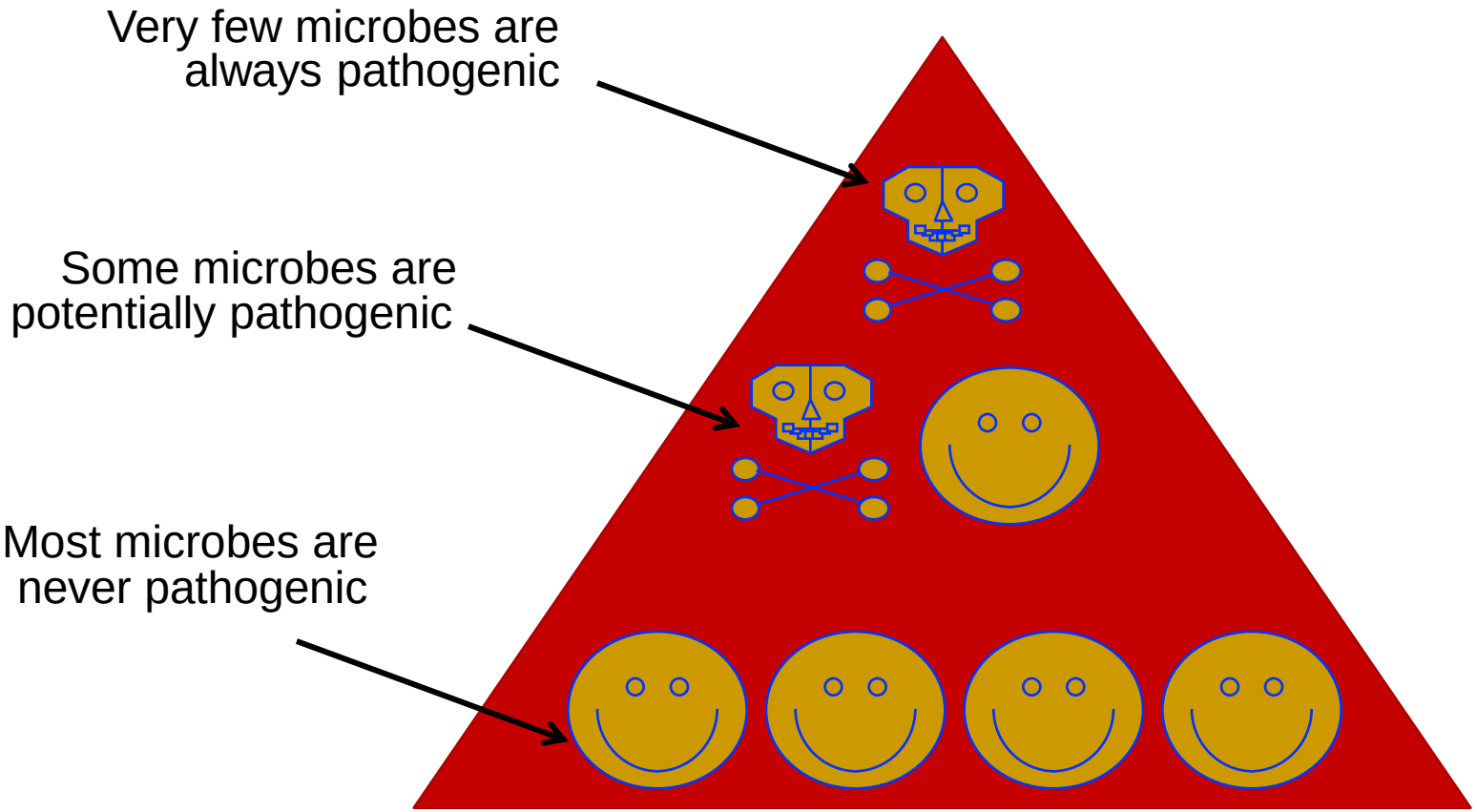
Antibiotic Resistance in Enterobacteriaceae- What is New from My Centre?

Prof KN Prasad, MD
Department of Microbiology,
Sanjay Gandhi Postgraduate Institute of Medical Sciences,
Lucknow

Microbes and humans

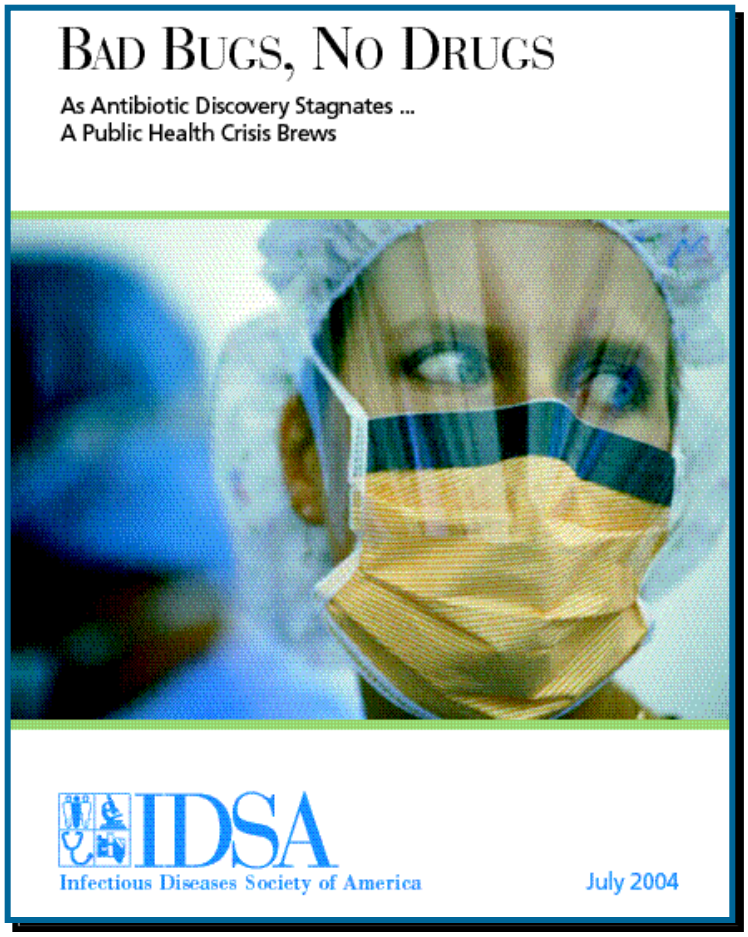


Bad Bugs, know your resistance

Problematic pathogens that "escape" the activity of drugs

"ESKAPE" include

- *Enterococcus faecium*
- *Staphylococcus aureus*
- *Klebsiella spp*
- *Acineto baumannii*
- *Pseudo aeruginosa*
- *Enterobacter* spp / *E. coli*

