of two chromogenic media.


Infections

Carriage

outpatients 13.8%
inpatients 27.1%

>100 million people carrying NDM-1
In Asia
Massive sanitation problems in India

New Delhi sewage system can only cater for 60% of the population

Unhealthy
Much of India lacks basic sanitation.

72.0% of the population don’t have access to improved sanitation facilities such as those linked to public sewers and septic systems.

58.0% of the population practice open defecation.

43.5% of children under 5 years of age are underweight for age.

20.3% of deaths among children under 5 are caused by diarrheal diseases.

11.0% of the population don’t have access to water from safe sources.

*Refers to sustained access to improved sources including household connections, public standpipes and protected wells or springs. Source: World Health Organization.
Upstream genetic context of NDM-1 gene

**E. coli** Japan (AP012208)

**K. pneumoniae** Sweden (FN396876)

**E. coli** Spain (HQ451074)

**A. baumanii** Germany (HQ857107)

**E. coli** Canada (JF503991)

**E. coli** Canada (JF14412)

**E. coli** Kenya (JF785549)

**E. coli** Spain (JF922606)
GC% change in NDM-1 gene environment
The GC% change happens within the NDM-1 gene
NDM-1 gene environment is identical to that of aminoglycoside gene *aphA6*
$\text{bla}_{\text{NDM-1}}$ is a gene fusion between $\text{aphA6}$ and $\text{bla}_{\text{MBL}}$
Figure 3

(a) In-frame deletion event

(b) rolling circle replication of ISCR27 starting at oriS moves bla_{MBL} into aphA6 forming bla_{NDM-1}
Implications

1. *aphA6* is a gene found almost exclusively in *Acinetobacter baumanii* in the form of a composite transposon flanked by IS*Aba125* elements. Therefore very likely that the fusion event happened in *Acinetobacter baumanii*.

2. *Acinetobacter baumanii* is a hospital associated pathogen therefore this event very likely happened in an Indian hospital.

3. NDM-1 has a lipidation signal and is partially membrane associated-this lipidation signal is weakened by the fusion enabling NDM-1 to be both membrane bound and soluable.

4. NDM-1 has been genetically engineered naturally to remove its original promoter and replace it with a promoter that we know functions excellently in numerous bacterial pathogens and environmental bacteria.
GC% change in NDM-1 gene environment

Sequence here is only 64% identical to *Acinetobacter baumanii* and this means that the DNA Associated with NDM-1 was collected by ISCR16 before ISAb125.
Insertion sequence common regions (ISCR elements)

Direction of rolling circle replication

1. \( \text{bla}_{\text{OXA-45}} \) - terIS - ISCR5 - oriIS
2. \( \text{eltA/B} \) - terIS - IS91 - oriIS

Co-transposed genes

### ISCR Elements

- **ISCR1**: TGGTTTATACTTCCTATAACCC
- **ISCR2**: GCGTTTATTCTTCCTATACGT
- **ISCR3**: GCGTTTGAACCTTCTATACGC
- **ISCR4**: GCGTTTGAATTCCTATACGC
- **ISCR5**: GCGTTTGAACCTTCTATACGC
- **ISCR14**: GCGTTTGAACCTTCTATACCC
- **ISCR15**: GCGTTTGAACCTTCTATACCC
- **ISCR16**: GCGTTTGAACCTTCTATACCC
- **IS1294**: GTTTTTCAATTCCTATACGT
- **IS91**: GGGATTTAAATTCCTATCGAT
- **IS801**: CATTTTGAAATTCCTATGCGAT
Genomic data-searching results

**Percent identities of ISCR ORF’s**

<table>
<thead>
<tr>
<th>ISCR4</th>
<th>ISCR5</th>
<th>ISCR14</th>
<th>ISCR15</th>
<th>ORF</th>
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<tbody>
<tr>
<td>77</td>
<td>88</td>
<td>86</td>
<td>87</td>
<td>ISCR3</td>
</tr>
<tr>
<td>***</td>
<td>75</td>
<td>76</td>
<td>77</td>
<td>ISCR4</td>
</tr>
<tr>
<td>***</td>
<td>95</td>
<td>95</td>
<td></td>
<td>ISCR5</td>
</tr>
<tr>
<td>***</td>
<td>96</td>
<td></td>
<td></td>
<td>ISCR14</td>
</tr>
</tbody>
</table>
Several MBL’s are likely mobilised by ISCR elements

**G+C% ISCR elements**

<table>
<thead>
<tr>
<th>ISCR</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>ISCR1</td>
<td>54%</td>
</tr>
<tr>
<td>ISCR2</td>
<td>59.6%</td>
</tr>
<tr>
<td>ISCR3</td>
<td>68.7%</td>
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<tr>
<td>ISCR4</td>
<td>69%</td>
</tr>
<tr>
<td>ISCR5</td>
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</tr>
<tr>
<td>ISCR14</td>
<td>69.6%</td>
</tr>
<tr>
<td>ISCR15</td>
<td>68.8%</td>
</tr>
</tbody>
</table>
The end of the antibiotic era??

An obituary- On the Death of antibiotics!

Abdul Ghafur K

We come across MDR or even pan resistant Gram negative bugs quite often and such bugs are reported in almost all major centers in India and most of international centers though to a lesser extent than India. We Indians are the leaders in antibiotic resistance. Many of MDR superbugs are from bacterial cultures taken at the time of admission to the hospital. By the time a patient is being admitted to a tertiary care centre, that patient has already visited many other hospitals and doctors and has received multiple courses of different antibiotics. These patients are literally walking culture plates of superbugs and you don’t have to be Nostradamus to predict their clinical outcome.

When we are called to manage patients with severe infections due to pan resistant bugs, we do really wonder whether we are living in pre-Alexander Fleming years without antibiotics and a resistance gene with a glamorous name. The overuse of antibiotics is embedded in our Indian tradition. Why should we Indians worry? We can always depend on honey, yoghurt and cow’s urine. At any rate within a few years these products may be more useful than antibiotics!
Acknowledgements

Molecular Resistance

• Tim Walsh
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• M. Sharma
• U. Chaudhary
• M. Thirunarayan
• P. Krishnan
• J. Bell
• R Jones

Proverbs 3:6 In all your ways acknowledge Him and he will make your paths straight.
Modified Hodge test
Castanheira et al 2011 AAC, 53. 1274-8
How to detect NDM-1 producers

FIG. 1. Strategy for identification of NDM-1 producers as a source of clinical infections and for detecting carriers of NDM-1 producers. *, this culture medium can be used for surveillance of outbreaks of infections with NDM-1 producers after validation of its detection sensitivity for the specific strain responsible for an outbreak. **, Etest MBL is reliable when the MIC of imipenem is not too low.

Nordmann et al 2011 AAC