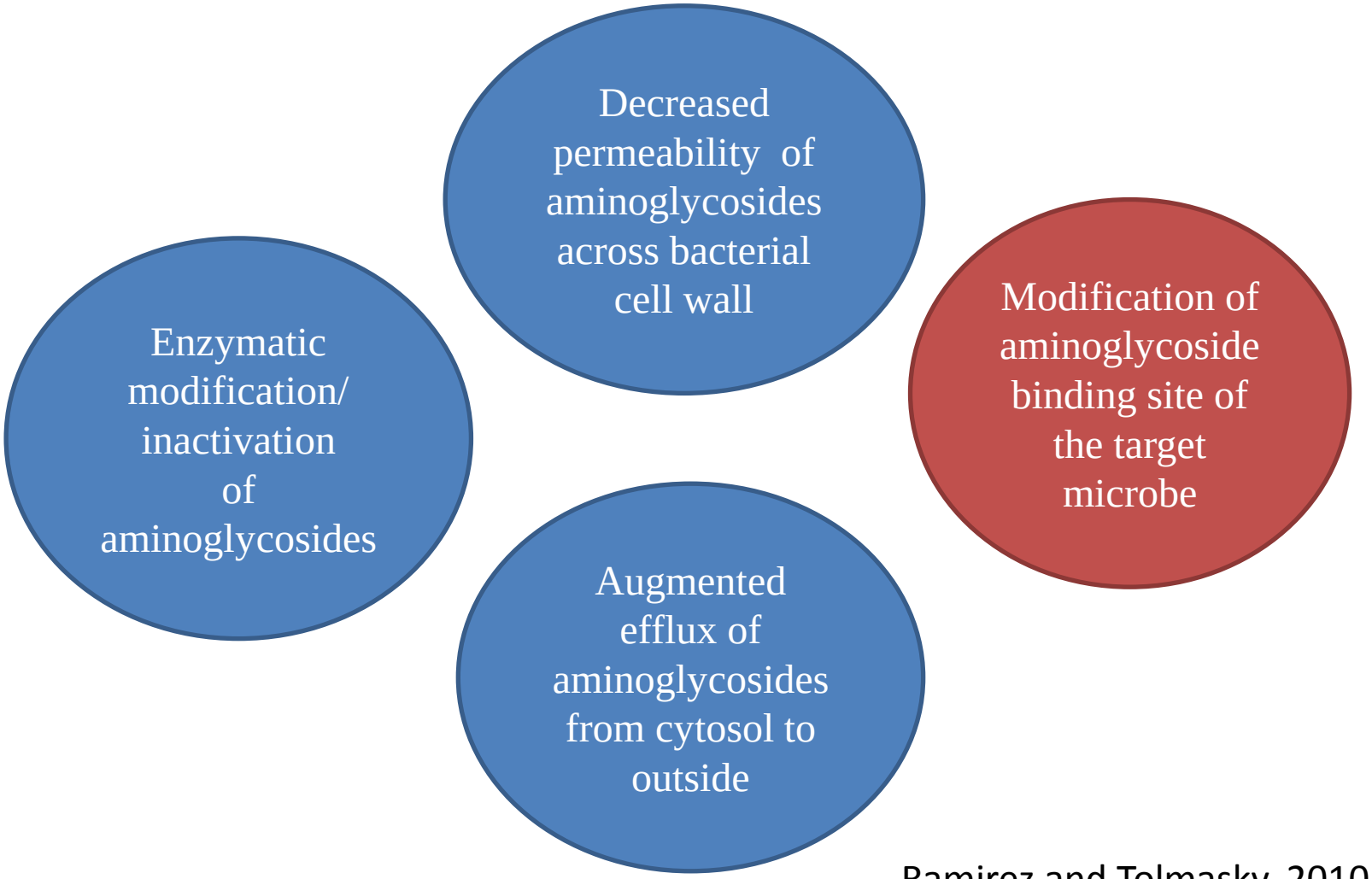


What is New from My Centre

- 16S rRNA methyltransferases (16S-RMTases)- new mechanisms conferring high level resistance to clinically relevant aminoglycosides

- Carbapenemases, especially New Delhi metallo-betalactamase (NDM) and its variants

Aminoglycoside Resistance Mechanisms



Ramirez and Tolmasky, 2010

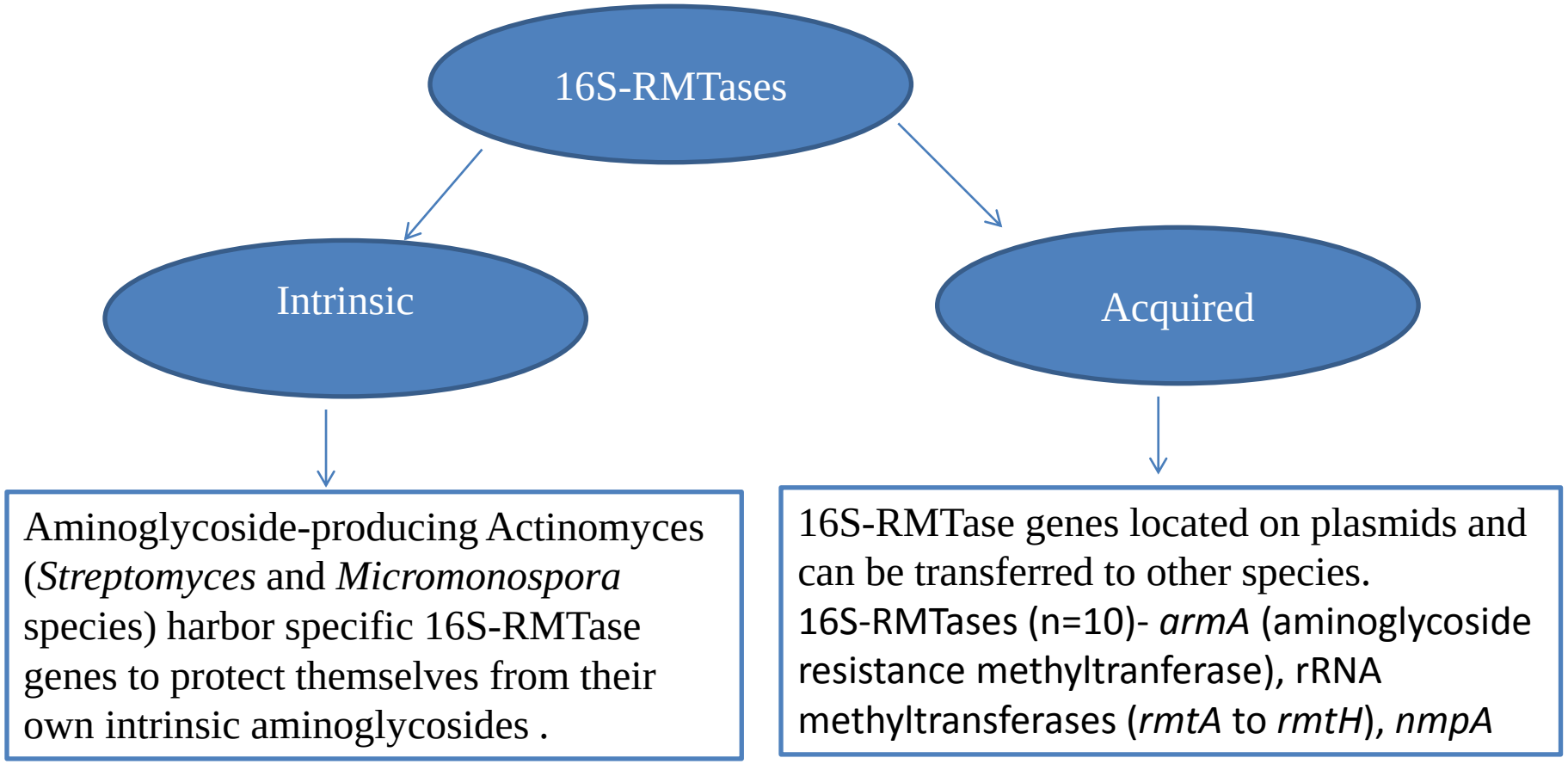
Aminoglycoside-modifying enzymes

- Based on their molecular mechanisms, these enzymes are divided into 3 groups.

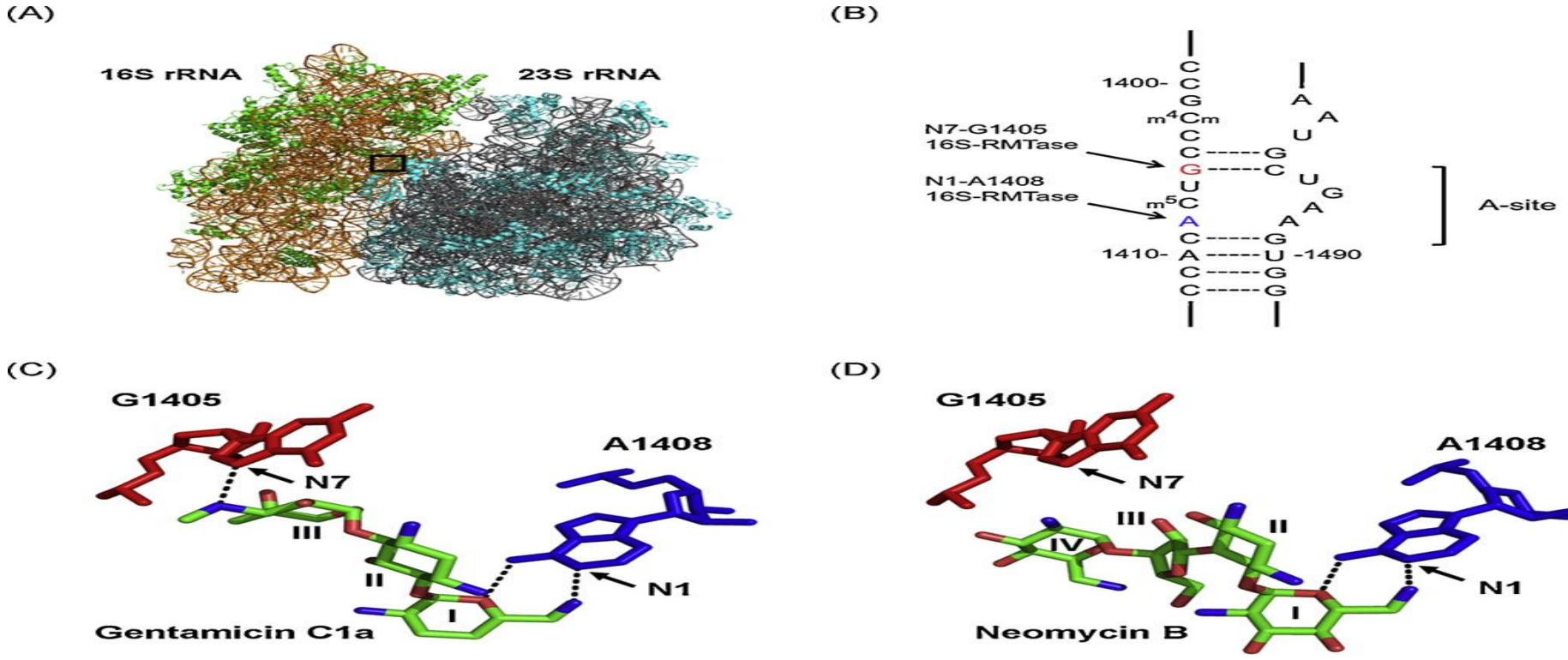
Acetyltransferase	Adenyltransferase	Phosphotransferase
Acetylation of amine group	Adenylation of hydroxyl group	Phosphorylation of hydroxyl group

Ramirez and Tolmasky, 2010

16S rRNA methyltransferase (16S-RMTase)

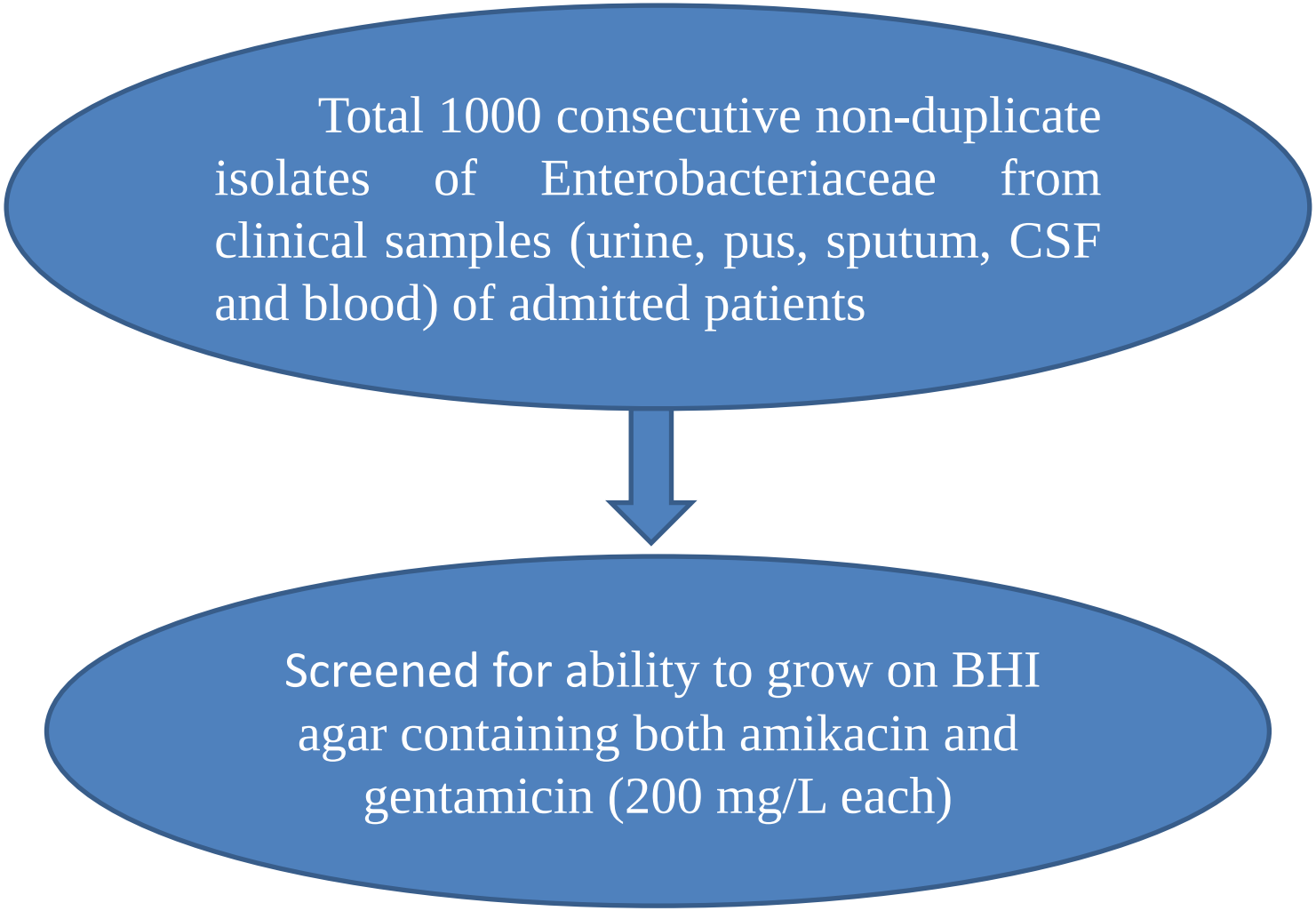


Most aminoglycosides bind to the decoding aminoacyl-tRNA recognition site (A-site) of the 16S rRNA that composes bacterial 30S ribosome, and subsequently interfere with bacterial growth through blocking of protein synthesis

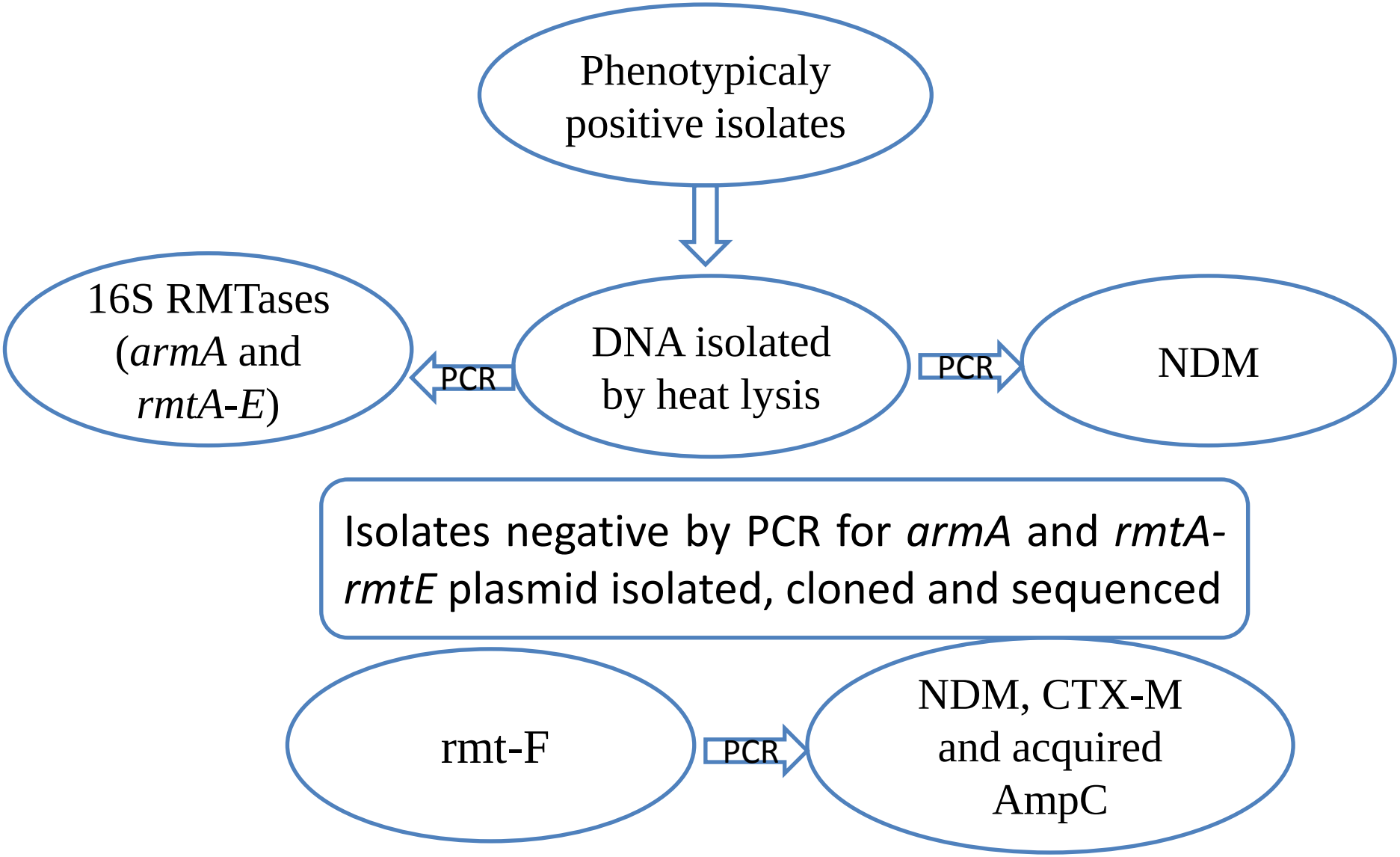


Materials and Methods

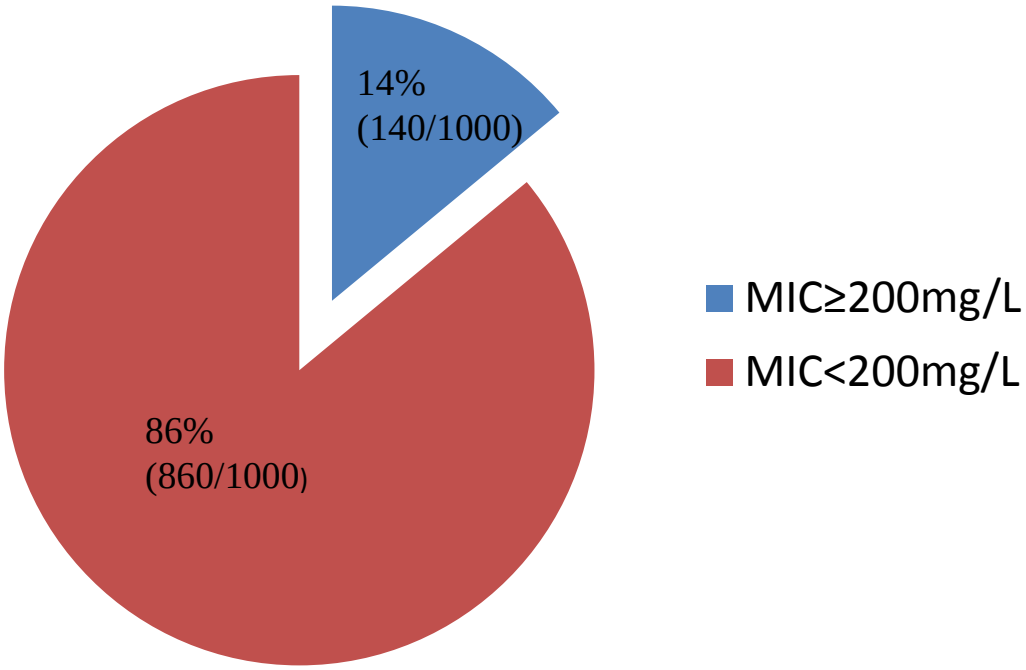
Bacterial isolates and antimicrobial susceptibility testing



Polymerase Chain reactions.

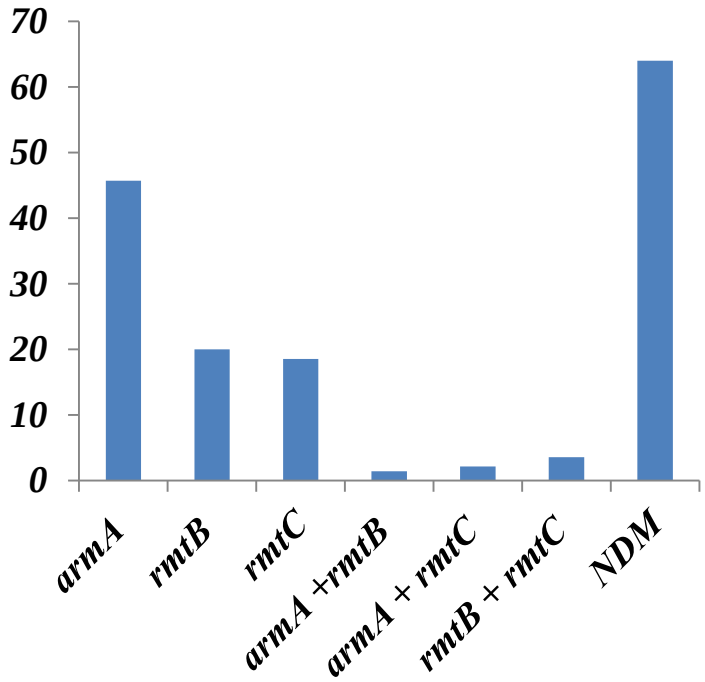


Results



Prevalence

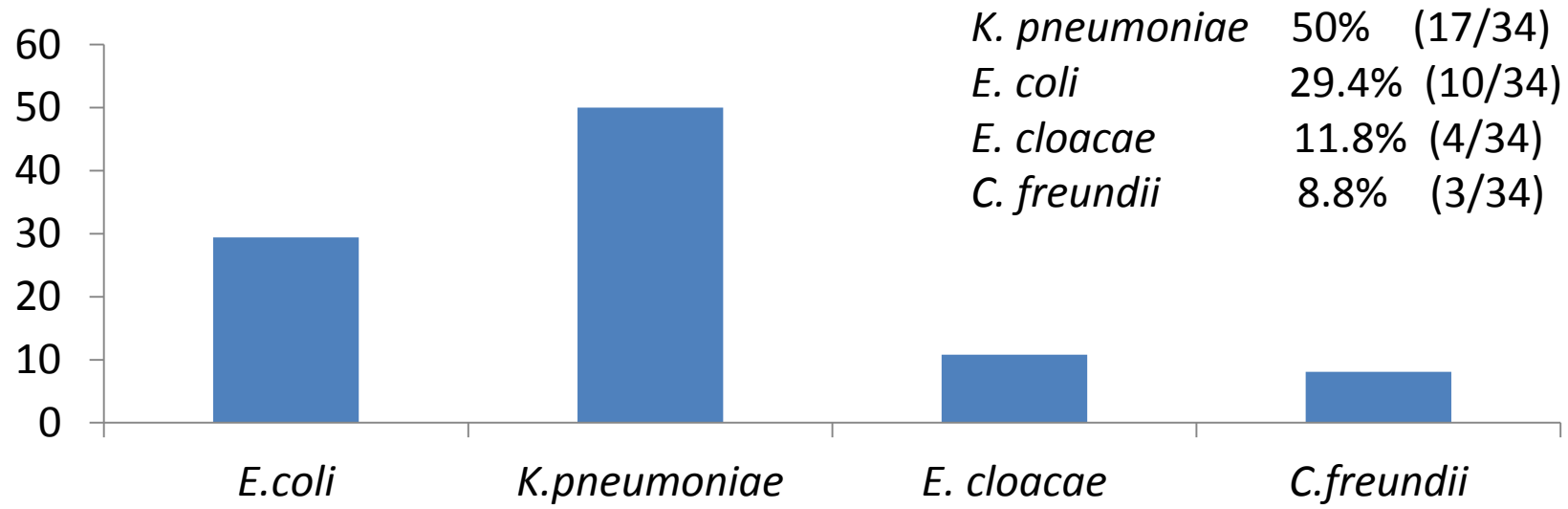
16S-RMTases	Prevalence
armA	45.7% (64/140)
rmtB	20% (28/140)
rmtC	18.6% (26/140)
armA +rmtB	1.4% (2/140)
armA + rmtC	2.1% (3/140)
rmtB + rmtC	3.6% (5/140)
NDM	64% (90/140)



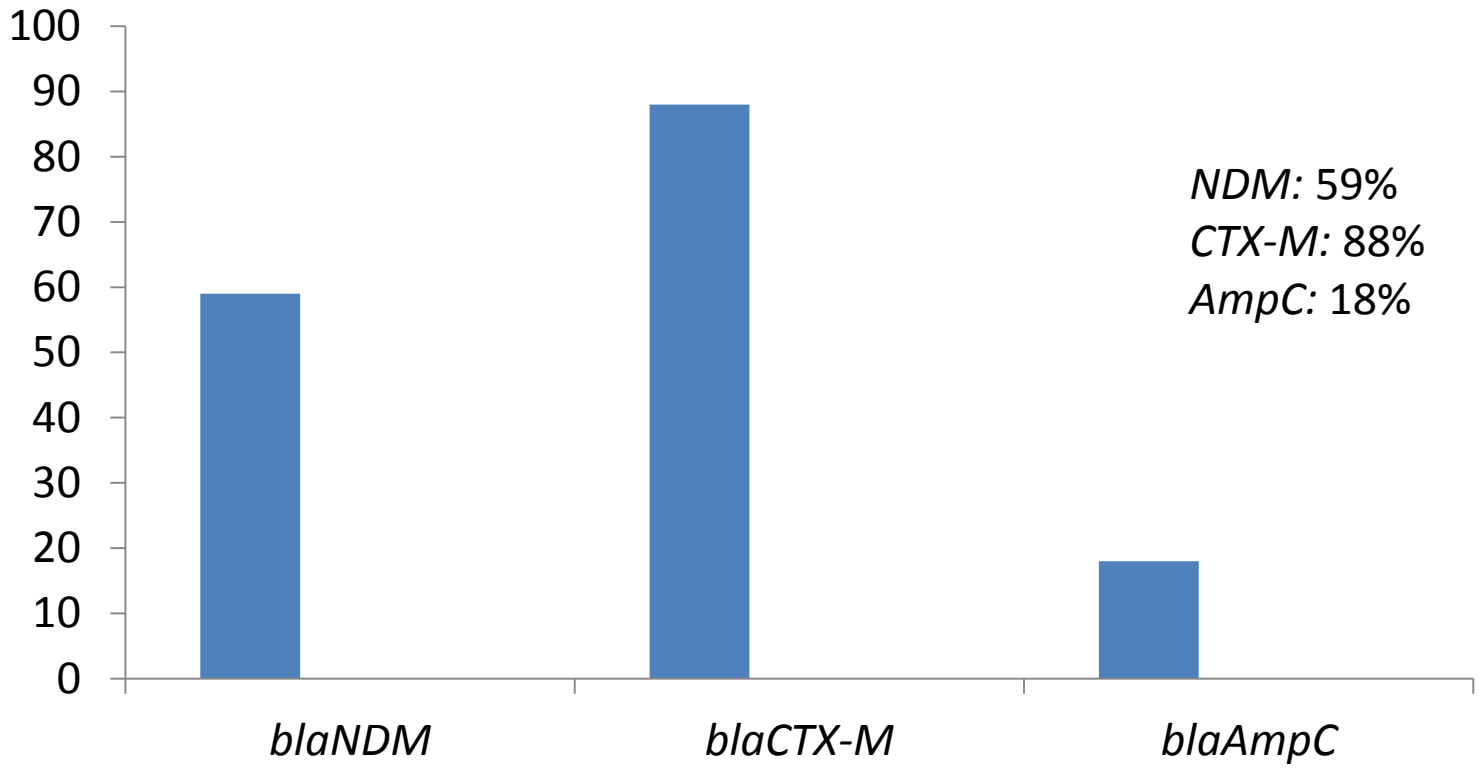
rmtD, *rmtE*, *rmtA*, *npmA* were not found; **novel *rmtF* was detected in 34 (24.3%) isolates**

Distribution of *rmtF* in different Enterobacteriaceae spp.

Total 34 (24.3%) isolates were found to carry *rmtF*

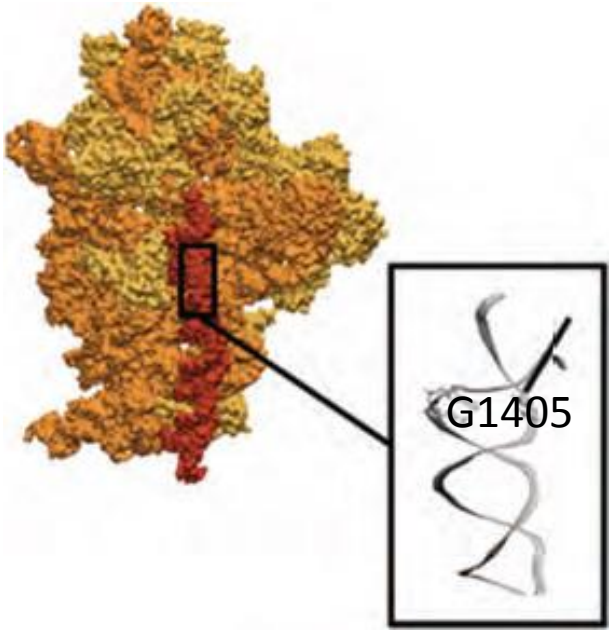


Additional antibiotics resistance genes in *rmtF* bearing clinical isolates

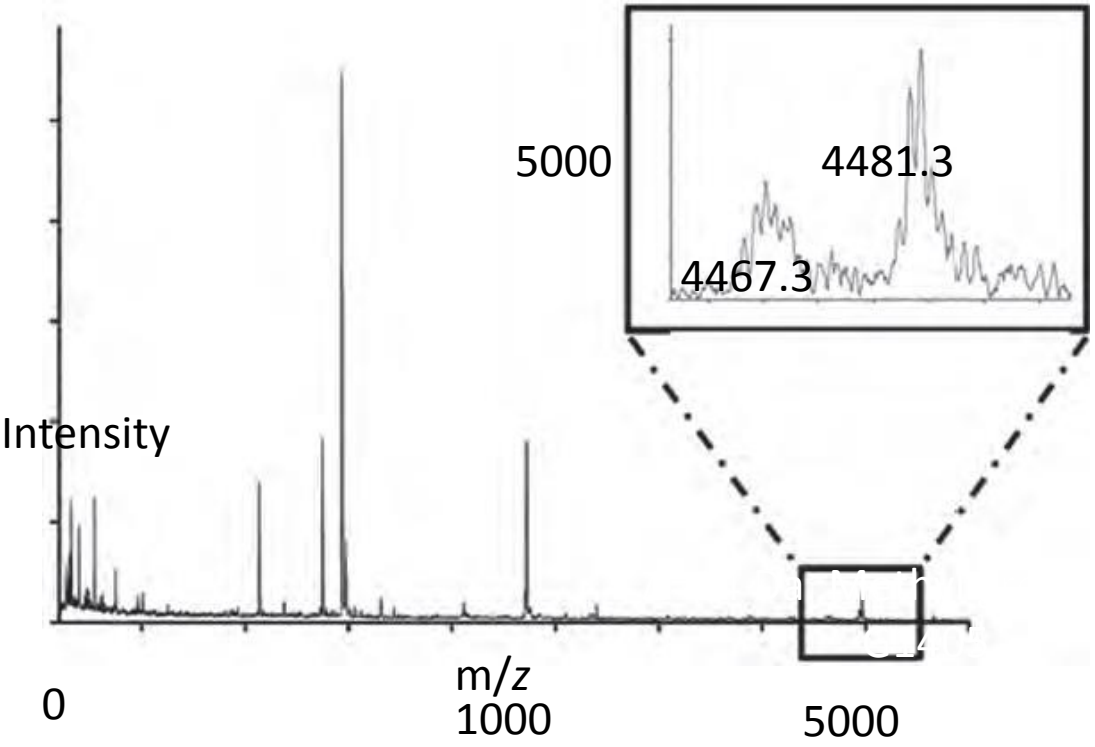


Four rmtF-bearing *K. pneumoniae*: tigecycline resistant (MICs 4–16 mg/L)
Colistin resistance in one *K. pneumoniae* (4 mg/L) & one *E. cloacae* (16 mg/L)

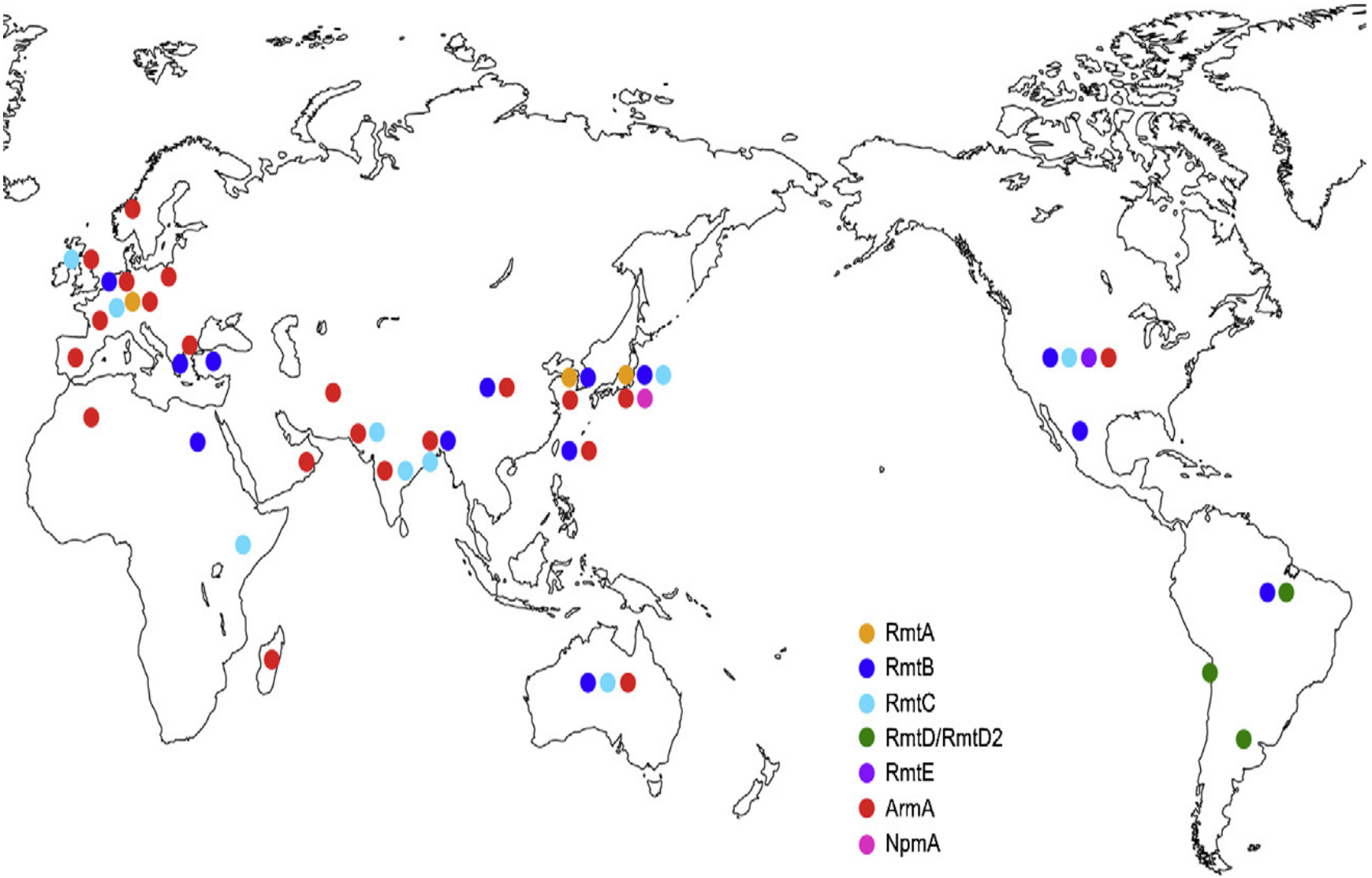
MALDI mass spectrometry- RmtF confers resistance by adding a methyl group at position G1405 of 16S rRNA



Three-dimensional structure of 30S ribosomal subunit- enlarged view shows amino glycosides binding pockets



MALDI mass spectrometry from *E. coli* expressing rmtF



Yachino J, Arakawa Y 2012. Worldwide distribution of acquired 16S-RMTases.

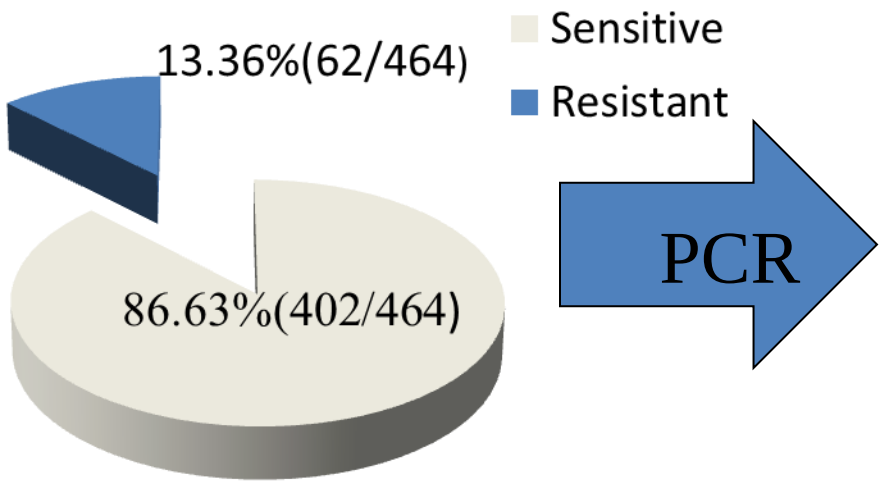
Conclusions

16S RMTase enzyme mediated resistance is common in North Indian Enterobacteriaceae clinical isolates

i) Novel *rmtF* identified first time in 34 strains, ii) rmtF causes methylation at G1405 of 30s ribosomal subunit of 16S rRNA and iii) rmtF positive strains co-produced NDM, CTX-M and AmpC

16S RMTase, *rmtB* reported first time in India

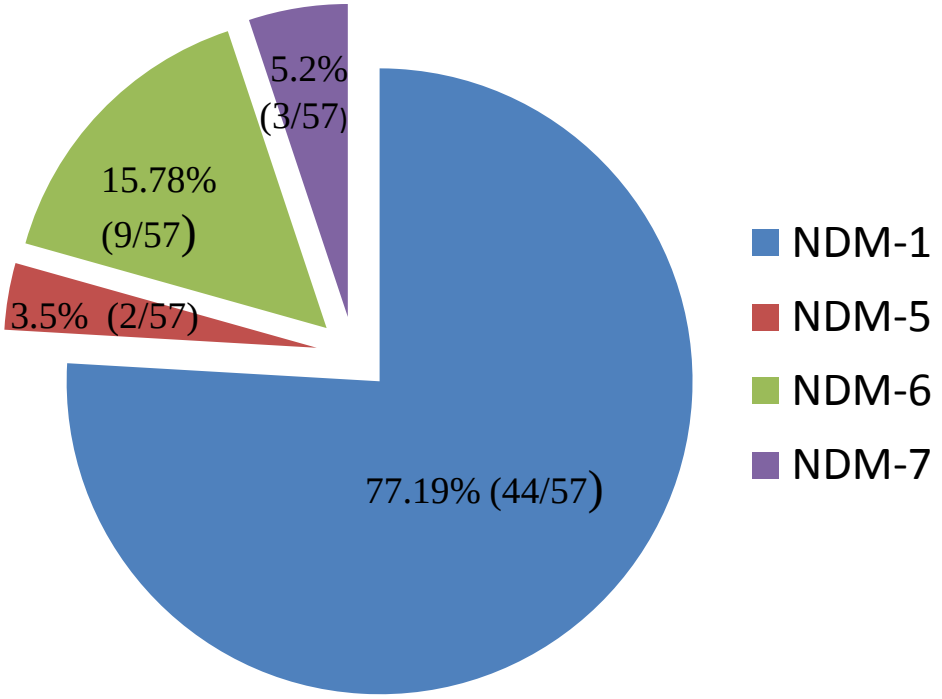
Carbapenem resistance in Enterobacteriaceae (n=464)

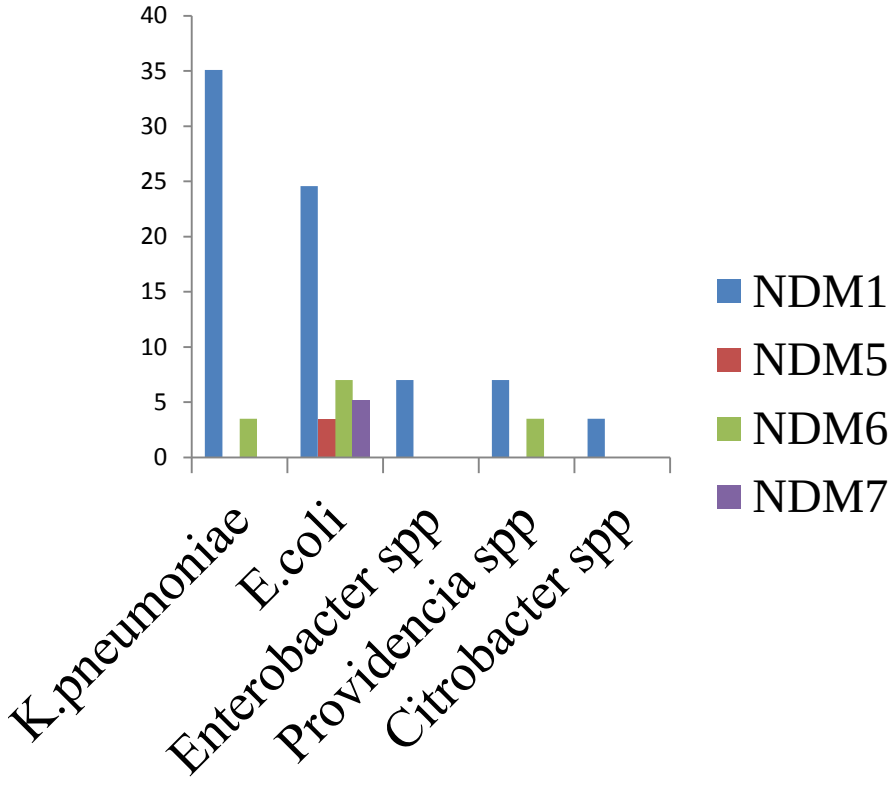


PCR

57 (12.2%) isolates were NDM positive

Prevalence of NDM variants



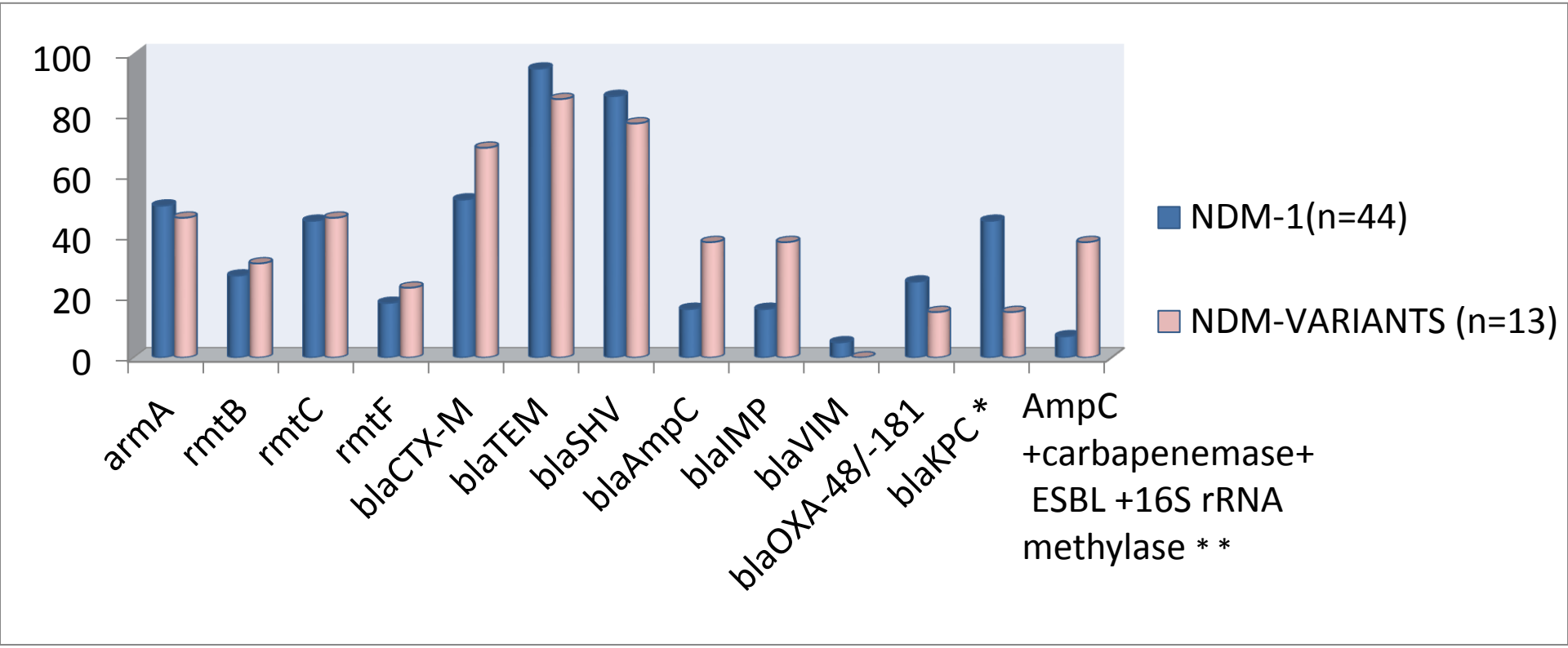


NDM variants were more frequently found in *E. coli*

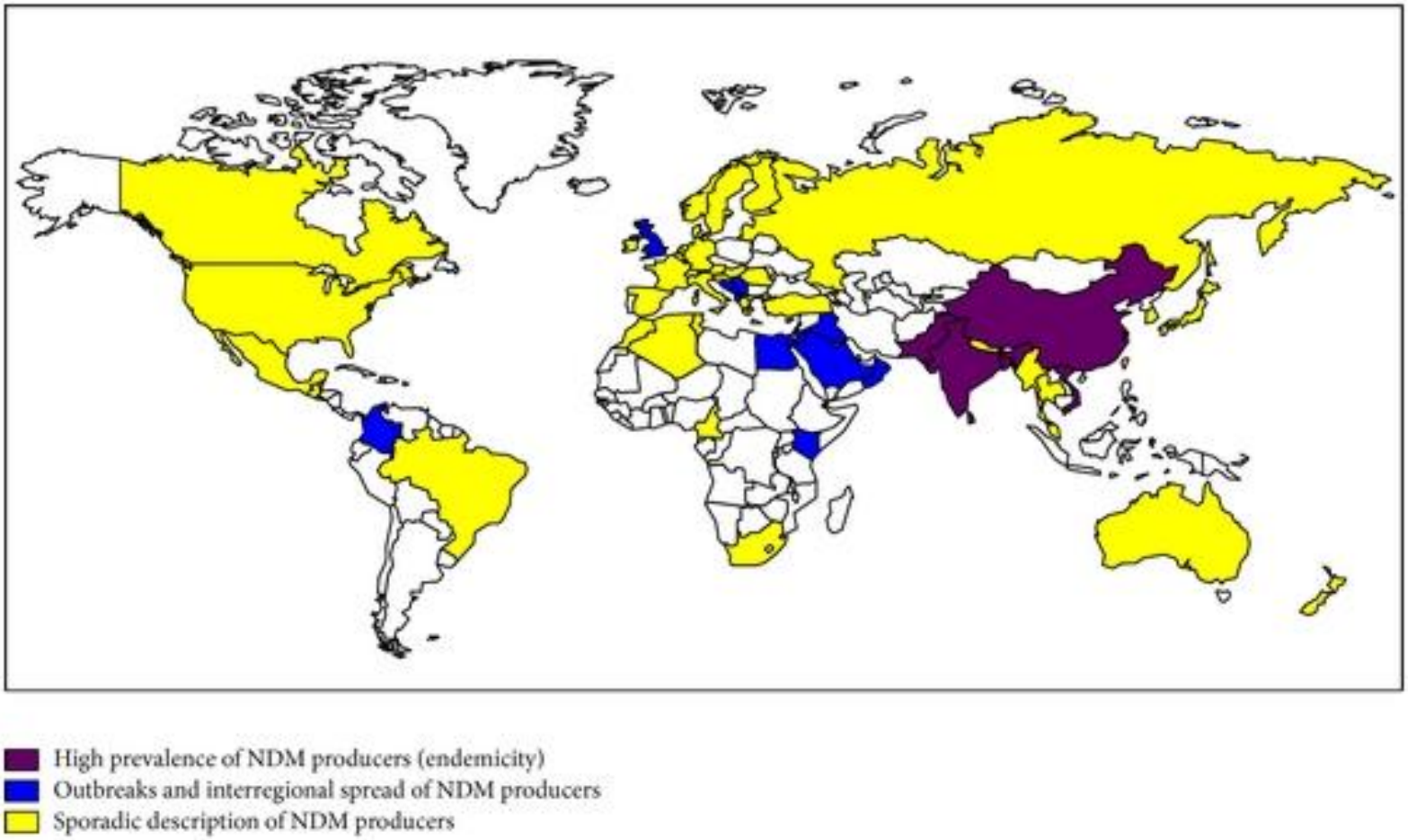
Assessment of phenotypic resistance to carbapenem by cloning *bla*$_{\text{NDM-1}}$ and *bla*$_{\text{NDM}}$ variants in *E. coli* Topo10.

Cloning and expression experiment showed the hydrolytic activity of NDM to carbapenem in the following order: NDM7 (MIC 128 µg/ml)- NDM5 (MIC 64 µg/ml)- NDM6= NDM1 (MIC 32 µg/ml)

Additional resistant genes in *bla*$_{NDM}$ positive isolates



** Associated with NDM variants (*P* = 0.003)



World wide distribution of NDM producers

Dortet et al, 2014; BioMed Research International

Incidence of NDM-1 in India

New-Delhi – 2010
291 environnemental samples
18% NDM-1

Mumbai, New-Delhi, Pune
1443 Enterobacteriaceae
39 CRE (3%)
15 NDM-1 only (35%)

Mumbai 2013
74 Enterobacteriaceae
38% NDM-1* only
16% NDM-1* +OXA-48-like
7% NDM-1* +VIM

Mumbai 2012
113 Enterobacteriaceae
76% NDM-1* only
15% NDM-1*+OXA-48-like
4% NDM-1*+VIM

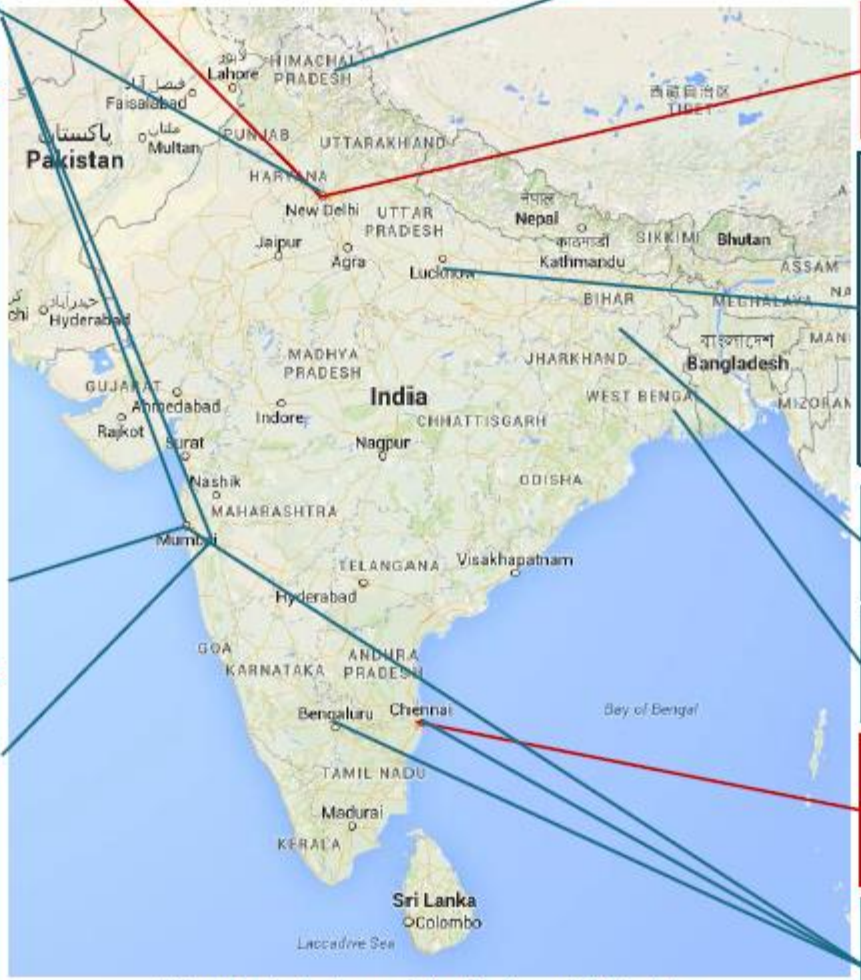
Mumbai 2010
57 CRE
86% NDM-1

Mumbai 2009
24 CRE
92% NDM-1

Pune 2012
300 E. coli:
15% CRE
44% NDM-1* only
55% NDM-1* +OXA-48

Pune 2012-outbreak
K. pneumoniae NDM-1 only

Pune 2010
885 Gneg
20% CRE
11% NDM-1



« * »: doubt about correct identification of NDM alleles
« (only) »: when other carbapenemases have been screened
Red: community samples
Blue: hospital samples

Kashmir valley-2011-2012
1625 Gneg
6% IMI^{R}
29% classA, 15% classB
9% NDM-1* only

Haryana – 2009
198 Enterobacteriaceae
24% CRE
55% NDM-1

Lucknow -2012
464 Enterobacteriaceae
12% CRE
77% NDM-1
22% other NDM variants (2 NDM-5, 8 NDM-6, 3 NDM-7)
45% of NDM with KPC
25% of NDM with OXA-48-like

Varanasi - 2010
780 Enterobactariaceae
7% NDM-1

Kolkata-2007-2011
105 Enterobacteriaceae
14% NDM-1 only

Chennai – 2009
3521 Enterobacteriaceae
4% CRE
31% NDM-1

Mumbai, Chennai, Bangaluru
252 Enterobacteriaceae
5% NDM-1 only

Conclusions

NDM variants (NDM5, NDM6 and NDM7) are emerging among clinical isolates of Enterobacteriaceae

NDM variants (especially NDM7) are more efficient in hydrolyzing carbapenems

NDM variants plasmids also carried resistant genes to four different other classes of antibiotics

Probably exposure to higher doses of carbapenem give rise to mutation of bla_{NDM1} leading to emergence of NDM variants

References for further reading:
Hidalgo et al. J Antimicrob Chemother 2013; 68: 1543-50.
Rahman et al. Int J Antimicrobial Agents 2014; 44: 30-37.



Thank you for your kind attention